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Phylogeography in sexual and parthenogenetic European Oribatida

Dissertation zur Erlangung des akademischen Grades eines Doctor rerum naturalium an der Georg-August Universität Göttingen

vorgelegt von

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Summary

Oribatid mites (Acari, Oribatida) are a species rich group which may form the oldest group of Chelicerata, as indicated by fossils from Devonian sediment (~380 million years) and molecular clock data (~570 million years). They are ubiquitous soil living arthropods and important decomposers. In oribatid mites parthenogenesis is common, about 10% of the individuals reproduce parthenogenetically, and they likely radiated while being parthenogenetic. Their high abundance (up to 400.000 individuals per square meter in temperate and boreal forest soils) and their species richness (10.000 described species) render oribatid mites ideal model organisms to answer evolutionary and ecological questions. Using molecular markers I investigated the genetic diversity of two sexual and two parthenogenetic European oribatid mite species.

(1) Genetic diversity in soil living microarthropods

Pleistocene glaciations shaped the genetic and species diversity of Europe. Using the mitochondrial gene of the cytochrome c oxidase (*COI*) I investigated the genetic structure of the soil living oribatid mite species *Steganacarus magnus*. The high intraspecific genetic variance of *COI* at nucleotide (32% uncorrected p-distance) and protein level (5% uncorrected p-distance) suggests that the climatic change had no strong influence on *S. magnus*. It survived the last ice ages in cryptic refugia, radiated in the Miocene or earlier and colonized Europe after the last ice age from cryptic refugia.

(2) Cryptic species complex in *Steganacarus magnus*

Intraspecific variance of mitochondrial DNA higher than 3% indicates a cryptic species complex. The high intraspecific distance in *COI* of the oribatid mite species *S. magnus* (up to 32%) indicates the existence of cryptic species. Using one mitochondrial (*COI*) and one nuclear marker (elongation factor 1 alpha; *ef 1α*) I investigated if there is a cryptic species complex in *S. magnus*. The results suggest that *S. magnus* does not comprise a cryptic species complex; phylogenetic trees of the genes studied were different indicating recombination between lineages.

(3) Post- and pre-glacial colonization of Europe by sexual and parthenogenetic oribatid mite species

Belowground organisms could have survived low temperatures undamaged and therefore survived the ice age in cryptic refugia. Using the molecular marker *COI* I investigated the colonization events of two sexual (*Achipteria coleoptrata*, *S. magnus*) and two parthenogenetic (*Nothrus silvestris*, *Platynothrus peltifer*) European oribatid mite species. Each oribatid mite species showed a different colonization pattern of Europe. *A. coleoptrata*, *S. magnus* and *P. peltifer* had high nucleotide divergences (19% *A. coleoptrata*, 31% *S. magnus*, 20% *P. peltifer*) but only the two sexual oribatid mite species also had high protein divergences (3% *A. coleoptrata*, 4% *S. magnus*). *N. silvestris* had low nucleotide (2%) and protein (0%) divergences. The results indicate that *A. coleoptrata*, *S. magnus* and *P. peltifer* radiated in the Miocene and survived the Pleistocene ice ages in cryptic refugia. IN contrast, *N. silvestris* did not survive the ice age in cryptic refugia but colonized Central and Northern Europe thereafter and radiated in the Holocene. The high protein variance in sexual and the low variance in parthenogenetic oribatid mites provided hints on mechanisms responsible for the maintenance of sexual reproduction.

Zusammenfassung

Oribatiden (Hornmilben) sind eine sehr alte und artenreiche Gruppe der Chelicerata; Fossilfunde aus dem frühen Devon (vor ~380 Millionen Jahren) belegen den Ursprung der Gruppe. Molekulare Datierungen weisen jedoch auf eine weitaus ältere Entstehung der Oribatiden im Präkambrium hin (vor ~570 Millionen Jahren). Auffällig bei Hornmilben ist, dass Parthenogenese weit verbreitet ist; ca. 10% der Oribatidenarten reproduzieren parthenogenetisch und radiieren sogar, wobei aber nur 1% aller Metazoen sich parthenogenetisch reproduzieren. Sie kommen ubiquitär in Bodensystemen vor und sind wichtige Zersetzer. Der Artenreichtum (10.000 beschriebene Arten) und ihre hohe Abundanz (bis zu 400.000 Individuen pro Quadratmeter in bodensauren Wäldern der temperierten Breiten) machen Oribatiden zu idealen Modellorganismen für evolutionsbiologische und ökologische Fragestellungen. In dieser Arbeit wurde die genetische Diversität zweier sexueller und zweier parthenogenetischer europäischer Oribatiden mit molekularen Markern untersucht.

(1) Genetische Diversität von im Boden lebendem Mikroarthropoden

Der Artenreichtum und die genetische Diversität Europas wurden durch die letzte Eiszeit stark beeinflusst. Mit Hilfe des mitochondrialen Gens der Cytochromoxidase I (*COI*) wurden die Auswirkungen auf die im Boden lebende Hornmilbe *Steganacarus magnus* untersucht. Die hohe intraspezifische Varianz der *COI* auf Nukleotid- (32% unkorrigierte p-Distanz) und Proteinebene (5% unkorrigierte p-Distanz) zeigen deutlich, dass die pleistozäne Vereisung auf die im Boden lebende Art *S. magnus* keinen starken Einflüsse ausgeübt hat. Die hohe Nukleotidvarianz weist daraufhin, dass diese Art die Eiszeit in kryptischen Refugien überdauert hat, wogegen sie im Miozän oder sogar schon früher diversifiziert ist und sich im Holozän weiter verbreitet hat.

(2) Kryptischer Artenkomplex in *Steganacarus magnus*

Über 3% innerartliche Varianz in mitochondrialer DNS weist auf einen kryptischen Artenkomplex hin. Die innerartliche Varianz bei der sexuellen Oribatide *S. magnus* lag bei bis zu 32%, was auf einen kryptischen Artenkomplex hindeutet. Um festzustellen, ob es sich tatsächlich um einen kryptischen Artenkomplex handelt, wurden *COI* und das nukleare Gen des Elongationsfaktors 1 alpha (*ef 1α*) untersucht. Das Ergebnis zeigte keinen kryptischen Artenkomplex in *S. magnus*, da sich die Topologien der phylogenetischen Bäume unterschieden, was darauf hindeutet, dass Individuen der verschiedenen mitochondrialen Linien untereinander Nachkommen produzieren.

(3) Unterschiedliche vor- und nacheiszeitlichen Kolonisation von Europa durch sexuelle und parthenogenetische Oribatiden

Bodenorganismen können niedrige Temperaturen über einen längeren Zeitraum unbeschadet überdauern und sind daher nur wenig von den Eiszeiten beeinflusst worden. Um herauszufinden, ob parthenogenetische und sexuelle Bodenorganismen sich in ihren nacheiszeitlichen Kolonisationsmustern unterscheiden, wurde der mitochondriale Marker *COI* von zwei parthenogenetischen (*Nothrus silvestris*, *Platynothrus peltifer*) und zwei sexuellen Oribatidenarten (*Achipteria coleoptrata*, *S. magnus*) europaweit untersucht. Jede Oribatidenart zeigte ein anderes Muster der Kolonisation von Europa. *A. coleoptrata*, *S. magnus* und *P. peltifer* wiesen eine hohe Nukleotidvarianz auf (19% *A. coleoptrata*, 31% *S. magnus*, 20% *P. peltifer*), wobei nur die sexuellen Arten auch eine hohe Proteinvarianz hatten (3% *A. coleoptrata*, 4% *S. magnus*). *N. silvestris* zeigte eine geringe Nukleotid- (2%) und Proteinvarianz (0%). Die hohe Nukleotidvarianz weist auf einen präglazialen Ursprung hin (mit einer Radiation im Miozän) während die geringe Nukleotidvarianz auf

einen postglazialen Ursprung hinweist (und eine Radiation im Holozän). Die hohe Proteinvarianz bei sexuellen und die niedrige Proteinvarianz bei parthenogenetischen Oribatiden bietet Hinweise auf Mechanismen für die Aufrechterhaltung sexueller Reproduktion.

Chapter One

General Introduction

1.1 Phylogeography

Phylogeography explores the phylogenetic and geographical origin of genetic lineages of a taxon thereby combining biogeography, population genetics and phylogenetic analyses.

1.1.1 Ice ages

Since the beginning of the Quaternary about 3 million years ago the climate in Europe oscillated several times from cold (ice age) to warmer periods (interglacial) (Hewitt 2000, 2004). These ice ages shaped the biodiversity of the European flora and fauna and were named after their maximum extension (Biber, Donau, Günz, Saale and Weichsel). The last ice age began 115.000 years and ended about 10.000 years before present. The ice sheet covered North Europe including Northern Germany, Poland and the Baltic States; glaciers also covered part of the Alps, the Pyrenees, the Carpathians, the Apennines and the Balkan. Steppe, tundra and permafrost expanded in Central Europe (Fig. 1). The living space for animals and plants shrunk to areas south of the Balkan, the Alps and the Pyrenees (Hewitt and Ibrahim 2001). In the past twenty years, several phylogeographic studies investigated the role of these refugia for populations and the current distribution of species by analyzing genetic data in a spatial context (Beheregaray 2008).

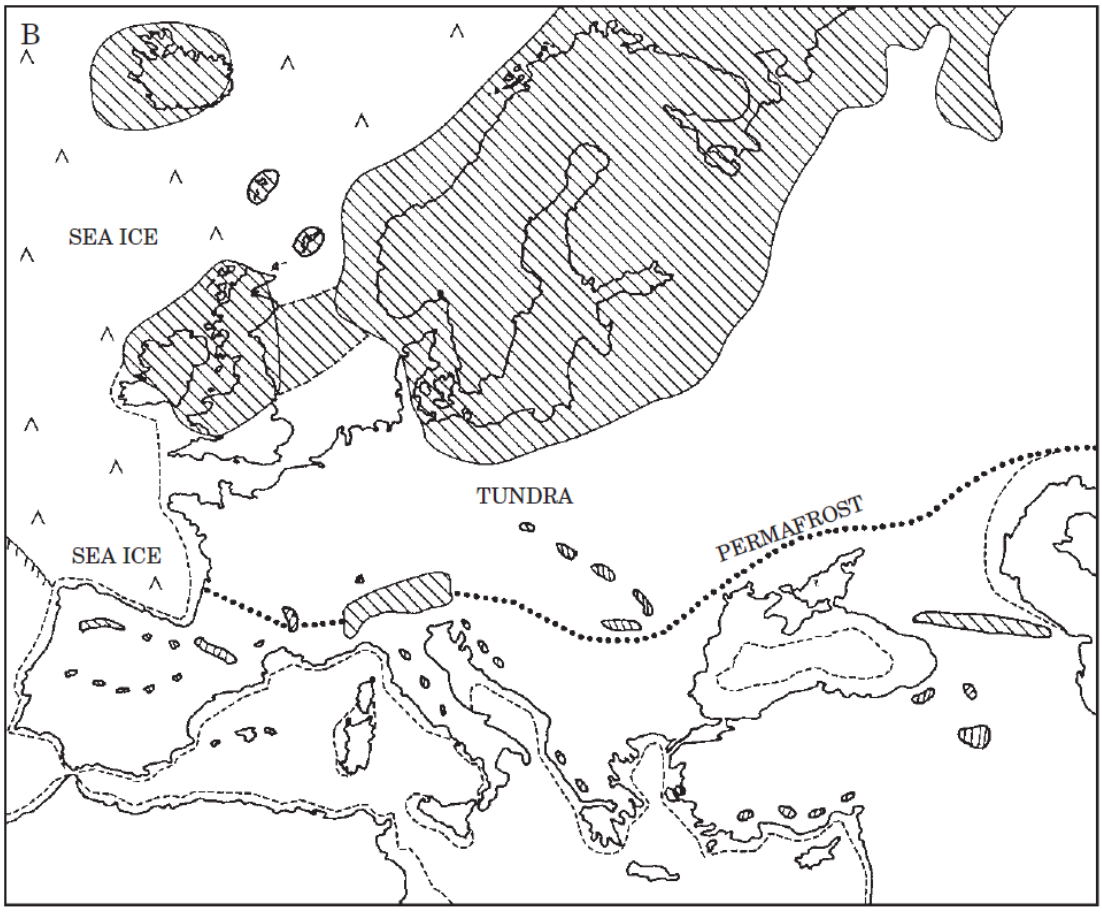

Figure 1: Map of Europe at the last glacial maximum (20.000 years before present). The shaded area represents the ice cover and the dotted line the Permafrost border (from Hewitt 1999).

1.1.2 Cryptic refugia

Cryptic refugia were areas in Central and Northern Europe, which were not covered with an ice sheet or had permafrost soil at the last glacial maximum (LGM) 20.000 years ago. Stewart and Lister (2001) recently discussed the existence of these Northern refugia for European biota. Their theory of cryptic refugia is supported by studies using pollen, molecular and radiocarbon analyses (Willis *et al.* 2000, Stewart and Lister 2001, Verovnik *et al.* 2005, Tollefsrud *et al.* 2008). However, these areas are difficult to detect, since presumably they were small, geographically isolated or located in wind protected valleys.

Survival of populations in central refugia implies that species expanding from southern refugia met populations that experienced a different demographic and ecological history for some period of time. Determining the extent of refugia at higher latitudes and their genetic contribution to present day populations is important to understand migration rates and the role of local adaptations to gene flow among populations, both of which are important factors to understand speciation processes.

1.1.3 Barcoding

DNA barcoding is a molecular technique which revolutionized taxonomy by allowing to delineate species on the basis of molecules. For DNA barcoding of metazoan animals a short gene fragment (~660bp) of the mitochondrial cytochrome c oxidase gene is commonly used (Hebert *et al.* 2003a-b, 2004 a-b, Ball *et al.* 2005, Hajibabaei *et al.* 2007). A divergence of over 3% in the nucleotide sequences is taken as indication for the existence of distinct species (Hebert *et al.* 2003a). The mitochondrial genome is maternally inherited and evolves with a faster rate than the nuclear genome (Wolstenholme 1992, Boore 1999). This makes mitochondrial genes a perfect tool for DNA barcoding.

1.1.4 Molecular markers

In this study I used the mitochondrial gene of the cytochrome c oxidase subunit I and the nuclear gene of the elongation factor 1 α. Both genes were used in several phylogeographic studies (Danforth *et al.* 1999, Verovnik *et al.* 2005) and in phylogenetic studies in oribatid mites (Heethoff *et al.* 2007, Domes *et al.* 2007a, Laumann *et al.* 2007, Dabert *et al.* 2010).

Cytochrome c oxidase subunit I

The enzyme cytochrome c oxidase (*COI*) is a large transmembrane protein complex and is located in the mitochondrial membrane in eukaryotes and in the cell membrane in prokaryotes. It is a heme-copper oxidase (Saraste 1990) and the terminal energy transfer enzyme in the respiratory chain (Mitchell 1966). It catalyzes the electron transfer from cytochrome c to molecular oxygen thereby reducing oxygen to water. The enzyme complex consists of 13 subunits in mammals and the catalytic center is located in the subunit I (Kadenbach and Stroh 1984; Blenkinsop *et al.* 1996; Arnold and Kadenbach 1997). However, only the subunits I-III are located in the mitochondrion, the other subunits are located in the nucleus. The subunit I of the cytochrome c oxidase consists of twelve transmembrane helices and contains two heme (heme a and heme a_3) and one copper (Cu_B) centers (Fig. 2).

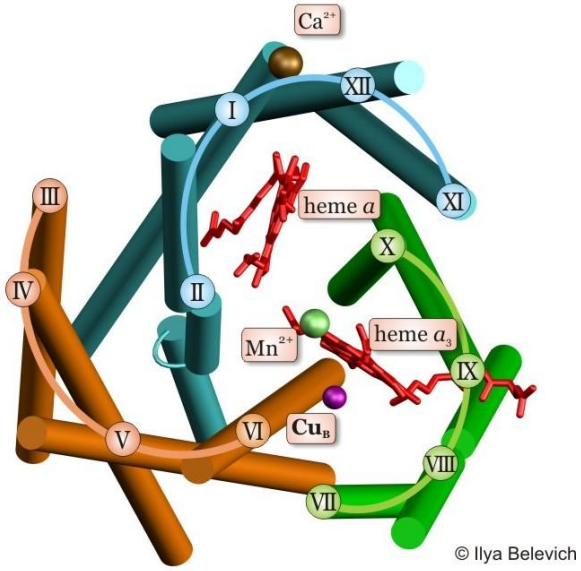

Figure 2: Model of the subunit one of the cytochrome c oxidase complex. The twelve helices are brown, green and blue. The catalytic center contains the two heme groups (red) and one copper atom (magenta).
Source: www.biocenter.helsinki.fi/bi/biophys/research_CcO_Str.html1.1.3.2

Elongation factor 1 α

The gene of the elongation factor 1 α (*ef 1α*) is located in the nucleus and is highly conserved (Keeling and Inagaki 2004). It is a single copy gene with a length of 1,430 bp (Klompen 2000) and a member of GTPase superfamily (Baldauf *et al*. 1996, Keeling *et al*. 1998, Keeling and Inagaki 2004). The e*f 1α* protein is associated with the ribosomes at the protein biosynthesis and catalyses the binding between the incoming amino acid and the growing polypeptide chain. It builds a binary complex with GTP which is associated with the aminoacyl-tRNA. This complex binds under GTP hydrolysis to the ribosome and thereby the elongation factor and GDP + P_i is set free.

1.2 *Oribatid mites*

Acari are one of the oldest, most abundant and diverse arthropod groups (Walter and Proctor 1999). Over 42,000 species are clustered in three major groups, the Opilioacaridae (20 species), the Parasitiformes (>10,000 species) and the Acariformes (>30,000 species) (Krantz 1978, Evans 1992, Walter and Proctor 1999, Krantz and Walter 2009). The Acariformes are grouped into Prostigmata, Astigmata, Oribatida and the paraphyletic Endeostigmata (O'Connor 1984, Walter 2001). The oribatid mites are the largest subgroup of the Acariformes with 10,000 described species (Schatz 2002, Subias 2004), but estimated numbers range from 50,000 (Travé *et al.* 1996) to 100,000 species (Schatz 2002). Oribatid mites are grouped into six subgroups, the two basal groups Palaesomata and Enarthronota, the small group Parhyposomata, the paraphyletic "Mixonomata", the "Desmonomata" with mainly parthenogenetic taxa and the higher Oribatida the Circumdehiscentiae (=Brachypylina) (Grandjean 1953, 1965, 1969). Fossils of oribatid mites were found in Devonian sediments 380 million years ago (mya) (Shear *et al.*1984, Norton *et al.* 1988a) but the origin of this group is dated back to 440 mya (Lindquist 1984) and molecular clock analyses date the origin of the Oribatida back to 570 mya (Schaefer *et al.* 2010).

Oribatid mites are soil-dwelling detritivorous and fungivourous microarthropods (Maraun and Scheu 2000, Schneider *et al.* 2004). Some species also prey on nematodes (Muraoka and Ishibashi 1976, Schneider *et al.* 2004, K. Heidemann unpublished data). In forests of the temperate and boreal zone they reach densities of up to 400,000 individuals per square meter (Maraun and Scheu 2000).

Achipteria coleoptrata (Linné, 1758)

The sexual oribatid mite species *Achipteria coleoptrata* (Oribatida, Brachypylina) (Fig. 3) has a size of 530-650 µm, is distributed throughout the Holarctic and lives in wet meadows and forests (Weigmann 2006, Subias 2009). In the laboratory it feeds on bark algae (*Desmococcus vulgaris*), grasses, herb litter and fungi (Hubert *et al.* 2001). The generation time is one year (Luxton 1981). Fossilis of Achipteriidae are known from Jurassic and Baltic amber (Labandeira *et al.* 1997, Krantz and Walter 2009).

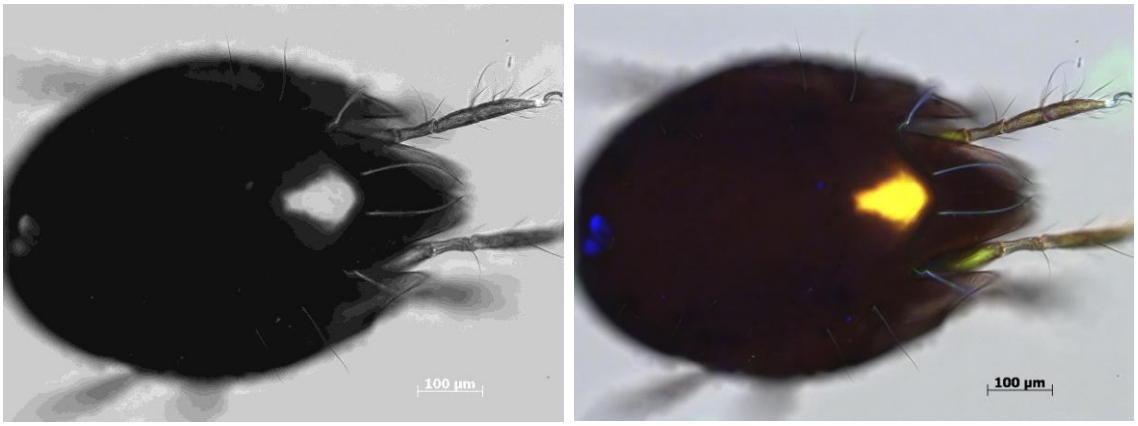

Figure 3: Scanning microscope image of *Achipteria coleoptrata* (left: black and white, right: fluorescence).

Steganacarus magnus (Nicolet, 1855)

The sexual oribatid mite species *Steganacarus magnus* (Oribatida, "Mixonomata") (Fig. 4) has a size of 700-1200 µm (Weigmann 2006) and is distributed throughout the Palearctic (Subias 2009). *S. magnus* has a generation time of one year from egg to adult (Webb 1977, 1989). Larval stages and nymphs live endophagous in lignified plant tissues. It is cold resistant, adult individuals can survive temperatures of up to -12°C and juveniles of up to -14°C undamaged (Webb and Block 1993); its supercooling point ranges between -7°C and -38°C (Block 1979, Krantz and Walter 2009). *S. magnus* is also tolerant against drought and heat (Siepel 1996). Generally, *S. magnus* functions as primary decomposer (Schneider *et al.* 2004) but he may also feed on nematodes (K. Heidemann unpublished data).

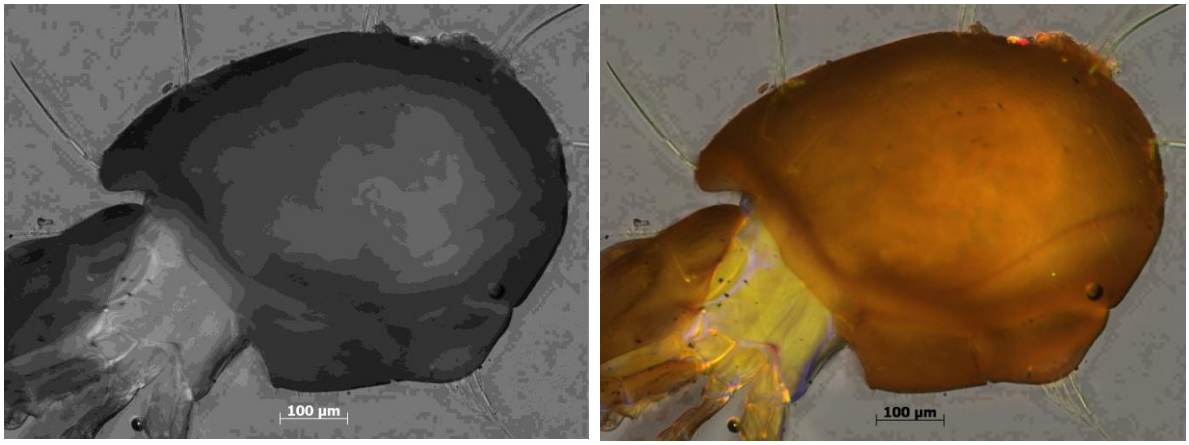

Figure 4: Scanning microscope image of *Steganacarus magnus* (left: black and white, right: fluorescence).

Nothrus silvestris (Nicolet, 1855)

The parthenogenetic oribatid mite species *Nothrus silvestris* (Oribatida, "Desmonomata") (Fig. 5) has a size of 710-810 µm and is distributed in acidic soils and mesophilic forests in the Holarctic and

Neotropis (Weigmann 2006, Subias 2009). It is assumed that *N. silvestris* is a primary decomposer and feeds on decaying plants and fungi (Siepel 1990). Recent analysis showed that *N. silvestris* also preys on nematodes (K. Heidemann unpublished data).

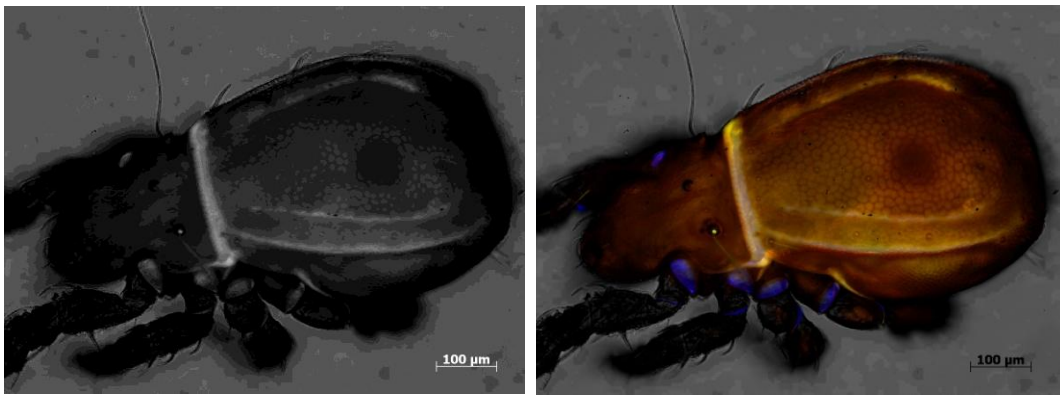

Figure 5: Scanning microscope image from *Nothrus silvestris* (left: black and white, right: fluorescence).

Platynothrus peltifer (C. L. Koch, 1839)

The parthenogenetic oribatid mite species *Platynothrus peltifer* (Oribatida, Desmonomata) (Fig. 6) is a cosmopolitan species (Subias 2009) with a size of 770-980 µm. It occurs in various terrestrial (Weigmann 2006) but also in freshwater and benthic habitats (Schatz and Gerecke 1996, Krantz and Walter 2009). *P. peltifer* is tolerant against salt, heat, drought (Siepel 1996) and metal contamination from smelters (Zaitsev and Van Straalen 2001). It is assumed that *P. peltifer* is a primary decomposer and feeds on decaying plant material and fungi (Siepel 1990, Schneider *et al.* 2004) but it also preys on nematodes (K. Heidemann unpublished data). The generation time of *P. peltifer* is one year in temperate European forests (Weigmann 1975, Schenker 1986, Krantz and Walter 2009).

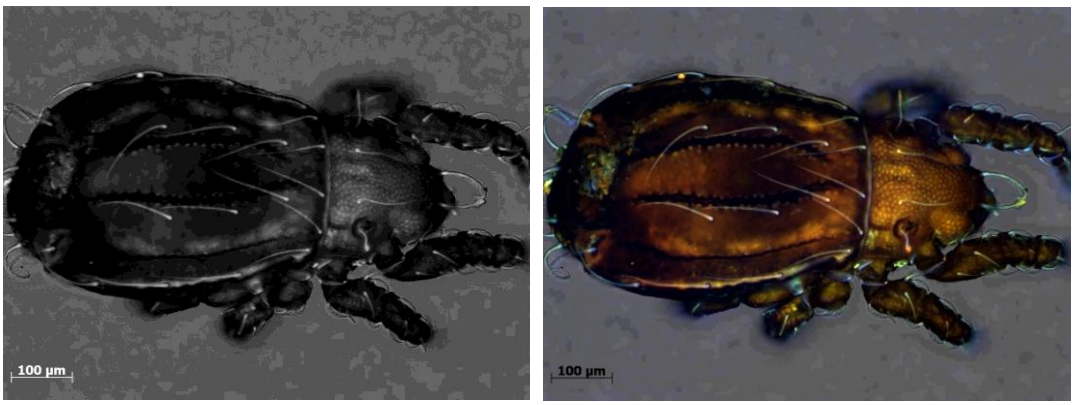

Figure 6: Scanning microscope image of *Platynothrus peltifer* (left: black and white, right: fluorescence).

1.3 *Sex versus parthenogenesis*

Sexual reproduction is the most widespread reproductive mechanism in animals; >99% of all metazoan taxa reproduce sexually (Bell 1982). Parthenogenetic reproduction is characterized by developing offspring from unfertilized eggs (Hughes 1989). Parthenogenesis includes different genetic mechanisms such as deuterotoky, arrhenotoky, pseudo-arrhenotoky and thelytoky (Bell 1982, Hughes 1989). Species reproducing by deuterotoky produce males and females from reduced eggs and diploidy is restored postmeiotically. Arrhenotoky is a reproductive mode where females develop from fertilized eggs and males from unfertilized eggs. In pseudo-arrhenotoky males and females develop from fertilized eggs and males become haploid later. These three mechanisms are not truly parthenogenetic, i.e. unisexual, since both sexes are needed and produced. In contrast, thelytoky is

characterized by the development of female offspring from unfertilized eggs and is called the "true" parthenogenesis as no males occur. Thelytoky is divided into two different forms, apomixis and automixis. In apomixis meiosis is suppressed and the offspring is genetically identical to the mother. In automixis meiosis is retained and the diploid status is restored -premeiotically- or by post-meiotic processes (Maynard Smith 1978, Hughes 1989), such as terminal or central fusion of meiotic products (Suomalainen *et al.* 1987, Stenberg and Saura 2009). In central fusion automixis the two central polar nuclei fuse (i.e. fusion of non sister chromatids). The maternal heterozygosity is restored, if the mother was heterozygous. In terminal fusion automixis the egg nucleus fuses with the second polar nucleus (i.e. fusion of sister chromatids) which results in homozygous offspring by a heterozygous mother (Wrench *et al.* 1994, Stenberg and Saura 2009, Heethoff *et al.* 2009). Parthenogenetic oribatid mites such as *P. peltifer* reproduce by automixis and restore diploidy by terminal fusion (Taberly 1987, Heethoff *et al.* 2009).

Nonsexual species have a number of advantages compared to sexual species, e.g. the twofold advantage of not producing males (Williams 1975, Maynard Smith, 1978, 1998, Tagg *et al.* 2005), faster colonization of habitats and easier establishment of new populations (Williams 1975; Bell 1982; Scheu and Schulz 1996; Lindberg and Bengtsson 2005, Schön 2007). However, for long-term survival sexual reproduction appears to be indispensible to counteract the accumulation of detrimental mutations (Mullers ratchet, Muller 1964; Mutational Load Theory, Kondrashov 1988, Butlin *et al.* 1999). However, this view is challenged by the existence of "ancient asexuals" (Maynard Smith 1978), which radiated while being parthenogenetic as is assumed to be in the case in the bdelloid rotifers with ~360 species (Welch and Messelson 2000), darwinulid ostracods with 36 species (Martens *et al.* 2003) and oribatid mites with more than 400 species (Norton *et al*. 1988b, Palmer and Norton 1990, Norton and Palmer 1991, Maraun *et al.* 2003).

1.4 *Objectives*

The present work investigated evolutionary aspects in parthenogenetic and sexual oribatid mites using molecular markers. Chapter II investigated the postglacial colonization of the sexual oribatid mite species *S. magnus* in Europe. I expected that *S. magnus* survived the last ice age in cryptic refugia and colonized Central and Northern Europe from there, since *S. magnus* is cold tolerant, small and has low dispersal ability.

Chapter III based on the high genetic diversity in *COI* in *S. magnus* (Chapter II) which could be a result of a cryptic species complex. To investigate if *S. magnus* comprises a cryptic species complex, mt (*COI*) and nuclear (*ef 1α*) were sequenced from the same individual from regions with different mt lineages.

In Chapter IV I investigated the postglacial colonization of two sexual (*A. coleoptrata* and *S. magnus*) and two parthenogenetic (*N. silvestris* and *P. peltifer*) oribatid mites species in Europe to detect cryptic refugia and to develop general postglacial colonization patterns for soil living oribatid mites based on the mt gene *COI*. I expected that parthenogenetic species have the advantage of faster colonization and that the genetic diversity of *COI* is similar in parthenogenetic and sexual oribatid mite species since mitochondria inherited maternally.

Chapter Two
Genetic diversity in a soil living microarthropod species: Cryptic species and reconstruction of the evolution of genetic complexity

Summary

Since the beginning of the Quaternary (~3 million years ago) ice ages shaped the biodiversity of Europe. The living space for many aboveground species shrunk to areas south of the Alps, the Pyrenees and the Balkans. If this was also true for soil living animals is unknown. I analyzed 180 individuals of the soil living oribatid mite species *Steganacarus magnus* from 47 locations in Europe and Central Asia using the mitochondrial gene of the cytochrome c oxidase (*COI*). The 180 sequences constituted 111 haplotypes for the nucleotide and 67 haplotypes for the protein. The maximum difference between populations was 31.8% in the nucleotide and 4.7% in the protein. Three different *COI* main lineages exist in *S. magnus* and coexist in the same sample of several Northern European locations. I conclude that the soil living oribatid mite *S. magnus* did not go through bottlenecks during the last ice ages contrasting the situation in aboveground animals and plants. The results suggest that individuals survived in multiple cryptic Northern refugia in Northern Germany, Poland and Scandinavia. The high genetic divergences between populations of *S. magnus* likely resulted at least from radiation in the Miocene (~20 mya).

2.1 Introduction

Climatic changes during the past 2.6 million years caused considerable range shifts in species' distributions in the Northern hemisphere and led to contractions and expansions in population sizes in both warm and cold adapted species. The effects of the last glacial maximum (LGM – 18-22 kya) on species' diversity has been intensively investigated for warm adapted, aboveground and freshwater animals and plants (Beheregaray 2008). The overall pattern has been characterized by genetic richness in Southern European countries and genetic paucity in Northern Europe (Hewitt 1999, Hewitt and Ibrahim 2001). This pattern supports the hypothesis that most taxa retreated during ice ages to refugia south of the Alps, whereas populations expanded rapidly into higher latitudes with post-glacial climatic warming (Hewitt 2000). Presumably, the Iberian Peninsula, Italy, Greece and the Balkan served as major glacial refuge areas where retreating populations met and escaped hostile climatic conditions in Central and Northern Europe.

Extending this view, radiocarbon (Stewart and Lister 2001), pollen (Willis *et al.* 2000; Tollefsrud *et al.* 2008, Kelly *et al.* 2010) and molecular data (Pfenninger *et al.* 2003; Verovnik *et al.* 2005; Tollefsrud *et al.* 2008) indicate that isolated areas in Central and Northern Europe also acted as refuges for species of the temperate and boreal zone (Bilton *et al.* 1998; Willis *et al.* 2000; Stewart and Lister 2001; Stewart 2003). These cryptic refugia (Stewart and Lister 2001; Provan and Bennett 2008), however, are difficult to detect since they presumably were small, geographically isolated or located in wind protected valleys or on ice free Nunataks (Schmitt 2009). Cryptic refugia resemble climatic islands in which conditions are more favorable than in surrounding areas and describes a species' distribution range during its contraction phase at glacial maxima (Stewart and Lister 2001; Stewart *et al.* 2010). Expansions and contractions of populations into refugia significantly affected the evolution and genetic variation of species (Stewart *et al.* 2010). Therefore, understanding the contribution of cryptic refugia during glacial and interglacial phases is important for understanding speciation, biodiversity and intraspecific variance of species of the Northern Hemisphere.

Oribatid mites (Acari, Oribatida) are among the most diverse and abundant soil living arthropods with about 10,000 described species (Schatz 2002) and up to 400,000 individuals per square meter of forest soil (Maraun and Scheu 2000). Fossils of oribatid mites were found in Devonian sediments (380 my ago; Shear *at al.* 1984, Norton *et al.* 1988a) and the origin of oribatid mites dates back to 440 my (Lindquist 1984) or even 570 my according to molecular dating (Schaefer *et al.* 2010).

The soil living oribatid mite species *Steganacarus magnus* (Nicolet, 1855) serves as excellent model organism to investigate the existence of cryptic refugia in Central and Northern Europe. This species requires ligneous plant tissues for development as larval stages and nymphs live endophagous in coniferous needles or leave petioles (Norton 1994). The dependence on host plants is rather unspecific and relatively loose as *S. magnus* predominantly feeds on dead organic material (Schneider *et al.* 2004). This implies that this species could persist in geographically isolated areas during glacial phases as long as habitat tolerances were met and plants producing ligneous needles or leaves were present. Presumably, *S. magnus* was directly affected by changing climatic conditions in its natural distribution range, as the species has a palearctic distribution (Subías 2009) and very limited dispersal abilities (Salomone *et al.* 2002). However, adults and juveniles survive temperatures of -12°C to -14°C (Webb and Block 1993) and the super-cooling point for this species is between -7°C and -38°C (Block 1979; Krantz and Walter 2009). Tolerance against cold temperatures, drought and heat (Siepel 1996; Krantz and Walter 2009) is common in oribatid mite species (R.A. Norton; personal communication). However, the distribution of *S. magnus* suggests that the species is not strictly cold-adapted but evolved a broad temperature tolerance. Another important character for using *S. magnus* as model organism to infer cryptic refugia is its small body size. (~1.2 mm; Weigmann 2006). Small species are

more likely to persist in cryptic refugia as their population density and habitat capacity exceeds that of large species (Stewart *et al.* 2010). Finally, *S. magnus* is relatively easy to sample, the species is common in temperate forests where it occurs in relatively high densities.

To detect intraspecific genetic diversity and to reconstruct phylogenetic relationships among haplotypes, I analyzed nucleotide and protein sequences of cytochrome oxidase I (*COI*) of 180 individuals of *S. magnus* from 44 sampling sites across Europe and three sampling sites in Central Asia. Sampling in Central Europe was extensive in order to detect cryptic refugia which were proposed for Central and Northern Europe (Stewart and Lister 2001; Tollefsrud *et al.* 2008). I expected that lineages which persisted in Central refugia express isolated haplotypes that are distinct from populations from Southern Europe and Central Asia and that genetic diversity within this species' range is highest in these former refuge areas (Stewart *et al.* 2010).

2.2 Materials and methods

2.2.1 Taxon sampling

Steganacarus magnus was sampled from 47 locations of Europe, Russia and China (Fig. 7, described in more detail in Table 1). Specimens were extracted from leaf litter using heat (Macfadyen 1961, Kempson *et al.* 1963). Animals were identified under a stereomicroscope, determined after Weigmann (2006) and stored in 75% ethanol at -20°C until preparation.

Figure 7: (a) Map of Europe and Asia (source: http:\\www.kelt.de), black dots with numbers are the sampling points; (b) Map of Europe from the last glacial maximum (modified from Hewitt 1999). Black dots with numbers represent the sampling points of oribatid mites used in this study, green shading marks refugia of coniferous trees and yellow shading those of deciduous trees, grey lines mark the expansion of glaciers, the dotted line represents the polar desert climate border and the dashed line the tundra and permafrost border (Hewitt and Ibrahim 2001, Alexander Kartographie 2006); (c) Map of Tenerife (source: Google Earth), white dots with number are the sampling points.

Table 1: Localities where *Steganacarus magnus* was sampled [ln: location number, country, location, coordinates, abbreviations of sampling locations (code) and name of collector (for details see Acknowledgements)]. Each location is marked by a colour symbol.

ln	country	location	coordinates	code	colour	litter collector
1	Austria	Villach	46.57° 13.85°	SM_A_1.1-2		K. Domes-Wehner
2	China	Nanjing	32.06° 118.78°	SM_CHINA_1.1-2		J. Shan
3	Czech Republic	Decin	50.78° 14.23°	SM_CZ_1.1-5		M. Rosenberger
4	Demark	Arhus 2	56.11° 10.21°	SM_DK_4.1-3		T. Bilde
5		Arhus 1	56.13° 10.19°	SM_DK_3.1-3		T. Bilde
6		Copenhagen	55.68° 12.58°	SM_DK_1.1-3		N. Eisenhauer
7		Hjørring	57.48° 9.96°	SM_DK_2.1-5		M. Rosenberger
8	England	Ascot	51.40° -0.68°	SM_GB_2.1-2		A. Milcu
9		Bedford	52.07° -0.44°	SM_GB_1.1-5		A. Milcu
10	Finland	Joensuu	62.58° 29.73°	SM_FIN_2.1		C. Platner
11		Lahti	60.99° 25.65°	SM_FIN_1.1-3		H. Setela
12	France	Haute Loire	44.99° 3.86°	SM_F_4.1-2		A. Jousset
13		Loire	45.56° 4.79°	SM_F_2.1-4		M. Maraun
14		Mont Blanc	45.82° 6.74°	SM_F_1.1-6		A. Jousset
15		Saint Isidore	45.27° -1.09°	SM_F_3.1-5		C. Digl
16	Germany	Black Forest	48.89° 8.43°	SM_D_6.1		K. Heidemann
17		Bonn	50.84° 7.14°	SM_D_9.1-4		R. Koller
18		Cuxhaven	53.86° 8.65°	SM_D_8.1-3		K. Heidemann
19		Goettingen	51.53° 9.96°	SM_D_2.1-9		K. Domes-Wehner
20		Kranichstein	49.89° 8.69°	SM_D_1.1-14		M. Rosenberger
21		Lake Constance	47.71° 9.37°	SM_D_3.1-3		K. Heidemann
22		Mecklen. Seenplatte	53.57° 12.33°	SM_D_4.1-9		I. Schaefer
23		Moerfelden	49.96° 8.55°	SM_D_5.1-3		K. Domes-Wehner
24		Uelzen	52.97° 10.52°	SM_D_7.1-4		H. Treptow
25	Hungary	Piliscaba	47.62° 18.83°	SM_HUN_1.1		C. Csjui
26	Ireland	Swords	53.45° -6.22°	SM_IRL_1.1-2		B. Eitzinger
27	Italy	Grosseto	42.63° 11.11°	SM_I_1.1-10		M. Maraun
28		Parma	44.20° 10.34°	SM_I_2.1-4		M. Maraun
29	Netherlands	Wageningen	51.97° 5.70°	SM_NL_1.1-3		O. Butenschoen
30	Norway	Narvik	68.44° 17.40°	SM_N_1.1-3		O. Butenschoen
31	Poland	Krakow	50.04° 19.84°	SM_PL_1.1-6		S. Scheu
32		Warsaw	52.33° 20.76°	SM_PL_2.1-3		A. Uvarov
33	Romania	Bagau	46.55° 26.72°	SM_RUM_3.1-4		T. Pasca
34		Busteni	45.41° 25.55°	SM_RUM_5.1-4		C. Ivanescu
35		Cluj	46.77° 23.52°	SM_RUM_4.1-3		T. Pasca
36		Sibiu 1	45.64° 23.74°	SM_RUM_1.1-3		S. Scheu
37		Sibiu 2	45.65° 23.70°	SM_RUM_2.1-4		S. Scheu
38		Sinaia	45.35° 25.56°	SM_RUM_6.1-3		C. Ivanescu
39	Russia	Altai Mountains	51.73° 85.76°	SM_RUS_1.1-4		M. Ackermann
40		Novosibirsk	54.97° 83.39°	SM_RUS_2.1-5		A. Uvarov
41	Scotland	Braemar	57.00° -3.40°	SM_GB_3.1-3		A. Jousset
42	Sweden	Stroemstad	58.87° 11.14°	SM_S_2.1-3		G. Kalinkat
43		Umea	63.83° 20.29°	SM_S_1.1		O. Butenschoen
44	Switzerland	Locarno	46.17° 8.77°	SM_CH_1.1		M. Scheu
45	Tenerife	Anaga Mountains 1	28.55° -16.20°	SM_E_2.1		S. Scheu
46		Anaga Mountains 2	28.53° -16.27°	SM_E_3.1-4		S. Scheu
47		Teno Mountains	28.32° -16.83°	SM_E_1.1-2		S. Scheu

2.2.2 DNA extraction and sequencing

Genomic DNA was extracted from single individual using the DNeasy® Blood and Tissue Kit (Qiagen; Hilden, Germany) following the manufacturer's protocol for animal tissue. A 514 bp region of cytochrome c oxidase subunit I (*COI*) was amplified using the primers *COI*arch1 (5´-GGTCAACAAATCATAAAGAYATYGG-3´) and *COI*arch2 (5´-TAAACTTCAGGGTGACCAAAAAATCA-3´) (Heethoff *et al.* 2007), the HotStarTaq® Master Mix Kit (Qiagen; Hilden, Germany) and the SuperHot Taq Mastermix (Genaxxon), respectively. The polymerase chain reaction (PCR) contained 0.5 µl of each primer (100 pmol/µl), 1 µl $MgCl_2$ (25mM), 12.5 µl of HotStarTaq® Master Mix (1.25 units HotStarTaq® polymerase, 100 µM of each dNTP and 7.5 mM $MgCl_2$ buffer solution; Qiagen, Germany) or SuperHot Taq Mastermix [2.5 units SuperHot Taq polymerase, 10 µM of each dNTP and buffer solution (20 mM Tris-HCI (pH 8.3), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Nonidet P40 and 0.5% Tween 20), Genaxxon; Ulm, Germany] containing polymerase, 3 µl template DNA and filled up to a total reaction volume of 25 µl with RNase free water. PCR parameters included a 15 min step at 95°C for polymerase activation followed by 36 cycles with 30s at 94°C for denaturation, 60s at 51°C for primer annealing and 60s at 72°C for elongation and a final 10 min step for elongation at 72°C. PCR products were visualized on 1% agarose gel and purified using the QIAquick® PCR Purification Kit (Qiagen; Hilden, Germany) following the manufacturer's protocol. The purified PCR products were sequenced in both directions by Macrogen Inc. (Seoul, Korea) and the Department of Experimental Phycology and Culture Collection of Algae (Georg-August University Göttingen, Germany).

2.2.3 Phylogeographic and population genetic analyses

Nucleotide sequences were edited and translated into amino acids using the invertebrate mitochondrial code implemented in SEQUENCHER v4.9 (Gene Codes) and aligned with ClustalX v1.81 (Thompson *et al.* 1997) using multiple alignment parameters: 10.0 for gap opening and 0.1 for gap extension for the nucleotide, default settings for the amino acids dataset. Phylogenetic trees were generated with Beast v1.5.4 (Drummond and Rambaut 2007), MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) and PAUP* (Swofford 1999) using the NJ algorithm without and with model of sequence evolution to identify monophyletic clusters in the dataset. The model of sequence evolution was estimated with Modeltest3.6 (Posada and Crandall 1998) in PAUP* and in MrModeltest (Nylander 2004) for the Bayesian analysis. The best fit model was GTR+I+G for both analyses. Model parameters were nst=6 and rates=invgamma for nucleotide sequences in BEAST and MrBayes, mtrev for the protein in Beast and aamodel=equalin for the protein alignment in MrBayes. Outgroups were *Hypochthonius rufulus* (C.L. Koch, 1835) (Oribatida, Enarthronota) and *Rhysotritia duplicata* (Grandjean, 1953) (Oribatida, Mixonomata). The Markov Chain Monte Carlo was run for ten million generations and sampled every 1000^{th} generation, the 50% majority consensus tree excluded the first 2,500,000 trees (burnin of 25%).

Standard diversity indices for the nucleotide sequences [haplotype number (N_h), haplotype diversity (H_d,) nucleotide diversity (Π_n), number of variable (N_{vs}) and invariable sites (N_{is}), parsimony informative sites (N_{pars}), the number of singletons (N_s) and the total number of substitutions (N_m)] and the McDonald-Kreitman (MK) test, to detect selection, were calculated in DNASP v5.0 (Rozas *et al.* 2003). The McDonald-Kreitman test can detect selection by examining the distribution of synonymous and non-synonymous substitutions among populations; it is robust against demographic and recombination events (McDonald and Kreitman 1991). The geographical structure of genetic diversity among and within populations and geographical clades were calculated with ARLEQUIN v3.01 (Excoffier *et al.* 2005) using analysis of molecular variance (AMOVA, 16,000 permutations). Only populations with two and more individuals were included in the analysis. Estimates for demographic

expansion (Tajima's D and Fu's F_s neutrality tests) and pairwise differences (F_{ST} 10,000 permutations) were also calculated in ARLEQUIN I calculating 10,000 permutations to test for significance.

The dataset exceeded the connection limit of TCS (Clement *et al.* 2000); therefore a parsimony based median-joining haplotype network (Bandelt *et al.* 1999) was generated in NETWORK v4.5 (Fluxus-Technology, Suffolk, UK) with default settings for nucleotide sequences and amino acids.

2.3 Results

A total of 182 individuals (180 *S. magnus*, one *R. duplicata* and one *H. rufulus*) from 47 localities in 19 countries were sequenced. The amplified *COI* fragments were 514 bp long and coded for 171 amino acids. The frequency of bases declined in the order 45.1, 25.7, 14.7 and 14.5% for T, A, C and G, respectively. The 180 *S. magnus COI* sequences formed 111 haplotypes for the nucleotide (61.7%) (Fig. 8) with 310 variable (60.7%); 275 of these were parsimony informative sites (Table A1). The amino acids formed 67 haplotypes (37.2%) (Fig. 9). The haplotype diversity (H_d) was very high with 0.98 in the nucleotide sequences.

2.3.1 Network

In the nucleotide haplotype network (Fig. 8) seven haplotypes were shared by individuals from different locations (D_1/D_2/D_9/F_2/I_1/I_2, D_1/D_2/D_9/DK_3/DK_4/I_2, D_1/I_1, D_8/GB_1/NL_1, D_4/DK_2, F_3/GB_1 and GB_3/NL_1). Sixteen haplotypes comprised two or more individuals from the same location (A_1.1-2, CZ_1.1-2, CHINA_1.1-2, D_2.6-7, D_4.1-2/D_4.6-7, D_5.1-3, D_7.2/D_7.4, F_1.1/F_1.4-6, F_3.3/F_3.5, F_4.1-2, FIN_1.1-2, N_1.1/N_1.3, PL_2.1/PL_2.3, RUM_1.1-2, RUM_3.1-2/RUM_3.4 and RUS_2.1-4). The other 88 haplotypes were single individuals.

The nucleotide haplotype network formed 30 subclades and eight isolated individual haplotypes which were separated each by high numbers of substitution steps. The different subclades formed three main clades (black, blue and red).

The black main clade consisted of 14 subclades, two isolated single individual haplotypes each from one location (GB_1.4, RUM_6.3) and one isolated haplotype of two individuals from one Chinese location (CHINA_1.1-2).

Subclade 1 comprised three smaller subclades 1a-c. Subclade 1a comprised eight haplotypes from seven locations [single individual haplotypes: one from one German location (D_1.12), one from another German location (D_2.2), one from one Danish location (DK_3.1), one from one Italian location (I_1.1), two from on French location (F_2.2, F_2.4); haplotypes of more than one individual: one haplotype of two individuals from one German location (D_2.6-7),.one of 13 individuals from six locations (two individuals from each one German location (D_1.1, D_9.1), five individuals from another German location (D_2.1, D_2.4-5, D_2.8-9), two individuals from one French location (F_2.1, F_2.3), three individuals from one Italian location (I_1.4, I_1.9-10), one individual from another Italian location (I_2.4)]. The most abundant haplotype was the centre of the subclade 1a, the other seven surrounding haplotypes were connected by one or two substitution steps to the Central haplotype.

Subclade 1b comprised six single individual haplotypes from four locations [two from one German location (D_1.5, D_1.14), two each from one location (D_9.2, I_1.2), two from one Italian location (I_2.1, I_2.2)] and one haplotype of six individuals from two locations [one individual from one German (D_1.2), five individuals from one Italian (I_1.3, I_1.5-8)]. Five single individual haplotypes were connected by one substitution step to the most abundant haplotype. The single individual I_2.2 was connected by six substitution steps to the most abundant haplotype.

Subclade 1c comprised four single individual haplotypes from three locations [two from one German (D_1.7, D_1.13), two each from one Danish (DK_1.3, DK_4.3)] and one haplotype of 15 individuals from six locations [seven individuals from one German (D_1.3-4, D_1.6, D_1.8-11), two individuals each one (D_2.3, I_2.3), two individuals from a third German (D_9.3-4), two individuals from one Danish (DK_3.2-3), two individuals from another Danish DK_4.1-2)]. Three single individuals were separated from the most abundant haplotype by one substitution step. The single individual haplotype D_1.13 was separated from the single individual haplotype D_1.7 by eleven substitution steps.
Subclade 2 comprised five single individual haplotypes from two locations on Tenerife [one from one location (E_2.1), four from another location (E_3.1-3.4)]. Subclade 3 comprised two single individuals from one location on Tenerife (E_1.1-2). Subclades 2 and 3 were separated from each other by 71 substitution steps. Subclade 4 comprised of three single individual haplotypes from one German location (D_3.1-3).
Subclade 5 comprised of two smaller subclades 5a and 5b. Subclade 5a comprised nine haplotypes from six locations [single individual haplotypes: three individuals each from one location (GB_1.2, GB_3.1, NL_1.2), two individuals from one English (GB_2.1-2), two individuals from one Irish (IRL_1.1-2); haplotypes of more than one individual: one haplotype of two individuals from two locations (F_3.1, GB_1.3), one haplotype of three individuals from two locations (GB_3.2-3 and NL_1.1)]. Subclade 5b comprised two haplotypes from three locations [one single individual haplotype from one German (D_8.3), one haplotype of five individuals from three locations (D_8.1-2, GB_1.1, GB_1.5, NL_1.3)].
Subclade 6 comprised three haplotypes from one French location [two single individuals (F_3.2, F_3.4), haplotype of two individuals (F_3.3, F_3.5)]. Subclade 7 comprised one haplotype of three individuals from one German location (D_5.1-3). Subclade 8 comprised six single individual haplotypes from two Romanian locations (RUM_5.1-4 and RUM_6.1-2). Subclade 9 comprised two haplotypes from one Polish location [one single individual haplotype (PL_2.2), one haplotype of two individuals (PL_2.1 and PL_2.3). Subclade 10 comprised six haplotypes from two Romanian locations [five single individual haplotypes from two locations (RUM_1.3 and RUM_2.1-4), haplotype of two individuals from one location (RUM_1.1-2)]. Subclade 11 comprised two single individual haplotypes each from one Scandinavian location (FIN_2.1, S_2.2).
The blue main clade formed three subclades and one isolated haplotype of two individuals from one Norwegian location (N_1.1, N_1.3). Subclade 12 comprised five haplotypes from three locations [single individual haplotypes: two haplotypes each from one location (D_4.5, HUN_1.1), two haplotypes from one Danish location (DK_1.1-2); haplotypes of more than one individual: one of four individuals one German location (D_4.1-2, 4.6-7)]. Subclade 13 comprised three haplotypes from two locations [two single individual haplotypes from two locations (CZ_1.5, F_1.2), haplotype of two individuals from one Czech location (CZ_1.1-2)]. Subclade 14 comprised five single individual haplotypes from four locations [three haplotypes each from one location (CZ_1.4, D_6.1, S_2.1), two haplotypes from one Polish location (PL_1.1, 1.3)].
The red main clade comprised nine subclades, six single individual haplotypes each from one location (D_4.4, D_5.5, F_1.3, RUM_3.3, RUS_1.4, RUS_2.5), two single individual haplotypes from one German location (D_7.1, 7.3) and two haplotypes of two individuals each from one location (D_7.2, D_7.4; A_1.1-2).
Subclade 15 comprised five haplotypes from five locations [single individual haplotypes: two each from one location (CH_1.1, S_2.3), two haplotypes from one Polish location (Pl_1.4-5); one haplotype of eight individuals from two locations (DK_2.1-5, D_4.3, 4.8-9). Subclade 16 comprised four haplotypes from three locations [single individual haplotypes: one from one German location (D_5.4), two from one Polish location (PL_1.2, 1.6); one haplotype of two individuals from one French location (F_4.1-2)]. Subclade 17 comprised three haplotypes from two Scandinavian locations [single

individual haplotypes: two each from one location (FIN_1.3, S_1.1); one haplotype of two individuals from one Finish location (FIN_1.1-2)]. Subclade 18 comprised one haplotype of four individuals from on French location (F_1.1 and F_1.4-6). Subclade 19 consisted of two smaller subclades 19a and 19b each from one Romanian and one single individual haplotype from one Romanian location (RUM_3.3). Subclade 19a comprised three single individual haplotypes from one Romanian location (RUM_4.1-3). Subclade 19b comprised one haplotype of three individuals from one Romanian location (RUM_3.1-2, 3.4). Subclade 20 comprised two single individual haplotypes each from one location (CZ_1.3 and N_1.2). Subclade 21 comprised three single individual haplotypes from one Russian location (RUS_1.1-3) and subclade 22 one haplotype of four individuals from another Russian location (RUS_2.1-4).

The southwest (subclades 2, 3), the southeast (subclades 8, 10, 19) and the Far East (subclade 21, 22, CHINA) refuge areas had no directly linkage to the subclades of Central and Northern Europe. Only the Italian locations were linked to Central French, Central German and Northern Danish locations. All other Central and Northern subclades were separated by large numbers of substitution steps to each other.

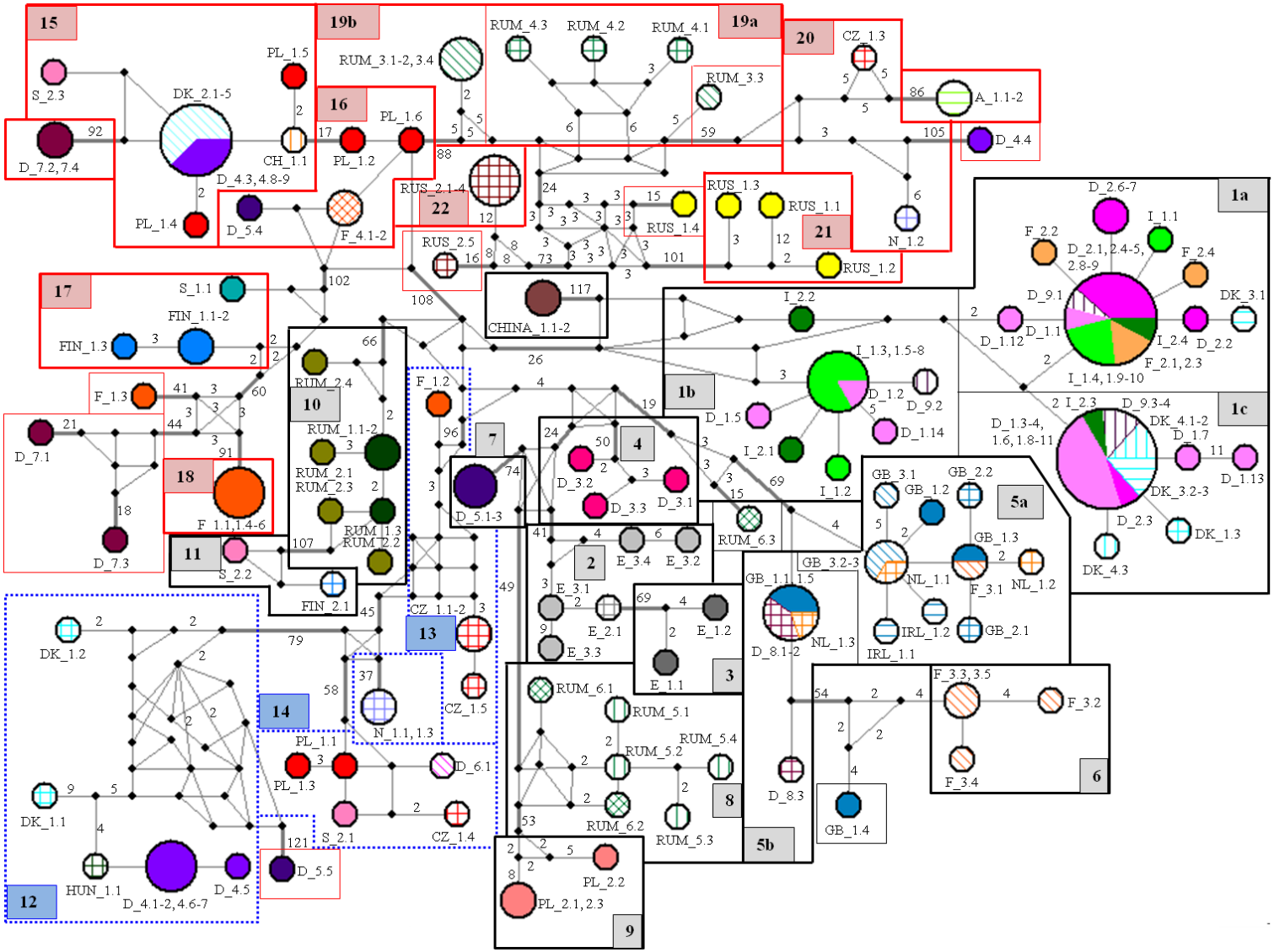

Figure 8: Median-joining haplotype network for the *COI* nucleotide of 111 haplotypes from *Steganacarus magnus*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one substitution step between haplotypes). Major subclades are marked by boxes.

The protein haplotype network (Fig. 9) comprised 67 haplotypes and 19 subclades, five single individual haplotypes each from one location (D_1.13, D_4.4, D_5.5, F_1.3, RUM_6.3), two single haplotypes from one German location (D_7.1, 7.3) and two haplotypes of two individuals each from one location (A_1.1-2, CHINA_1.1-2) which were separated by five or more amino acid changes (~3% divergence).

Subclade 1 comprised eight haplotypes from nine locations [single individual haplotypes: two each from one location (DK_4.3, I_2.2), two from one Italian location (I_1.1-2), two from one German location (D_1.13-14); haplotypes of more than one individual: one haplotype of ten individuals from five locations (three individuals each from one location (D_9.2, DK_1.3, I_2.1), two individuals from one German location (D_1.2, D_1.5), five individuals from one Italian location (I_1.3, 1.5-8)), one haplotype of 35 individuals from eight locations (ten individuals from one German location (D_1.1, 1.3-4, 1.6-12), nine individuals from another German location (D_2.1-9), three individuals from a third German location (D_9.1, 9.3-4), three individuals from one Danish location (DK_3.1-3), two individuals from another Danish location (DK_4.-2), four individuals from one French location (F_2.1-4), three individuals from one Italian location (I_1.4,1.9-10), one individual from another Italian location (I_2.3-4))].

Subclades 2 and 3 fused and comprised four haplotypes from three locations on Tenerife [single individual haplotypes: two from one location (E_3.2-3); haplotypes of more than one individual: one haplotype of two individuals from one location (E_1.1-2), one haplotype of three individuals from two locations (E_2.1, E_3.1, 3.4)]. Subclade 4 comprised one haplotype of three individuals of one German location (D_3.1-3). Subclades 5, 6 and the isolated individual GB_1.4 fused and comprised eight haplotypes from five locations [single individual haplotypes: three each from one location (D_8.3, GB_2.1, GB_3.1), two from one French location (F_3.2, 3.4); haplotypes of more than one individual: one of three individuals from two locations (F_3.3, 3.5; GB_1.4), one of four individuals each from one location (F_3.1, GB_1.3, GB_2.2, NL_1.2), one of eleven individuals from five locations (two individuals from one German (D_8.1-2), two individuals from one Scottish (GB_3.2-3), two individuals from one Irish (IRL_2.1-2), two individuals from one Dutch (NL_1.1, NL_1.3) three individuals from one British (GB_1.1-2, 1.5))].

Subclade 7 was identical with the nucleotide network. Subclades 8 and 9 fused and comprised four individuals [single individual haplotypes: two each from one location (PL_2.2, RUM_5.1); haplotypes of more than one individual: one of two individuals form one Polish location (PL_2.1, 2.3), one of five individuals from two Romanian locations (RUM_5.2-4, RUM_6.1-2)]. Subclade 10 comprised three haplotypes from two Romanian locations [single individual haplotypes: two from one Romanian (RUM_2.2, 2.4); one of five individuals from two Romanian locations (RUM_1.1-3, RUM_2.1, 2.3)]. Subclade 11 had no changes to the nucleotide network.

Subclade 12 comprised four haplotypes from three locations [single individual haplotypes: one from one German (D_4.5), two from one Danish (DK_1.1-2); one of five individuals from two locations (D_4.1-2, 4.6-7, HUN_1.1)]. Subclade 13 and the isolated Norwegian haplotype (N_1.1, 1.3) fused and comprised three haplotypes from five locations [single individual haplotype: one from one Polish (PL_1.3); haplotypes of more than one individual: one of two individuals from one Norwegian location (N_1.1 and N_1.3), one of four individuals each from one location (CZ_1.4, D_6.1, PL_1.1, S_2.1). Subclade 14 comprised two haplotypes from two locations [one single individual haplotype from one Czech (CZ_1.5), one of three individuals from two locations (CZ_1.1-2, F_1.2)].

Subclades 15 and 16 fused and comprised six haplotypes from seven locations [single individual haplotypes: two individuals each from one location (D_5.4, S_2.3), two from one Polish location (PL_1.4-5); haplotypes of more than one individual: one of four individuals from two locations (F_4.1-2, PL_1.2, 1.6), one of nine individuals from three locations (CH_1.1, D_4.3, 4.8-9, DK_2.1-5)]. The protein haplotype network of subclades 17 and 18 was identical to that of the nucleotide

network. Subclades 19, 20, the isolated Austrian haplotype (A_1.1-2) and the isolated haplotype RUS_1.4 fused and comprised four haplotypes from six locations [single individual haplotype: one from one Romanian (RUM_3.3); haplotypes of more than one individual: one of two individuals from one Austrian location (A_1.1-2), one of two individuals from two locations (CZ_1.3, N_1.2), one of seven individuals from three locations (RUM_3.1-2, 3.4, RUM_4.1-3, RUS_1.4)]. Subclade 21 was identical to that of the nucleotide network. Subclade 22 and the isolated haplotype (RUS_2.5) fused and comprised two haplotypes [one single individual (RUS_2.5), one haplotype of four individuals from one Russian location (RUS_2.1-4)].

The Chinese and the two German (D_7) subclades of the protein haplotype network were identical to the nucleotide haplotype network. The Chinese subclade was next to subclade 19/20 and not to subclade 1 as in the nucleotide network. The isolated individual D_1.13 was separated from the most abundant haplotype of the largest subclade by seven amino acid changes (more than 4% differences). In the nucleotide network the D_1.13 haplotype was separated from the D_1.7 haplotype by eleven substitution steps (2.1% divergence). Haplotypes of individuals from the same location existed in different subclades in the nucleotide and in the protein (CZ_1, D_4, D_5, N_1, RUM_6, RUS_1 and S_2). Among these subclades there were large numbers of substitution steps in the nucleotide and several amino acid changes in the protein.

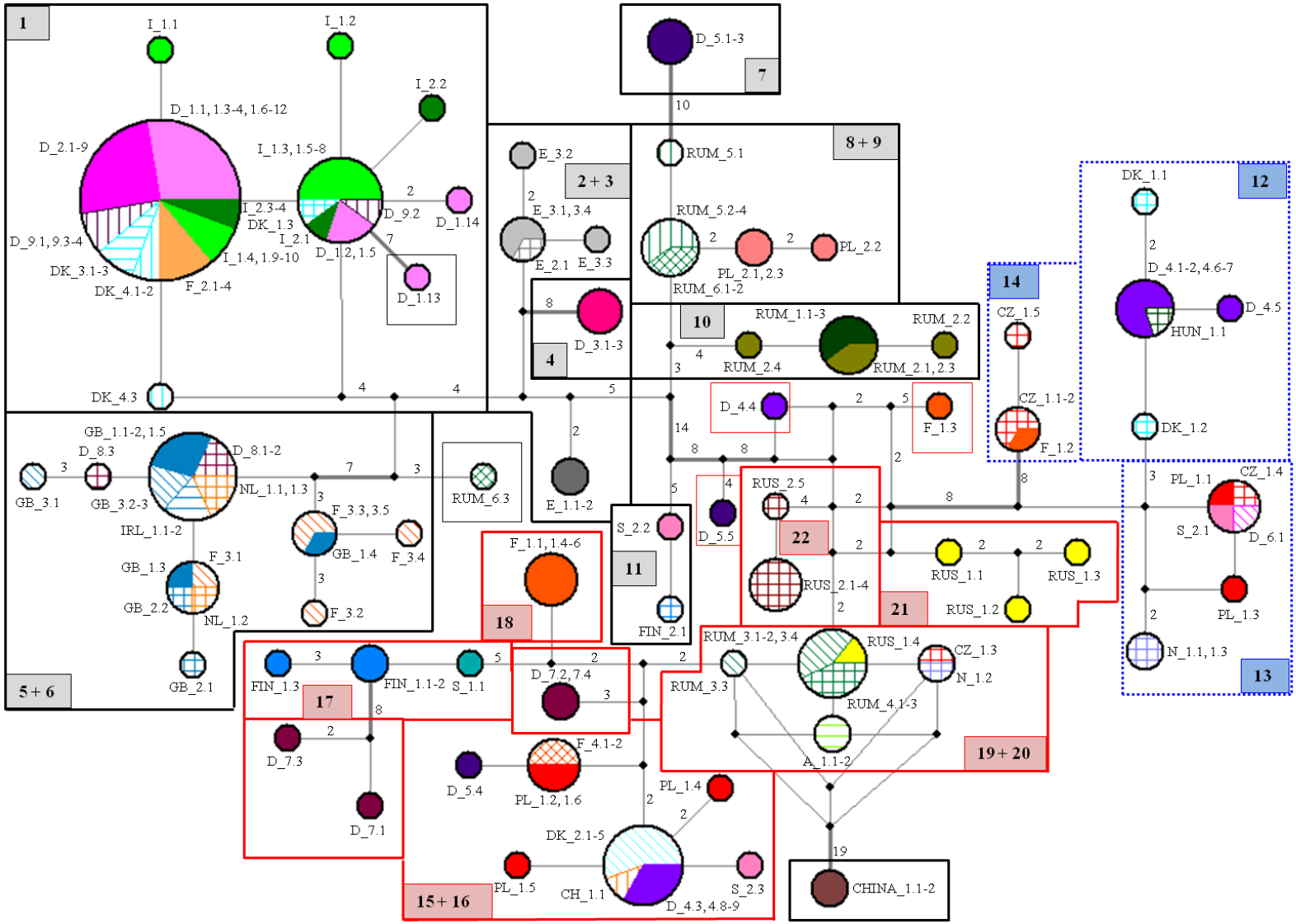

Figure 9: Median-joining haplotype network for the *COI* protein of 67 haplotypes from *Steganacarus magnus*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one amino acid change between haplotypes). Major subclades are marked by boxes.

2.3.2 Phylogenetic and population genetic analyses

In the Bayesian phylogenetic analysis of the nucleotide in BEAST the three main clades were monophyletic and supported by maximum posterior probabilities (Fig. 10). The blue and the black clade formed sister clades. In the other three phylogenetic analyses (NJ without and with model of sequence evolution and MrBayes) the black and blue clades were paraphyletic (Fig. A1-4). In all phylogenetic analyses the Chinese individuals were in the black clade which was supported by high bootstrap values and posterior probabilities. The red clade was only monophyletic in the Bayesian analysis of BEAST and paraphyletic in the other analyses. In the phylogenetic analyses of the protein the three main clades were monophyletic (Fig. 11, Fig. A5-7) and supported by high bootstrap and posterior probabilities. The Chinese individuals formed the basal group of the *S. magnus* protein sequences.

The minimum and maximum average pairwise differences for the nucleotide sequences between populations were 0.3% (D_2/F_2) and 31.8% (RUM_5/RUS_1) (Table 3; for the protein see Table A1). Excluding Russia and China from the analysis the maximum average pairwise difference was 30.2% (A_1/D_3). Within populations the minimum and maximum average pairwise differences were 0% (A_1, CHINA_1, DK_2 and F_4) and 25.8% (S_2). For the protein the minimum and maximum average pairwise differences between populations were 0% (A_1/RUM_3/RUM_4, CHINA_1/F_4, D_2/DK_3/DK4/F_2/RUM_1, D_9/RUM_1, GB_1/GB_2/IRL_1/NL_1, I_1/RUM_1, I_2/RUM_1) and 4.7% (D_3/CHINA_1); excluding Russia and China from the analysis the maximum average pairwise difference was 4.2% (FIN_1/PL_2). The minimum and maximum average pairwise differences within population were 0% (A_1, CHINA_1, D_2, D_3, DK_2, DK_3, DK_4, E_1, E_3, F_2, F_4, FIN_1, GB_1, GB_2, IRL_1, NL_1, RUM_1, RUM_3 and RUM_4) and 2.1% (D_5) for the protein (Table A2).

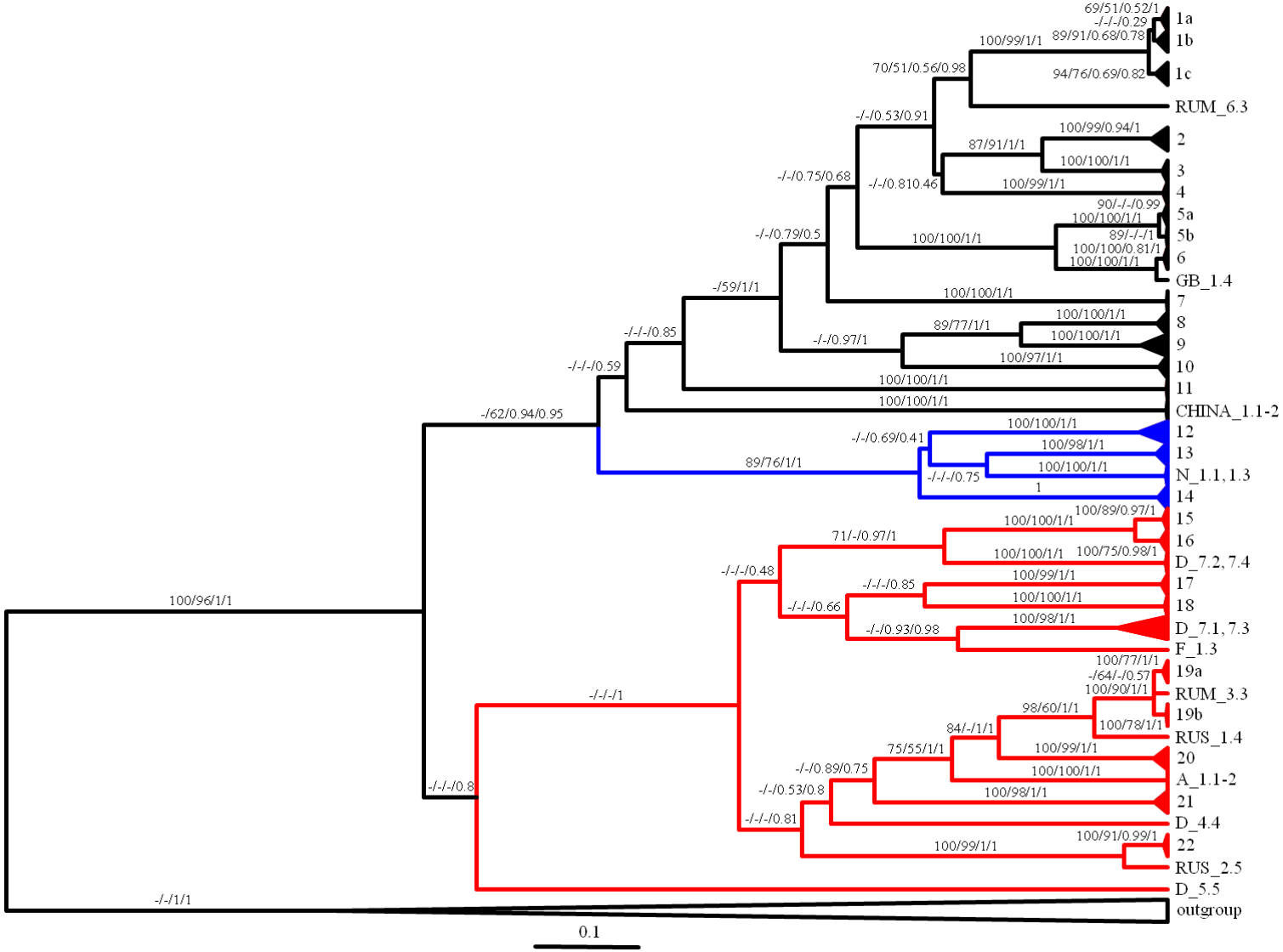

Figure 10: Bayesian phylogeny after $10x10^6$ generations from the 180 *COI* nucleotide sequences of *Steganacarus magnus* with Beast v1.5.4. Outgroups were *Hypochthonius rufulus* and *Rhysotritia duplicata.* Numbers on the branches are bootstrap values from NJ without and with evolution model (GTR+I+G) analysis and posterior probabilities from MrBayes and Beast. Branch colours represent the different clades (black, blue, red). Numbers at the end represent the different subclades as explained in Table 2.

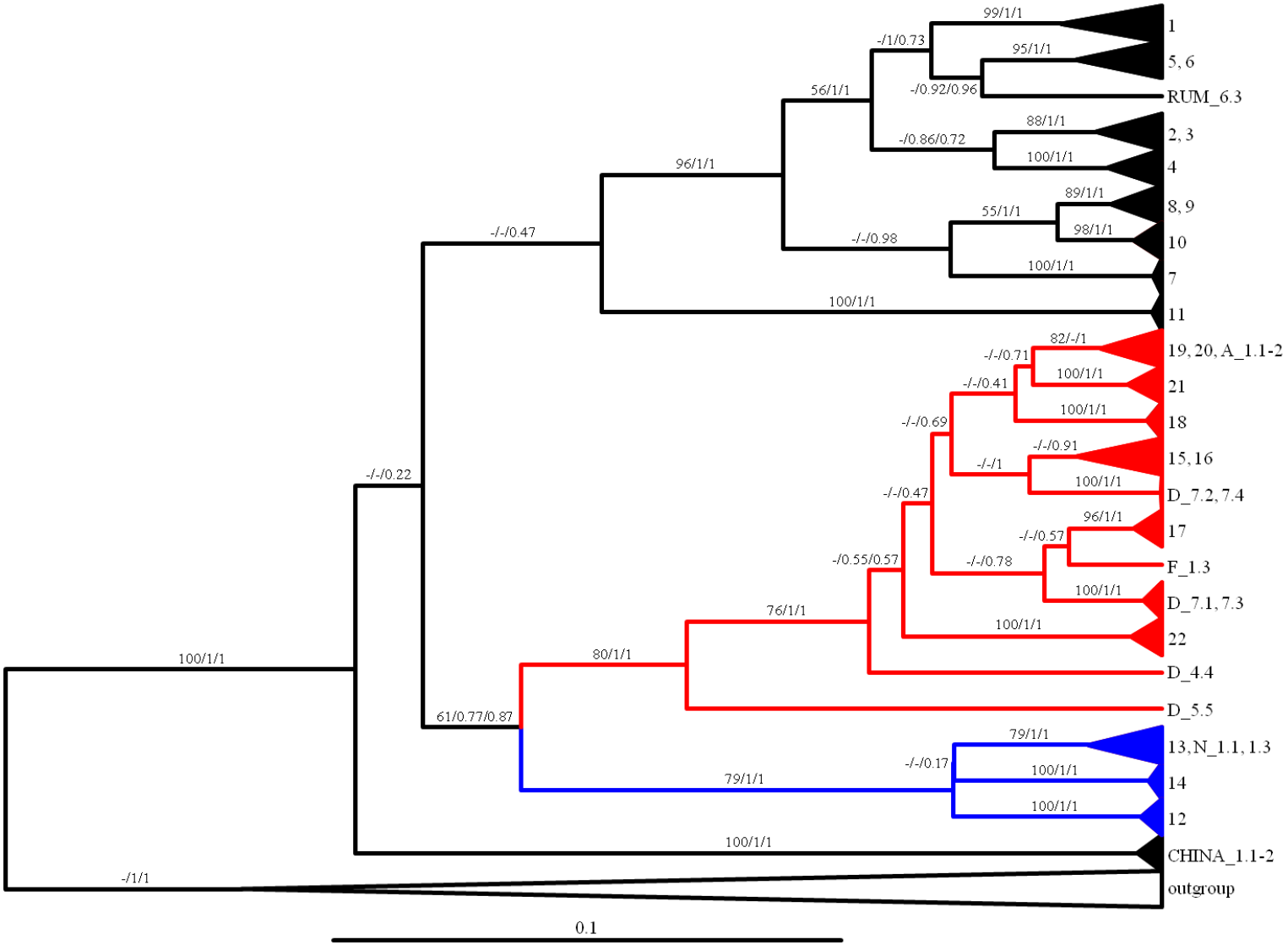

Figure 11: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 based on 180 *COI* protein sequences of *Steganacarus magnus*. Outgroups were *Hypochthonius rufulus* and *Rhysotritia duplicata*. Numbers on the branches are bootstrap values from NJ analysis and posterior probabilities from MrBayes and Beast. Branch colours represent the different clades (black, blue, red). Numbers at the end represent the different subclades as explained in Table 2.

Table 2: Subclades of Bayesian phylogenetic trees of *Steganacarus magnus* from the phylogenetic trees based on *COI* nucleotide sequences (#ind=number of individuals, pp=posterior probabilities, sampling site, individuals and ind. pop.=quantity of individuals from the population).

Clade I

Subclade	# ind	pp	sampling site		individuals	ind. pop.
1	**53**	**1**	**9**			
1a	**21**	**1**	KW	D_1	1, 12	2/14
			Goettingen	D_2	1-2, 4-9	8/9
			Bonn	D_9	1	1/4
			Arhus 1	DK_3	1	1/3
			Loire	F_2	1-4	4/4
			Grosseto	I_1	1, 4, 9-10	4/10
			Parma	I_2	4	1/4
1b	**12**	**0.82**	KW	D_1	2, 5, 14	3/14
			Bonn	D_9	2	1/4
			Grosseto	I_1	2-3, 5-8	6/10
			Parma	I_2	1-2	2/4
1c	**20**	**0.78**	KW	D_1	3-4 6-11	8/14
			Goettingen	D_2	3	1/9
			Bonn	D_9	3-4	2/4
			Parma	I_2	3	1/4
			Copenhagen	DK_1	1	3/3
			Arhus 1	DK_3	2-3	2/3
			Arhus 2	DK_4	1-3	3/3
isol. ind.	**1**		Sinaia	RUM_6	3	1/3
2	**5**	**1**	Anaga Mountains 1	E_2	1	1/1
			Anaga Mountains 2	E_3	4	4/4
3	**2**	**1**	Teno Mountains	E_1	2	2/2
4	**3**	**1**	Lake Constance	D_3	1-3	3/3
5	**18**	**1**				
5a	**12**	**0.99**	St. Isidore	F_3	1	1/5
			Bedford	GB_1	2-3	2/5
			Ascot	GB_2	1-2	2/2
			Braemar	GB_3	1-3	3/3
			Swords	IRL_1	1-2	2/2
			Wageningen	NL_1	1-2	2/2
5b	**6**	**1**	Cuxhaven	D_8	1-3	3/3
			Bedford	GB_1	1, 5	2/5
			Wageningen	NL_1	3	1/3
6	**4**	**1**	St. Isidore	F_3	2-5	4/5
isol. ind.	**1**		Bedford	GB_1	4	1/5
7	**3**	**1**	Moerfelden	D_5	1-3	3/5
8	**6**	**1**	Busteni	RUM_5	1-4	4/4
			Sinaia	RUM_6	1-2	2/3
9	**3**	**1**	Warsaw	PL_2	1-3	3/3
10	**7**	**1**	Sibiu 1	RUM_1	1-3	3/3
			Sibiu 2	RUM_2	1-4	4/4
11	**2**	**1**	Joensuu	FIN_2	1	1/1
			Stroemstad	S_2	2	1/3
isol. ind	**2**	**1**	Nanjing	CHINA_1	1-2	2/2

Table 2 continued

Clade II

Subclade	# ind	pp	sampling site		individuals	ind. pop.
12	**8**	**1**	Meckl. Seenpl.	D_4	1-2, 5-7	5/9
			Copenhagen	DK_1	1-2	2/3
			Piliscaba	HUN_1	1	1/1
13	**4**	**1**	Decin	CZ_1	1-2, 5	3/5
			Mont Blanc	F_1	2	1/6
isol. ind.	**2**	**1**	Narvik	N_1	1, 3	2/3
14	**5**	**1**	Decin	CZ_1	4	1/5
			Black Forest	D_6	1	1/1
			Krakow	PL_1	1, 3	2/6
			Stroemstad	S_2	1	1/3
isol. ind.	**1**		Moerfelden	D_5	1	1/5

Clade III

Subclade	# ind	pp	sampling site		individuals	ind. pop.
15	**12**	**1**	Logarno	CH_1	1	1/1
			Meckl. Seenpl.	D_4	3, 8-9	3/9
			Hjorring	DK_2	1-5	5/5
			Krakow	PL_1	4-5	2/6
			Stroemstad	S_2	3	1/3
16	**5**	**1**	Moerfelden	D_5	4	1/5
			Haute Loire	F_4	1-2	2/2
			Krakow	PL_1	2, 6	2/6
isol. ind.	**2**	**1**	Uelzen	D_7	2, 4	2/4
17	**4**	**1**	Lahti	FIN_1	1-3	3/3
			Umea	S_1	1	1/1
18	**4**	**1**	Mont Blanc	F_1	1, 4-6	4/6
isol. ind.	**2**	**1**	Uelzen	D_7	1, 3	2/4
isol. ind.	**1**		Mont Blanc	F_1	3	1/6
19	**7**	**1**	**2**			
19a	**3**	**1**	Cluj Napoca	RUM_4	1-3	3/3
isol. ind.	**1**		Bagau	RUM_3	3	1/4
19b	**3**	**1**	Bagau	RUM_3	1-2, 4	3/4
isol. ind.	**1**		Altai Mountains	RUS_1	4	1/4
20	**2**	**1**	Narvik	N_1	2	1/3
			Decin	CZ_1	3	1/5
isol. ind.	**2**	**1**	Villach	A_1	1-2	2/2
21	**3**	**1**	Altai Mountains	RUS_1	1-3	3/4
isol. ind.	**1**		Meckl. Seenpl.	D_4	4	1/9
22	**4**	**1**	Novosibirsk	RUS_2	1-4	4/4
isol. ind.	**1**		Novosibirsk	RUS_2	5	1/5
isol. ind.	**1**		Moerfelden	D_5	5	1/5

Table 3: Mean percentage pairwise differences of uncorrected p-distances for the *COI* nucleotide of *Steganacarus magnus* from 41 locations. The diagonal represents the within population differences (bold), among population differences are below the diagonal. Bold numbers in red are the minimum and maximum differences within and among populations. Bold numbers in pink are the maximum divergences excluding individuals from China and Russia. Locations with less than two individuals were excluded from the analysis.

	Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	A 1	0																										
2	CHINA 1	30	0																									
3	CZ 1	25.3	28.4	**16.1**																								
4	D 1	28.4	24.1	23.7	**1.3**																							
5	D 2	28.2	23.9	23.6	1.1	**0.3**																						
6	D 3	30.2	24.9	25.3	16.8	16.5	**0.9**																					
7	D 4	26	28.1	23	24.7	24.7	24.7	**16.3**																				
8	D 5	27.6	27.3	24.8	21.3	21.3	21.3	23.3	**18.3**																			
9	D 7	23.5	29.2	26.6	26.9	26.6	26.1	24.6	25.9	**16.2**																		
10	D 8	28	27	26.2	20.1	16.6	21.3	26.7	22.8	26.9	**0.3**																	
11	D 9	28.4	23.9	23.6	1.1	0.9	16.6	24.6	21.2	26.7	19.9	**1.1**																
12	DK 1	29.1	26.9	22.4	17.1	17.1	22.4	16.9	23.2	27.1	24	17	**18.2**															
13	DK 2	21.8	28.2	24.1	23.2	23.1	23.2	16	20.1	20.5	27.6	23.1	23.5	0														
14	DK 3	28.1	24.2	23.6	1	0.7	16.8	24.5	21.2	26.7	19.8	0.8	16.8	23.1	**0.8**													
15	DK 4	28.1	24.1	23.6	0.8	0.8	16.8	24.6	21.2	26.9	19.9	0.7	16.8	23	0.5	**0.1**												
16	E 1	28.7	27.2	22.8	18.8	18.6	21.2	27.3	22.4	29.4	22.8	18.7	24.8	26.8	18.7	18.7	**1.2**											
17	E 3	29.1	27.6	23.5	17.4	17.5	16.1	16.3	22.6	28.3	20.3	17.3	22.8	26.2	17.5	17.3	1.6	0										
18	F 1	25.1	28.9	24.7	26.2	26	26.3	26	26.8	23.1	28.8	26	28	22.6	26.1	26.2	29.9	29.7	**15.6**									
19	F 2	28.3	23.9	23.7	1.2	0.3	16.5	24.7	21.4	26.7	19.7	0.9	17.1	23.2	0.8	0.9	18.7	17.5	26.1	**0.2**								
20	F 3	29.5	25.8	26.1	20.7	20.2	20.1	27.2	23.8	28.7	10	20.6	24.7	27.7	20.4	20.6	22	19.9	29.4	20.2	**5.3**							
21	F 4	21.2	28.4	23.8	22.7	22.5	23	17.2	19.7	20.7	27.4	22.6	23.2	3.9	22.5	22.4	27.1	25.9	22.2	22.6	27.6	0						
22	FIN 1	23.5	27.6	28.6	25.1	24.9	25.3	25.9	26.2	21.8	27.8	24.9	28.3	20.8	25	25.0	29.7	28	22.6	24.9	28.3	20.6	**0.4**					
23	GB 1	28.4	26.7	26.4	20.5	20	21.5	27	23.2	27.5	3	20.4	24.4	28	20.2	20.4	22.3	20.5	29.1	20.1	8.9	27.7	28.1	**5.4**				
24	GB 2	28.1	27.2	26.8	21.1	20.6	21.8	27.6	23.4	27.4	1.5	20.9	25	28.4	20.8	20.9	22.7	20.8	29.3	20.6	10.2	28	27.8	3.2	**0.4**			
25	GB 3	28	27.2	26.8	21.1	20.6	21.8	27.5	23.4	27.3	1.4	20.9	24.9	28.4	20.8	21	22.5	20.8	29.1	20.7	10.2	28	27.8	3.2	0.7	**0.7**		
26	I 1	28.7	23.7	23.7	1.4	1.1	16.3	24.7	21.2	26.7	20.2	1.1	17.2	23.1	1.3	1.4	16.9	17.2	25.9	1.1	20.8	22.7	24.8	20.6	21.1	21.2	**0.9**	
27	I 2	28.4	23.7	23.6	1.2	1	16.5	24.6	21.1	26.7	20	1	17	23.1	1.1	1.0	17	17.4	25.9	1	20.6	22.5	25	20.4	21	21	1.1	**1.3**
28	IRL 1	27.7	27.2	26.5	20.9	20.4	21.6	27.3	23.3	27.2	1.3	20.7	24.6	28.3	20.6	20.7	22.5	20.6	28.9	20.4	10.1	27.9	27.7	3.1	0.6	0.5	20.9	20.8
29	N 1	25.7	28.5	19.4	25.7	25.7	26.4	24.1	25.5	27	26.4	25.7	23.5	26.1	25.6	25.6	27.6	25.9	27.1	25.8	26.8	25.1	27.7	26.8	27.2	27.1	25.7	25.6
30	NL 1	28	27.2	26.5	20.8	20.3	21.5	27.2	23.1	27.1	1	20.6	24.6	28.2	20.5	20.6	22.5	20.7	28.9	20.4	10.1	27.8	27.7	3.1	0.8	0.8	20.9	20.7
31	PL 1	23.6	28.7	21.8	23.7	23.6	23.5	18.7	21.4	22.7	27	23.6	22.9	9.5	23.6	23.6	26	24.9	24.2	23.6	26.7	9.8	23.2	27.2	27.7	27.7	23.7	23.5
32	PL 2	29.8	23.9	23.8	16.4	16.1	18.9	24.8	21.9	25.0	21	16.2	21.9	24.3	16.2	16.2	20.4	19.7	26.7	16.1	20	24.3	26.7	20.7	21.1	20.9	16.3	16.1
33	RUM 1	28.2	25.2	24.3	17.5	17.7	18.9	24.7	21.6	26.4	19.1	17.5	22.2	24.3	17.7	17.6	21.2	18.6	27.3	17.7	19.3	25	25.6	19.3	19.5	19.3	17.3	17.1
34	RUM 2	28.1	25.3	24.4	17.6	17.7	18.9	24.8	21.6	26.6	19.2	17.5	22.3	24.3	17.7	17.6	21.2	18.7	27.3	17.7	19.5	25	25.7	19.4	19.6	19.5	17.3	17.2
35	RUM 3	18.7	28.8	22.5	26.8	26.8	25.7	23.6	25.7	21.8	27	26.6	27	19.5	26.7	26.7	28.1	27.7	22.5	26.9	28.1	19.1	23	27.4	27.6	27.5	26.5	26.6
36	RUM 4	18.7	28.3	22.3	27.2	27.2	25.6	23.3	26.2	22.2	27.6	27.1	26.7	19.9	27.2	27.2	28.7	28	23.1	27.3	28	19.7	22.6	27.8	28.1	28	27	27
37	RUM 5	29.1	26.6	24.1	17.8	17.9	19.2	25.4	21.4	26.7	22.1	17.7	23.1	25.4	17.5	17.6	21.1	18	26.8	17.9	20.9	25	25.6	21.9	22.3	22.1	18	17.7
38	RUM 6	29.3	26.5	23.9	16.6	16.6	18.4	25.3	21.2	26.8	20.4	16.4	22.5	25	16.4	16.3	20.2	17.6	27.4	16.7	20.1	24.6	26.1	20.5	20.8	20.6	16.6	16.4
39	RUS 1	21.8	30.3	26.9	29.2	28.9	27.4	26.8	28.4	24	30.2	29	29.7	23.4	29	29.1	31	30.7	24.1	28.8	31.3	22.2	24.4	30.6	30.6	30.7	29.1	29
40	RUS 2	24.4	30.5	28.4	30	29.5	29.4	26.6	28.4	23.2	29.3	29.7	29	25.6	29.7	30	30	31.7	24	29.6	31	25.3	26.3	30	30.3	30.2	29.6	29.7
41	S 2	26.6	29.4	22.4	24.6	24.8	23.7	22.5	23.7	25.4	26.8	24.7	24.3	17.8	24.8	24.8	25.5	24.2	25.8	24.8	26.2	19.2	26	27	27.6	27.5	24.7	24.7

Table 3 continued

Population		28	29	30	31	32	33	34	35	36	37	38	39	40	41
28	**IRL 1**	**0.4**													
29	**N 1**	26.8	**18**												
30	**NL 1**	0.7	26.8	**1**											
31	**PL 1**	27.6	24.1	27.5	**14.4**										
32	**PL 2**	20.9	25.1	21	23.9	**2**									
33	**RUM 1**	19.4	26.7	19.3	24	16	**0.3**								
34	**RUM 2**	19.5	26.8	19.4	24	16	0.6	**1.1**							
35	**RUM 3**	27.4	23.7	27.2	21.3	27.2	27.9	27.8	**1.8**						
36	**RUM 4**	27.9	23.3	27.8	21.7	27.8	27.7	27.7	3	**0.9**					
37	**RUM 5**	22	25.1	22	25.1	12.8	16.1	16.3	29.4	29.3	**0.6**				
38	**RUM 6**	20.5	25.1	20.4	24.6	14.6	16.4	16.6	28.7	28.6	6.1	**11.6**			
39	**RUS 1**	30.5	27.3	30.4	24.9	30.7	30.4	30.6	19.5	20	**31.8**	31.2	**12.7**		
40	**RUS 2**	29.9	28.2	29.9	26.6	29.3	27.8	28.1	23	22.7	30.2	30.4	23.5	**2.8**	
41	**S 2**	26.5	24.8	27.3	17.9	24.5	22.7	22.8	24.5	24.5	24.5	24.3	27.8	27.1	**25.8**

The results of the AMOVA showed that the nucleotide variation among countries (16.2%), the variation among samples within countries (57.5%) and the variation within samples (26.4%) were significant and high (Table 2). Also, the protein variation among samples within countries (67.7%) and the variation within samples (28%) were significant and high. In contrast, variation among countries (4.2%) was not significant (Table 4). The neutrality test (Tajima's D) was only significant for two populations in the nucleotide (F_3 Tajima's D=-1.26, p=0.0033 and RUS_2 Tajima's D=-1.25, p=0.0081) (Table A3) and for three populations in the protein (CZ_1 D=-1.4, p<0.01, D_1 D=-1.54, p=0.04 and I_2 D=-2.86, p<0.01) (Table A3). Fu's F_s were not significant for the nucleotide dataset but significant for one population (I_2 FS = -0.32, p = 0.04) in the protein dataset (Table A3 and A4).

Table 4: Results of the AMOVA on the variation in nucleotide and protein sequences among countries, among populations, within populations and in total. Each population was considered as a separate group. Populations with less than two individuals were excluded from the analysis. Significant level is p<0.05 (d.f.: degree of freedom). Black letters are for the nucleotide and red letters are for the protein.

Source of variance	d.f.	sum of squares	variance components	percent of total variation	fixation indices
among countries	16 / 16	3668.05 / 87.71	9.16 Va* / 0.07 Va	16.15 / 4.24	FCT: 0.16* / 0.04
among populations	24 / 24	3797.55 / 126.74	32.6 Vb* / 1.1 Vb*	57.5 / 67.74	FSC: 0.69* / 0.71*
within population	133 / 133	1986.6 / 60.42	14.94 Vc* / 0.45 Vc*	26.35 / 28.02	FST: 0.74* / 0.72*
total	173 / 173	9452.2 / 273.87	56.7 / 1.62		

The McDonald-Kreitman test showed that the differences between 233 of 820 population comparisons (28.4%) were significant (Table 5; for more details see Table A5). All computed neutrality indices were 0 (Table A6) and indicated purifying selection.

Table 5: Results of the McDonald-Kreitman test of *Steganacarus magnus* from 41 locations (38 European, 3 East Asian). *. 0.01<P<0.05 (90 populations), **: 0.001<P<0.01 (80 populations), ***: P<0.001 (63 populations)

	Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	A 1																											
2	CHINA 1	-																										
3	CZ 1	*	*																									
4	D 1	-	-	*																								
5	D 2	-	-	*	-																							
6	D 3	-	-	*	*	-																						
7	D 4	-	**	-	**	**	*																					
8	D 5	*	-	-	-	-	-	-																				
9	D 7	-	***	-	**	***	***	-	-																			
10	D 8	-	-	**	**	-	-	**	-	***																		
11	D 9	-	-	**	-	-	-	**	-	***	-																	
12	DK 1	-	-	-	-	-	**	-	-	-	**	-																
13	DK 2	-	-	*	-	-	-	-	-	-	-	-	-															
14	DK 3	-	-	*	-	-	-	**	-	***	-	-	-	-														
15	DK 4	-	-	*	-	-	-	**	-	***	-	-	-	-	-													
16	E 1	-	-	*	*	-	-	-	-	***	-	-	***	-	-	-												
17	E 3	-	-	**	*	-	-	*	-	***	-	-	**	-	-	-	-											
18	F 1	-	*	-	*	*	**	-	-	-	**	**	-	*	*	*	**	**										
19	F 2	-	-	*	-	-	-	**	-	***	-	-	-	-	-	-	-	-	*										
20	F 3	**	*	**	-	-	-	***	-	***	-	-	*	*	-	-	-	-	**	-									
21	F 4	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	**								
22	FIN 1	**	*	-	-	-	-	-	*	-	*	-	-	**	-	**	-	-	*	-	-	**							
23	GB 1	***	***	***	-	-	-	***	-	***	-	-	-	***	-	-	-	-	***	-	-	***	**						
24	GB 2	-	-	**	**	-	-	**	-	***	-	-	**	-	-	-	-	-	**	-	-	-	*	-					
25	GB 3	*	*	*	***	-	-	**	-	***	*	-	**	*	-	***	-	*	*	**	-	*	**	-	-				
26	I 1	-	-	*	-	-	-	**	-	***	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	**			
27	I 2	-	-	*	-	-	-	**	-	***	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-		
28	IRL 1	-	-	**	**	-	-	**	-	***	-	-	**	-	-	-	-	-	**	-	-	-	-	-	-	-	-	-	
29	N 1	**	-	-	-	-	-	-	*	-	*	-	-	**	-	-	-	*	-	-	**	**	*	***	*	-	-	-	
30	NL 1	-	-	*	**	-	-	**	-	***	-	-	*	-	-	-	-	-	**	-	-	-	-	-	-	-	-	-	
31	PL 1	*	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	*	-	-	*	-	*	**	-	-	-	-	
32	PL 2	-	-	**	-	-	-	*	-	***	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	-	-	-	
33	RUM 1	-	-	-	*	-	-	-	*	**	-	-	**	-	-	-	-	-	*	-	-	-	-	-	-	*	-	-	
34	RUM 2	-	-	-	-	-	-	-	-	**	-	-	**	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	
35	RUM 3	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	***	-	-	***	-	-	-	-	
36	RUM 4	-	-	-	-	*	**	-	*	-	-	*	-	-	*	-	*	-	*	-	***	-	-	***	-	-	-	-	
37	RUM 5	-	-	*	-	-	-	*	-	***	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	*	-	-	
38	RUM 6	-	-	**	-	-	-	**	-	***	-	-	-	-	-	-	*	-	**	-	-	-	-	-	-	-	-	-	
39	RUS 1	-	***	-	**	***	***	-	-	-	***	***	-	-	***	***	***	***	*	***	***	-	-	***	***	***	***	***	
40	RUS 2	-	**	-	-	**	***	-	-	-	*	**	-	-	**	*	**	*	-	**	*	-	-	***	*	-	***	**	
41	S 2	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	

Table 5 continued

Population		28	29	30	31	32	33	34	35	36	37	38	39	40
29	N 1	*												
30	NL 1	-	*											
31	PL 1	-	**	-										
32	PL 2	-	-	-	-									
33	RUM 1	-	-	-	-	-								
34	RUM 2	-	-	-	-	-	-							
35	RUM 3	-	**	-	-	-	-	-						
36	RUM 4	-	**	-	-	-	-	-	-					
37	RUM 5	-	-	-	-	-	-	-	-	-				
38	RUM 6	-	-	-	-	-	-	*	-	-	-			
39	RUS 1	***	*	***	-	***	***	***	*	-	***	***		
40	RUS 2	**	-	*	-	*	**	**	-	-	*	-	-	
41	S 2	-	-	-	-	-	-	-	-	-	-	-	-	-

2.4 Discussion

The structure of the population of *S. magnus* in Europe is genetically exceptionally complex. Remarkably, the high genetic variance in *COI* nucleotides of up to 31.8% did not only result from variations of the four times degenerated third codon position (Baker 2000), rather, variations in the first and second codon positions were also high. Fourfold degenerate sites do not affect the protein sequence and are assumed to have a slower saturation and less sensitivity to transversion-transition bias (Li 1993). These variations applied to both Southern and Northern populations and therefore were not confirming to the pattern of Southern richness and Northern paucity in aboveground animals and plants (Hewitt 1999, Hewitt and Ibrahim 2001). The extensive variation in both the *COI* nucleotide and the protein in populations from Central and Northern Europe suggests that *S. magnus* survived Pleistocene glaciations in cryptic refugia. The lack of star-like haplotypes (one big haplotype with several smaller nearly connected haplotypes) in the median-joining network (Fig. 8) resulted from deep splits of the several *COI* lineages which must have originated before the Pleistocene. Only in one *COI* lineage star-like haplotypes indicated postglacial re-colonisation of Central Europe (Avise 1994, 2004, Conroy and Cook 2000, Jolly *et al.* 2005, Mortimer and van Vuuren 2007); individuals from Italy, France, Germany and Denmark shared the same haplotype in subclade 1a. Survival in cryptic refugia is supported by the fact that individuals from locations which are geographically close together (D_1/D_5) or from the same location (CZ_1, D_4, D_5, DK_1, F_1, N_1, PL_1 and S_2) were genetically very different.

Coexistence of very different genetic lineages of *S. magnus* at the same location suggests that these lineages do not exclude each other. Results of the two different analyses for detecting mitochondrial clusters of *S. magnus* (Fig. 8, 9 and Fig. 10, 11) were identical. The substitution rate of the mitochondrial *COI* gene of *Steganacarus* has been estimated as 2.15% per million years (Salomone *et al.* 2002). This rate suggests that major radiations of lineages of *S. magnus* occurred in the Miocene or earlier (>20 my ago). However, nucleotide sequences of mtDNA will be saturated after 10-20 my (Avise 2004, Schaefer 2009) and with such highly saturated sequences, it becomes difficult to estimate divergence times even if the sequences evolved clock-like (Heethoff *et al.* 2007). Time estimates therefore have to be interpreted with some caution, but that such large genetic distances (up to 31.8%) evolved over at least twenty million years seems to be certain. Representatives of the three lineages (black, blue and red) survived in multiple Northern refugia, including Sweden, Finland, Norway, Great Britain, the Netherlands, Germany, Poland, Czech Republic, Austria and at Nunataks (Schmitt 2009) such as the Mont Blanc region (Fig. 12). Schmitt (2009) postulated a genetic linkage between the North-eastern Alps and the North-western Carpathians. I only found indications for such a linkage in

the protein in specimens from the Eastern Alps and the Carpathians, however, and in the nucleotide these specimens were separated by a large number of substitutions.

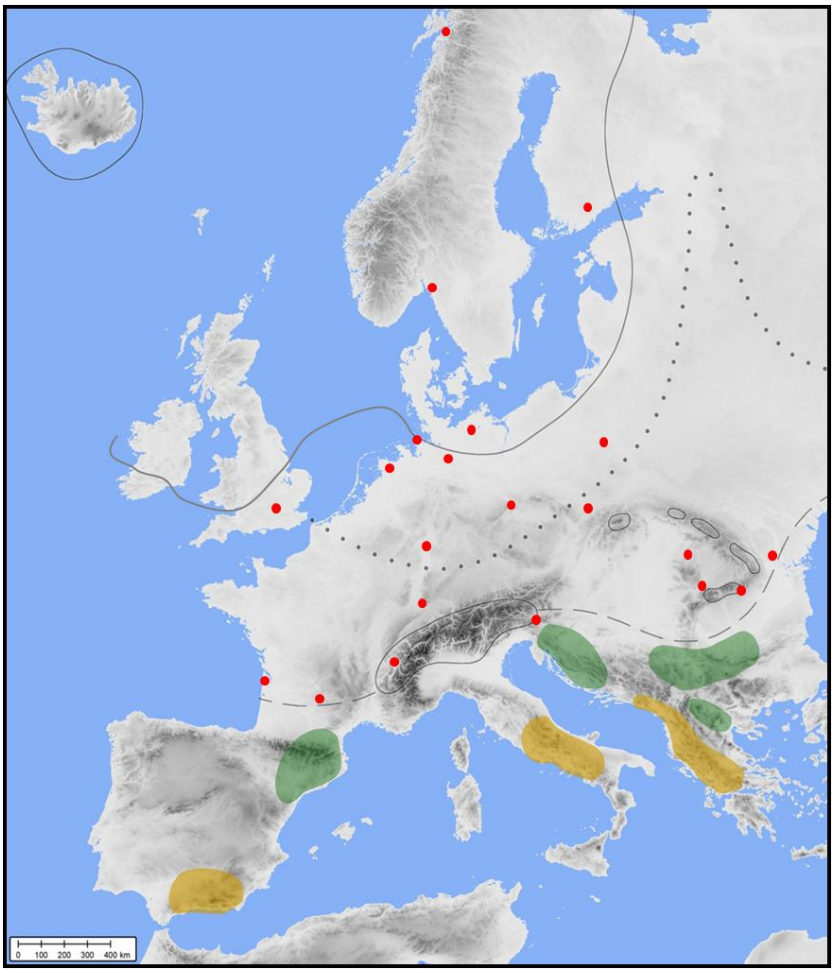

Figure 12: Map of Europe at the last glacial maximum. Red dots represent cryptic refugia as indicated by results of this study. Green areas represent refugia of coniferous trees, yellow areas refugia of deciduous trees; grey lines depict the maximum expansion of glaciers, the dotted line the border of arctic vegetation and the dashed line the border of tundra vegetation and permafrost (modified from Hewitt 1999, Hewitt and Ibrahim 2001, Alexander Kartographie 2006).

The phylogenetic analyses of the nucleotide and the protein were largely consistent, however, the blue clade changed its sister clade in the protein. In the nucleotide the black clade formed the sister clade of the blue, whereas in the protein it was the red clade. This shift in the clade arrangement likely resulted from the four times degenerated third codon position in the mitochondrial code (Xia *et al.* 1996, Baker 2000). Substitutions on these positions were synonymous and had no effect on the protein structure and therefore varied strongly. This variation in synonymous substitutions renders phylogenetic analyses difficult resulting in wrong phylogenetic relationships. The protein phylogeny is much more conservative and therefore more trustworthy e.g., back mutations are unlikely to be included.

This is the first study investigating nucleotide and protein divergences in *COI*. The exceptionally high molecular divergence in *COI* nucleotide and protein indicates that *S. magnus* comprises a cryptic species , as in barcoding literature sequence divergences in the COI fragment of >3% is sufficient to describe new species)Hebert *et al* 2003a-b, 2004b Hogg and Hebert 2004, Johnson *et al*. 2008). The translated nucleotide sequence of the investigated COI fragment is fully functional and amplification of *COI* pseudogenes can therefore be excluded (Bensasson *et al.* 2001, Williams and Knowlton 2001,

Song *et al.* 2008, Buhay 2009). Intraspecific *COI* divergences typically is less than 2%, whereas interspecific divergences generally exceeds 4% (Peek *et al.* 1997, Johnson *et al.* 2008) The *COI* gene is highly conserved at the protein level (Johnson *et al.* 2008) and the high protein divergence (up to 4.7%) strongly indicates the existence of cryptic species in *S. magnus*. Gene duplications and heteroplasmy of the *COI* gene are excluded, since the complete mitochondrion of *S. magnus* was sequenced by Domes *et al.* 2008 and Domes-Wehner 2009 and neither gene duplications nor heteroplasmy were detected. In contrast to Hurst and Jiggins (2005) the high genetic variance is not an effect of inherited symbionts such as *Wolbachia* or *Cardinium*, since none of these symbionts has ever been found in *S. magnus*. However, *Wolbachia* and *Cardinium* can trigger parthenogenesis and were found in an unidentified oribatid mite (Cordaux *et al.* 2001) and in some populations of *Oppiella nova* (Weeks *et al.* 2003).

To study the structure of a cryptic species complex of *S. magnus* in more detail analyses including several nuclear genes from the same individual need to be investigated to detect recombination of several mitochondrial lineages (e.g. using the elongation factor 1 α and heat shock protein). Individuals of *S. magnus* are morphologically coherent, characters indicative of a species complex are lacking, suggesting that there is gene flow, i.e. *S. magnus* forms a biological species. There are variations in body size and colour but these are unlikely due to be based on genetic differences but rather to variations in habitat characteristics and food availability (Weigmann 2006).

High mobility of species may contribute to genetic dissimilarity of specimens occurring locally (Schäffer *et al.* 2010). For the dispersal of soil microarthropods wind and water are likely to be important, but not common in oribatid mites (Seyd 1962, Starý and Block 1998). Oribatid mite species might attach to nest-dwelling vertebrates (Miko and Stanko 1991, Lebedeva *et al.* 2006); another species of the genus *Steganacarus* (*Steganacarus striculus* C.L. Koch, 1836) has been found in bird plumage; in fact, several oribatid mite species are distributed by birds (Lebedeva *et al.* 2006) and same taxa showed active dispersal by phoresy on arthropods (Norton 1980, Townsend *et al.* 2008). Wind dispersal (Hawes *et al*. 2007), e.g. of eggs deposited in litter materials or as aerial plankton (Washburn and Washburn 1984), is possible but not known for this species and not common in oribatid mites (Seyd 1962, Starý and Block 1998). *S. magnus* is unlikely to be dispersed easily. At a single site or at locations close to each other genetically very different individuals exist, separated from each other by high numbers of substitutions. In contrast to the high nucleotide divergence the protein divergence is low. Explanations for the lower protein variance are the four times degenerated third codon position (Xia *et al*. 1996, Baker 2000) and the fact that the *COI* is under purifying selection (Li *et al.* 1985). Purifying selection eliminates non-synonymous substitutions and tolerates synonymous substitutions (Xia *et al.* 1996). However, non-synonymous substitutions take place in the *COI* gene of *S. magnus* and non-synonymous substitutions could be present in a small number since 60% of the amino acids of the *COI* barcoding region do not interact with another protein (Ballard and Melvin 2010).

High frost tolerance of *S. magnus* (Block 1979, Webb and Block 1993) likely contributed to the high genetic diversity in *S. magnus* as this may have allowed several lineages to survive Pleistocene glaciations in Central and Northern Europe. Thereafter, representatives of other lineages arrived contributing to the local coexistence of very different genetic lineages. Dispersal by man colonizing Central and Northern Europe in the Holocene likely contributed to the high genetic variance in local populations (Dlugosh and Parker 2008).

As stated above, major splits in *S. magnus* likely occurred in the Tertiary, presumably before the Miocene with the diversity of the lineages increasing fastly. At the beginning of the Miocene (~23 Ma) the climate and vegetation in Europe and Asia changed. The evergreen forests dominating in the early and mid Triassic changed to coniferous and deciduous forests and the climate became dryer and cooler

(Mai 1989, 1991, 1996, Kvaček 2000). Today, early to mid Miocene evergreen forests only exist in the western Mediterranean such as the Canary Islands and East Asia (Kvaček 2000, Kvaček *et al.* 2000). Radiation in *S. magnus* in the Miocene may be related to these vegetation changes. In fact, *S. magnus* dominates in forests of deciduous and conifer trees and may have dispersed with the expansion of temperate and boreal forest in the Miocene. Then, during the Pleistocene cooling *S. magnus* survived in various glacial refugia from where it started to expand with the recent Holocene warming and expansions of temperate and boreal forest.

High genetic differences in *COI* nucleotide sequences were also found in other species of *Steganacarus* (up to 28.6%; Salomone *et al.* 2002) and other arthropods, such as the oribatid mites *Platynothrus peltifer* (up to 25%; Heethoff *et al.* 2007), *Scutovertex sculptus* (up to 11%; Schäffer *et al.* 2010) the springtail *Friesea grisea* (17.7%; Torricelli *et al.* 2010), the harvestman *Aoraki denticulata* (up to 19.2%; Boyer *et al.* 2007) and the intertidal copepod *Tigriopus californicus* (up to 23%; Edmands 2001). High genetic variation in soil and marine animals but not in those living above the ground may indicate that species in these habitats stay more constant but accumulate neutral mutations whereas species above the ground evolved more vigorously and radiated, presumably parallel to that of higher plants. In fact, the radiation of the most diverse group insects, such as Diptera, Chrysomelidae (Coleoptrata) and Aculeata (Hymenoptera), occurred in the Neozoic (Grimaldi and Engel 2005). In contrast, radiation in soil organisms, such as oribatid mites and Collembola occurred much earlier (Schaefer 2009, Schaefer *et al*. 2010, Schäffer *et al*. 2010, Torricelli *et al*. 2010). However, to prove whether this scenario applies in general other soil living animals, such as Collembola, nematodes and earthworms, need to be investigated. This will also prove if other soil animal taxa also survived the Pleistocene glaciation in refugia in Central and Northern Europe as is the case in oribatid mites.

2.5 Conclusion

The results of this study suggest that microarthropods in soil, such as the oribatid mite species *S. magnus*, survived the Pleistocene glaciations in cryptic refugia contrasting the pattern proposed for the majority of aboveground animals and plants. A multitude of lineages presumably survived in refugia including Scandinavia, Central and Eastern Europe, and also in Alpine regions of Austria. Remarkably, today individuals of very different *COI* lineages coexist at localities which are geographically close together or even at the same locality. The exceptionally high genetic variation in *S. magnus* suggests that the major lineages of this species split in the Tertiary and radiated with the expansion of the temperate and boreal forests in the Miocene. With cooling in the Pleistocene populations shrunk but survived in multiple refugia in Northern and Central Europe, and also in Southern locations. From these refugia they expanded with warming following the temperate and boreal forests colonizing Central and Northern Europe in the Holocene. With this expansion representatives of very different lineages met and colonized the same or geographically close locations. To support this scenario nuclear genes, such as elongation factor 1α, need to be investigated to allow reconstruction of mixing processes due to sexual reproduction.

Chapter Three

Is there a cryptic species complex in the oribatid mite *Steganacarus magnus* (Nicolet, 1855) (Acari, Oribatida)?

Summary

DNA barcoding is a popular tool for species determination and delineation in molecular ecology and the mitochondrial cytochrome c oxidase (*COI*) gene is most common marker used for barcoding and detection of cryptic species. However, several studies revealed problems when using mitochondrial DNA for barcoding approaches due to nuclear amplified pseudogenes, symbionts and gene duplications. The soil-living oribatid mite *Steganacarus magnus* is a member of a morphological difficult to determine species complex and displays extraordinary high genetic diversity among populations in Europe (Chapter II). In this study, 77 individuals of 14 populations throughout Europe were analyzed to clarify if *S. magnus* consists of cryptic species or if gene-flow is maintained between individuals. To preclude the above mentioned problems and to detect sexual recombination between individuals the nuclear elongation factor 1 alpha (*ef 1α*), a conservative single copy gene, and the *COI* were sequenced from 37 individuals of the oribatid mite *S. magnus*. Phylogenetic trees generated with nucleotide and protein sequences of both genes revealed that sexual reproduction occurred between individuals with distinct mitochondrial lineages. The maximum of the uncorrected mean pairwise differences of the *COI* sequences between the populations was 28.4% in the nucleotide and 3.1% in the protein. The maximum of the pairwise differences *ef 1α* sequences were 19.6% in the nucleotide and 3.7% in the protein. Despite this high variability the results suggest that *S. magnus* does not consist of cryptic species and that the high genetic diversity is not maintained by speciation processes, but by neutral evolution at the third codon position in the mitochondrial gene (*COI*). The results support the hypothesis that the soil-system is evolutionary old and has endured ecological and climatic changes for tens of millions years. However, if sexual reproduction of different lineages combines different ecotypes and therefore provides selective advantage over other lineages remains to be tested.

3.1 Introduction

DNA barcoding is a well established tool for discovery of new species and biodiversity assessment without using morphology (Ball *et al.* 2005, Hebert *et al.* 2003a, Hajibabaei *et al.* 2007, Dasmahaptra *et al.* 2010). For invertebrates, the first 650 bp of the 5'-end of the mitochondrial gene cytochrome c oxidase (*COI*) has become the most widely used marker ("Folmer fragment", Folmer *et al.* 1994, Hebert *et al.* 2003a-b, 2004a-b, Hajibabaei *et al.* 2006, Rock *et al.* 2008, Derycke *et al.* 2010). For vertebrates another mitochondrial (mt) gene, part of cytochrome b, is used (Taberlet *et al.* 1994, Bilton *et al*. 1998, Conroy and Cook 2000, Ursenbacher *et al.* 2006). The method of DNA barcoding revolutionized taxonomy and species delineation of undescribed species and of species which are difficult to determine morphologically; further, it has been successful in detecting cryptic species, overlooked by traditional taxonomic methods (Burns *et al.* 2007). However, the method received much criticism based on theoretical (Hickerson *et al.* 2006), methodological (Will and Rubinoff 2004, Cameron *et al.* 2006, Song *et al.* 2008) and empirical grounds (Hurst and Jiggins 2005).

Oribatid mites (Acari, Oribatida) are among the most diverse and abundant soil living arthropods with about 10,000 described species (Schatz 2002) and up to 400,000 individuals per square meter of forest soil (Maraun and Scheu 2000). Fossils of oribatid mites were found in Devonian sediments (380 my ago; Shear *at al.* 1984, Norton *et al.* 1988a) and the origin of oribatid mites dates back to 440 my (Lindquist 1984) or even 570 my according to molecular dating (Schaefer *et al.* 2010).
The soil-living oribatid mite *Steganacarus magnus* is a member of a morphologically difficult to determine species complex (Steganacaridae, Weigmann 2006) and displays extraordinary high genetic diversity among populations in Europe (up to 31.8%; Chapter II). The extraordinary high genetic diversity indicates a cryptic species complex in *S. magnus*. Reasons for this high genetic diversity are that many morphologically identical species live together in a small area (cryptic species complex) or neutral evolution, i.e. large population size of *S. magnus* and polymorphism established coincidentally. One way to overcome problems with using mtDNA as barcoding gene is to compare mitochondrial markers with nuclear markers (Roe and Sperling 2007, Moniz and Kaczmarska 2009, Cicconardi *et al.* 2010, Leo *et al.* 2010) to investigate recombination between different mitochondrial lineages and to detect cryptic species (co-cladogenesis of both genes). The same phylogenetic tree in both genes suggests that the species in fact comprises a cryptic species complex. In contrast, different phylogenetic trees in the mtDNA and the nuclear gene provide evidence for recombination and sex and therefore indicate that lineages studied originated from a single biological species.

Mitochondria are maternally inherited cell organelles with a circular genome of about 14-19 kp in bilaterian organisms (Wolstenholme 1992, Boore 1999). The mitochondrial genome evolves with a faster rate than the nuclear genome (Chantangsi *et al.* 2007) and 'reproduces' without sexual recombination. Migration, speciation and splitting events that occurred several million years ago can be detected even in morphologically coherent taxa.

To investigate if *S. magnus* comprises a cryptic species complex, mt (*COI*) and nuclear genes (*ef 1a*) were sequenced from the same individuals. Sampling in regions with different mt lineages (Chapter II) was extended (Northern Germany, Poland, Czech Republic). In total, 77 individuals of eleven countries throughout Europe were analyzed.

3.2 Materials and methods

3.2.1 Taxon sampling

Throughout Europe, 77 individuals of *Steganacarus magnus* (Oribatida, Mixonomata) were sampled from 14 locations in eleven countries (Fig. 13, Table 6). Specimens were extracted from leaf litter using life extraction by heat (Macfadyen 1961, Kempson *et al.* 1963). *Hypochthonius rufulus* (Oribatida, Enarthronota) was sampled as outgroup taxon. Animals were identified under a stereomicroscope, determined after Weigmann (2006) and stored in 75% ethanol at -20°C until DNA extraction.

Figure 13: Map of Europe (http://de.wikipedia.org/wiki/Europa). Black dots with numbers are the sampling points of *Steganacarus magnus.*

Table 6: Localities where *Steganacarus magnus* was sampled [ln: location number, country, location, coordinates, abbreviations of sampling locations (code), number of sequenced individuals per gene (*COI, ef 1α*)] and name of collector (for details see Acknowledgements). Each location is marked by a colour symbol.

ln	country	location	coordinates	code	*COI*	*ef 1α*	colour	litter collector
1	Austria	Villach	46.57° 13.85°	A 1	1-2	3		K. Domes-Wehner
2	Czech Republic	Decin	50.78° 14.23°	CZ 1	1-5	1-2		M. Rosenberger
3	Denmark	Copenhagen	55.68° 12.58°	DK 1	1-3	2		N. Eisenhauer
4	England	Bedford	52.07° -0.44°	GB 1	1-5	2-6		A. Milcu
5	France	Loire	45.56° 4.79°	F 1	1-4	1, 5		M. Maraun
6	Germany	Colonge	50.83° 7.18°	D 3	-	1		M. Maraun
7		Kranichstein	49.89° 8.69°	D 1	1-14	3, 13, 15-18		M. Rosenberger
8		Mecklen. Seenplatte	53.57° 12.33°	D 2	1-9	3, 6-9		I. Schaefer
9	Hungary	Piliscaba	47.62° 18.83°	HUN1	1	1		C. Csjui
10	Italy	Grosseto	42.63° 11.11°	I 1	1-10	4-7		M. Maraun
11	Poland	Krakow	50.04° 19.84°	PL 1	1-6	1-2, 5, 7		S. Scheu
12	Romania	Bagau	46.55° 26.72°	RUM 1	1-4	1-2		T. Pasca
13		Cluj	46.77° 23.52°	RUM 2	1-3	1, 3		T. Pasca
14	Switzerland	Locarno	46.17° 8.77°	CH 1	1	1		M. Scheu

3.2.2 DNA extraction and sequencing

Genomic DNA was extracted from single individuals using the DNeasy® Blood and Tissue Kit (Qiagen; Hilden, Germany) for animal tissue following the manufacturer's protocol.

In total, two PCR reactions of 76 individuals were performed using the primers 40.71F (5´-TCN TTY AAR TAY GCN TGG GT-3´) and 52RC (5´-CCD ATY TTR TAN ACR TCY TG-3´) for elongation factor-1 a (Klompen 2000) and COIarch1 (5´-GGT CAA CAA ATC ATA AAG AYA TYG G-3´) and COIarch2 (5´-TAA ACT TCA GGG TGA CCA AAA AAT CA-3´) (Heethoff *et al.* 2007) for COI, amplifying fragments of 598 bp and 463 bp, respectively.

The PCR contained 0.5 µl of each primer (100 pmol/µl), 1 µl $MgCl_2$ (25mM), 12.5 µl of HotStarTaq® Master Mix (1.25 units HotStarTaq® polymerase, 100 µM of each dNTP and 7.5 mM $MgCl_2$ buffer solution; Qiagen, Germany) or SuperHot Taq Mastermix [2.5 units SuperHot Taq polymerase, 10 µM of each dNTP and buffer solution (20 mM Tris-HCI (pH 8.3), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Nonidet P40 and 0.5% Tween 20), Genaxxon; Ulm, Germany] containing polymerase, 5 µl template DNA for *ef 1α* and 3 µl for *COI*; reaction volumes were filled up to 25 µl with RNase free water. PCR parameters for *COI* included a 15 min step at 95°C for polymerase activation followed by 36 cycles of 30s at 94°C for denaturation, 60s at 51°C for primer annealing and 60s at 72°C for elongation and a final 10 min step for elongation at 72°C parameters. PCR parameters for *ef 1α* included a 15 min step at 95°C for polymerase activation followed by 9 cycles of 50s at 95°C for denaturation, 70s at 46°C for primer annealing and 120s at 72°C for elongation, followed by 34 cycles of 50s at 95°C for denaturation, 70s at 50°C for primer annealing and 120s at 72°C for elongation and a final 10 min step for elongation at 72°C. PCR programme are given in Table 7. PCR products were visualized on 1% agarose gel and purified using the QIAquick® PCR Purification Kit (Qiagen; Hilden, Germany) following the manufacturer's protocol. The purified PCR products were sequenced in both directions by Macrogen Inc. (Seoul, Korea).

Table 7: PCR conditions of the elongation factor 1α (*ef 1α*) and of the cytochrome c oxidase subunit I (*COI*).

	ef 1α		***COI***	
	temperature (°C)	**time**	**temperature (°C)**	**time**
initial denaturation step	95	15 min	95	15 min
denaturation	95	50 sec	94	30 sec
annealing	46	70 sec	1 min	60 sec
elongation	72	2 min	72	60 sec
number of cycles	9		36	
denaturation	95	50 sec	-	-
annealing	50	70 sec	-	-
elongation	72	2 min	-	-
number of cycles	34		-	
final elongation	72	10 min	72	10 min

3.2.3 Phylogenetic, population genetic and statistical analyses

Nucleotide sequences were edited and translated into amino acids using the invertebrate mitochondrial and the nuclear standard eukaryotic codes for *COI* and *ef 1α* implemented in SEQUENCHER v4.9 (Gene Codes) (starting at the third position). Ambiguous positions were corrected using electropherogramms and corrected sequences were aligned with ClustalX v1.81 (Thompson *et al.* 1997), using multiple alignment parameters: 10.0 for gap opening and 0.1 for gap extension for the nucleotide, default settings for the amino acid dataset. Phylogenetic trees were generated with Beast v1.5.4 (Drummond and Rambaut 2007), MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) and Neighbor-Joining analyses without and with model of sequence evolution in PAUP* (Swofford 1999) to identify monophyletic clusters in the dataset. The best model of sequence evolution was estimated with Modeltest3.6 (Posada and Crandall 1998) for PAUP* and MrModeltest (Nylander 2004) for the Bayesian analysis. The best fit model for the *COI* dataset was GTR+I+G for the analyses and K80+G for MrBayes and TrNef+G for PAUP*. Model parameters were nst=6 and rates=invgamma for *COI* nucleotide sequences and nst=2 and rates=gamma for *ef 1α* nucleotide sequences. All protein alignments in MrBayes were analyzed with aamodel=equalin and in Beast with mtrev for mitochondrial protein and WAG for nuclear protein sequences. One individual of *Hypochthonius rufulus* (C. L. Koch 1835) (Oribatida, Enarthronota) was selected as outgroup for *S. magnus*. The Markov Chain Monte Carlo was run for ten million generations and sampled every 1000th generation, the 50% majority consensus tree excluded the first 2,500,000 trees (burnin of 25%) for MrBayes. Burnin for the Bayesian approach in Beast was 1000 (burnin of 10%).

Standard diversity indices (haplotype number (N_h), haplotype diversity (H_d,) nucleotide diversity (Π_n), number of variable (N_{vs}) and invariable sites (N_{is}), parsimony informative sites (N_{pars}), the number of singletons (N_s) and the McDonald-Kreitman (MK) test, to detect selection, were calculated with DNASP v5.0 (Rozas *et al.* 2003). The McDonald-Kreitman test detects selection by examining the distribution of synonymous and non-synonymous substitutions among populations; it is robust against demographic and recombination events (McDonald and Kreitman 1991). The geographical structure of genetic diversity among and within populations and geographical clades were calculated with ARLEQUIN v3.01 (Excoffier *et al.* 2005) using analysis of molecular variance (AMOVA, 16.000 permutations). Only populations with two and more individuals were included for the analysis. Estimates for demographic expansion (Tajima's D and Fu's F_s neutrality tests) and pairwise differences (F_{ST} 10.000 permutations) were also calculated in ARLEQUIN. Significance was calculated with 10.000 permutations for nucleotide sequences and amino acids for both genes.

A parsimony based median-joining haplotype network (Bandelt *et al.* 1999) was generated in NETWORK v4.5 (Fluxus-Technology, Suffolk, UK) with default settings for nucleotide sequences and amino acids of both genes.

3.3 Results

Amplification of *ef 1α* proved to be difficult; both genes were amplified from 28 individuals, from 39 individuals only *COI* and from 9 individuals only *ef 1α* was amplified.

The 67 *COI* sequences consisted of 47 haplotypes (70.2%) for the nucleotide (Fig. 18) with 309 variable (51.8%) and 277 (46.4%) parsimony informative sites (Table A7), the amino acid sequences of *COI* consisted of 29 haplotypes (43.3%) (Fig. 19). Variance in the 37 *ef 1α* sequences was even higher, the nucleotide dataset consisted of 35 haplotypes (94.6%) (Fig. 20) with 218 variable (47.3%) and 127 (27.5%) parsimony informative sites (Table A8); the protein formed 33 haplotypes (89.2%) (Fig. 21). Haplotype diversity (H_d) of the nucleotide sequences was high being 0.980 in *COI* and 0.997 in *ef 1α*.

3.3.1 Phylogenetic analyses

Phylogenetic trees (NJ without and with model of sequence evolution, MrBayes and Beast, Fig. A8-11) for the *COI* nucleotide sequences uniformly separated three monophyletic clades. One clade contained populations from Italy, Germany, France, Great Britain and one individual from Denmark (black clade), the second comprised most individuals from Eastern Europe, i.e. Czech Republic, Hungary, eastern Germany and eastern Denmark (blue clade), and the third included all populations from southern east Europe (Romania, Austria and Switzerland) as well as four individuals from eastern Germany and one individual from Czech Republic (red clade) (Fig. 14). The black clade was well supported in all analyses, with 97% and 93% bootstrap support for NJ without and with model of sequence evolution, respectively and posterior probabilities of 1 for the two Bayesian approaches (MrBayes and Beast). The blue clade was also well supported with respective bootstrap values of 98% and 88% and posterior probabilities of 1 in the Bayesian analyses. The red was only recovered in the Bayesian analyses with posterior probabilities of 1 but was paraphyletic in the NJ analyses. In all phylogenetic analyses the black and the blue clade formed sister clades and this was supported by high bootstrap values and posterior probabilities (94%, 91%, 1 and 0.87).

The phylogenetic trees based on the amino acid sequences of *COI* confirmed the three main clades with high statistical support (Fig. 15, A12-14), but in contrast to the trees based on nucleotide sequences, the blue and the red clade grouped as sister clades and the blue clade became paraphyletic in the Bayesian approach of MrBayes. However, in all other analyses the blue clade was monophyletic and supported by a high bootstrap values and posterior probabilities (76% and 1).

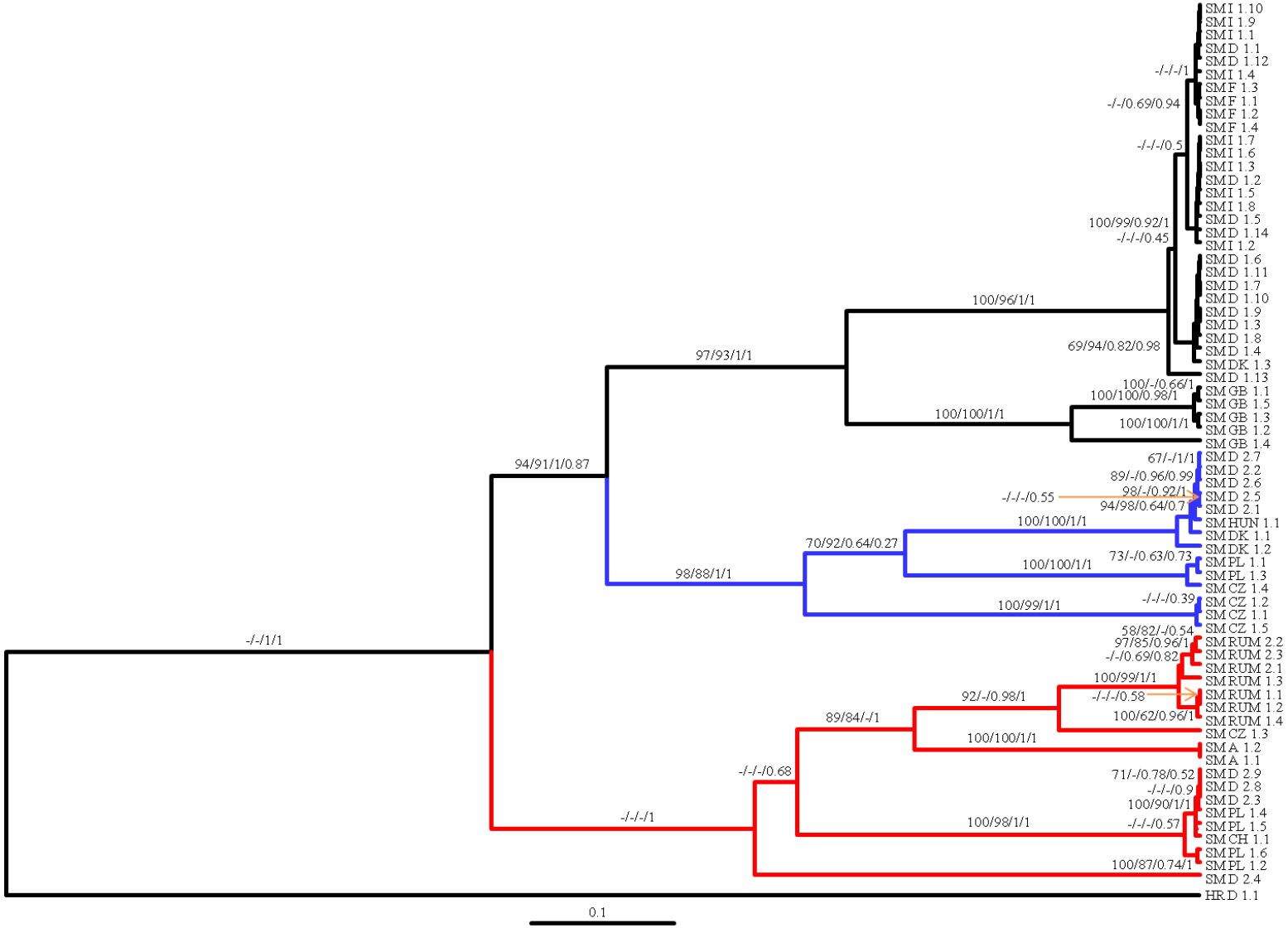

Figure 14: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 based on 67 *COI* nucleotide sequences and *Hypochthonius rufulus* as outgroup (HR_D_1.1). Branch colours show the different clades (black, blue, red). Numbers on branches are bootstrap values from NJ analysis without and with evolution model (TVM+I+G) and posterior probabilities from MrBayes and Beast.

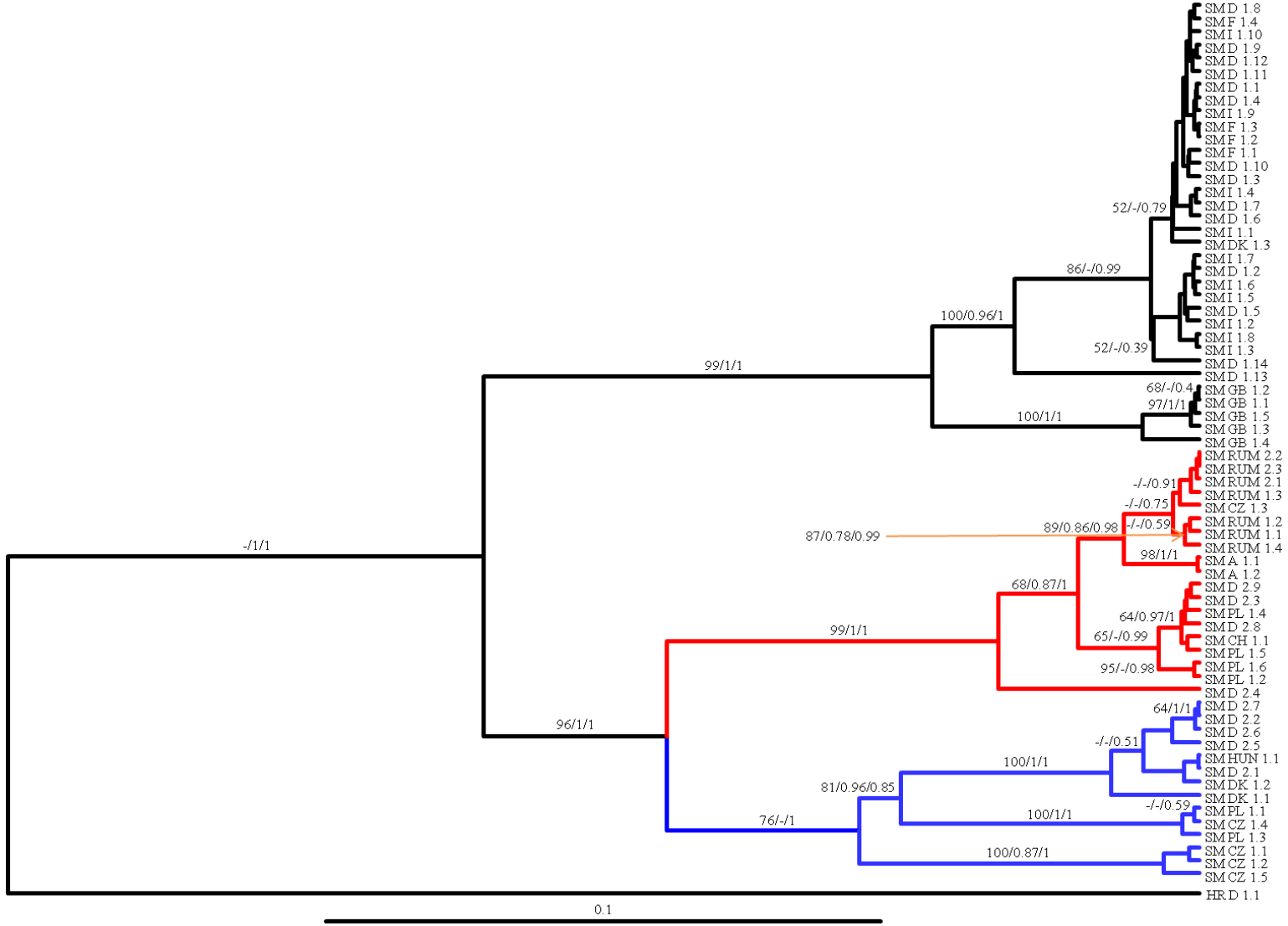

Figure 15: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 based on 67 *COI* protein sequences and *Hypochthonius rufulus* as outgroup (HR_D_1.1). Branch colours show the different clades (black, blue, red). Numbers on branches are bootstrap values from NJ analysis without evolution model and posterior probabilities from MrBayes and Beast.

The phylogenetic tree based on the nucleotide sequences of *ef 1α* (Fig. 16, A15-18) recovered the same monophyletic clades as those formed in the analysis of the *COI* gene. Again, the black clade was very well supported by high bootstrap values and posterior probabilities of 100% and 1, respectively. The blue clade was also well supported by bootstrap values of 93% and 88% and posterior probabilities of 0.95 and 1, however, it included one individual of the red clade. The other individuals of the red clade also formed one clade which was supported with high bootstrap values and posterior probabilities (91%, 63%, - and 1).

Importantly, one *ef 1α* haplotype (PL_1.2) that was in the red clade in the *COI* trees, was recovered in a well supported blue subclade (bootstrap values of 99% and 100% and posterior probabilities of 0.99 and 1).

The three main clades in the phylogenetic analyses based on the amino acid sequences of the *ef 1α* were paraphyletic and weakly supported (Fig. 17). However, the haplotypes in the paraphyletic subclades corresponded to the subclades in the other phylogenetic trees (Fig. A19-21). Notably, in accordance with the nucleotide based trees of *ef 1α*, haplotype PL_1.2 clustered within the blue subclade.

The red and the blue clade only formed sister clades in the Bayesian approach of Beast which were supported by a posterior probability of 1. In the other phylogenetic analyses the black clade formed the sister clade of the blue (Fig. 17, A18). In the other phylogenetic approaches the black clade formed the sister clade of the blue (Fig. A15-17), or the red clade was paraphyletic (Fig. A17). In each of the three phylogenetic trees (NJ, MrBayes and Beast) of the *ef 1α* protein the black, blue and red clades were paraphyletic (Fig. 17, Fig. A19-21).

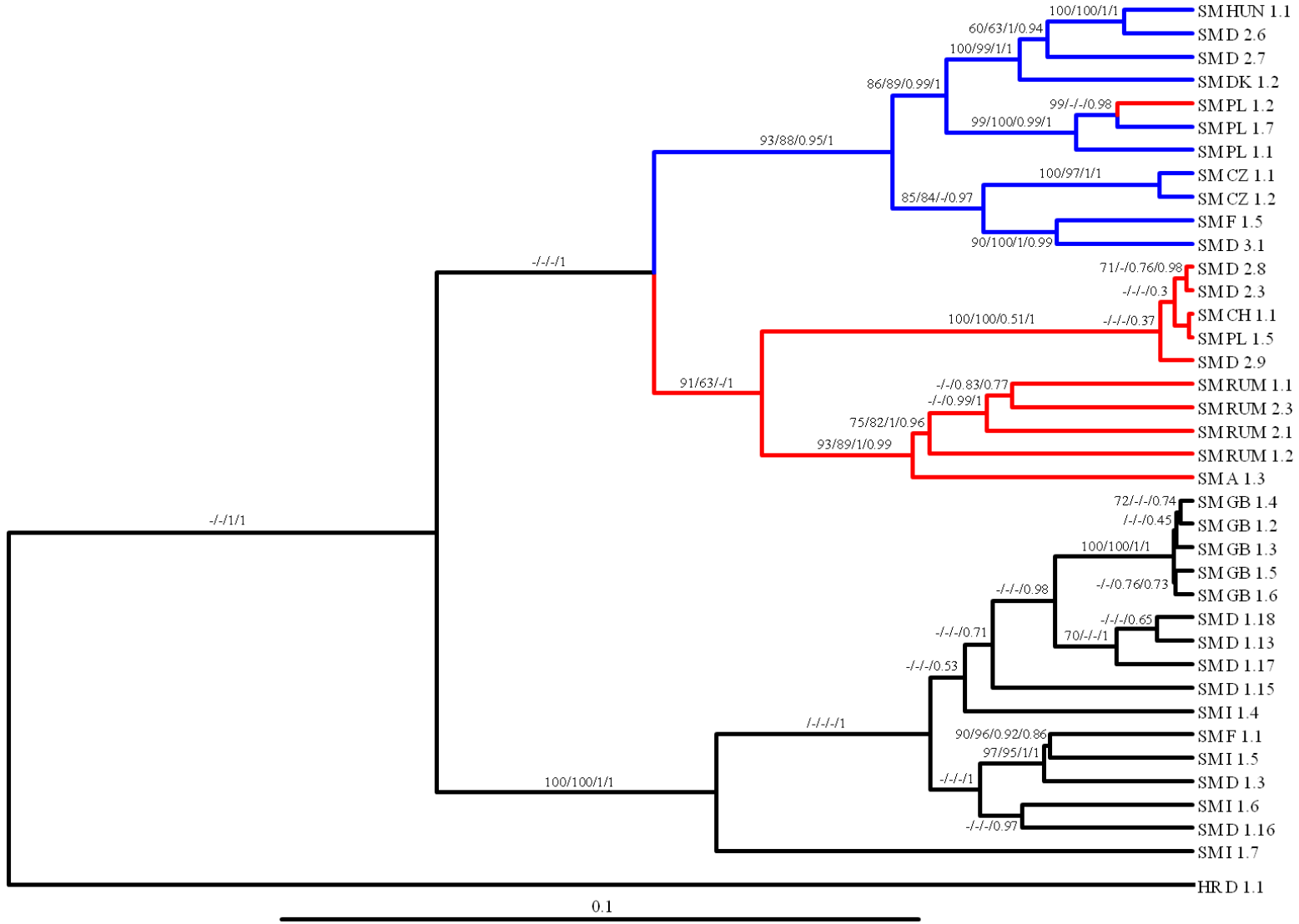

Figure 16: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 based on 37 *ef 1α* nucleotide sequences and *Hypochthonius rufulus* (HR_D_1.1) as outgroup. Numbers on the branches are bootstrap values from NJ analysis without and with model of sequence evolution (TrNef+G) and posterior probabilities from MrBayes and Beast. Branch colours show the different clades as identified in phylogenies based on *COI* sequences (black, blue, red; Figs. 14, A8-11).

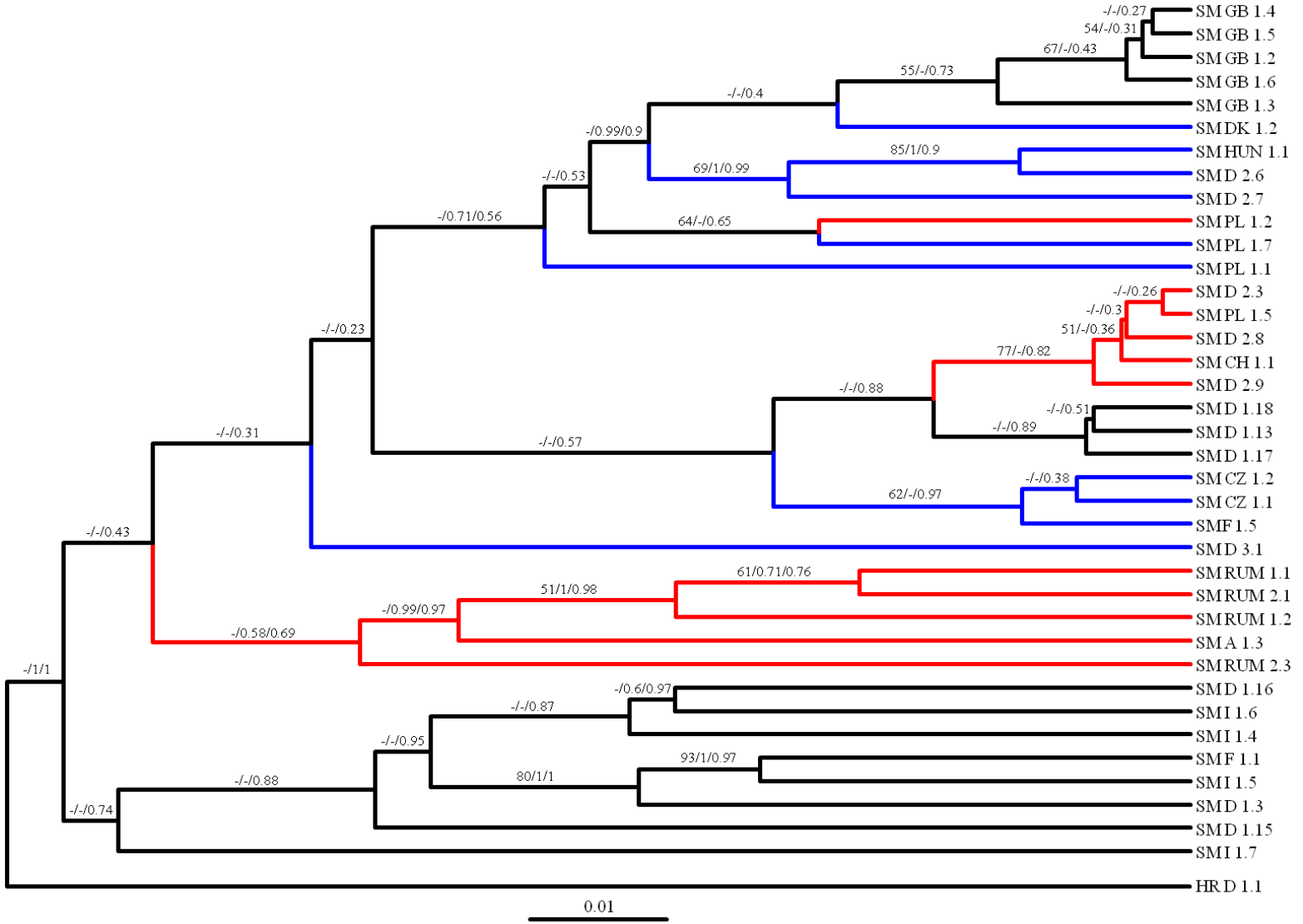

Figure 17: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 based on 37 *ef 1α* protein sequences and *Hypochthonius rufulus* (HR_D_1.1) as outgroup. Numbers on the branches are bootstrap values from NJ analysis without model of sequence evolution and posterior probabilities from MrBayes and Beast. Branch colours show the different clades as identified in phylogenies based on *COI* sequences (black, blue, red; Figs. 15, A12-14).

3.3.2 Network

All Network analyses recovered the three main clades (black, blue and red) from the phylogenetic trees representing three distinct mitochondrial lineages (Fig. 18, 19). The nucleotide dataset of *COI* consisted of 47 haplotypes and the three main clades were separated by 127 and 130 numbers of substitution steps (Fig. 18).

The black clade consisted of 34 sequences representing 20 haplotypes. As in the phylogenetic analyses, this clade comprised populations of Central Europe (Central Germany, France, Great Britain), Italy and one individual from Denmark. All individuals in this clade were closely related, except for the population from Great Britain that was separated from the other haplotypes by >110 substitution steps. Individuals from France, Italy and Germany shared identical or closely related haplotypes.

The blue clade comprised 14 individuals with twelve haplotypes from Central-East Europe, four individuals with four haplotypes from Czech Republic, five individuals with three haplotypes from northeast Germany, a single individual from Hungary, two individuals with different haplotypes from Denmark and two individuals with two haplotypes from Poland. The high number of haplotypes indicates that the mitochondrial lineages in these populations have been separated for a longer time than those in populations of the black clade, which is also demonstrated by long branches in the phylogenetic tree (Fig. 14).

The red clade consisted of 15 haplotypes comprising the same 19 individuals forming the red clade in the phylogenetic tree (Fig. 14); the individuals originated from Southeast Europe and four individuals from Northeast Germany, four individuals from Poland and one individual from the Czech Republic.

There were only three individuals sharing the same haplotype (D_2, RUM_1 and A_1) all other haplotypes were isolated from each other by high numbers of substitution steps and long branches in the phylogenetic tree. In addition to the genetic distance between lineages within the main clades, the three main clades were separated by substantial distances; the black clade was separated from the blue clade by at least 127 substitution steps and the blue clade was separated by at least 130 substitution steps from the red clade.

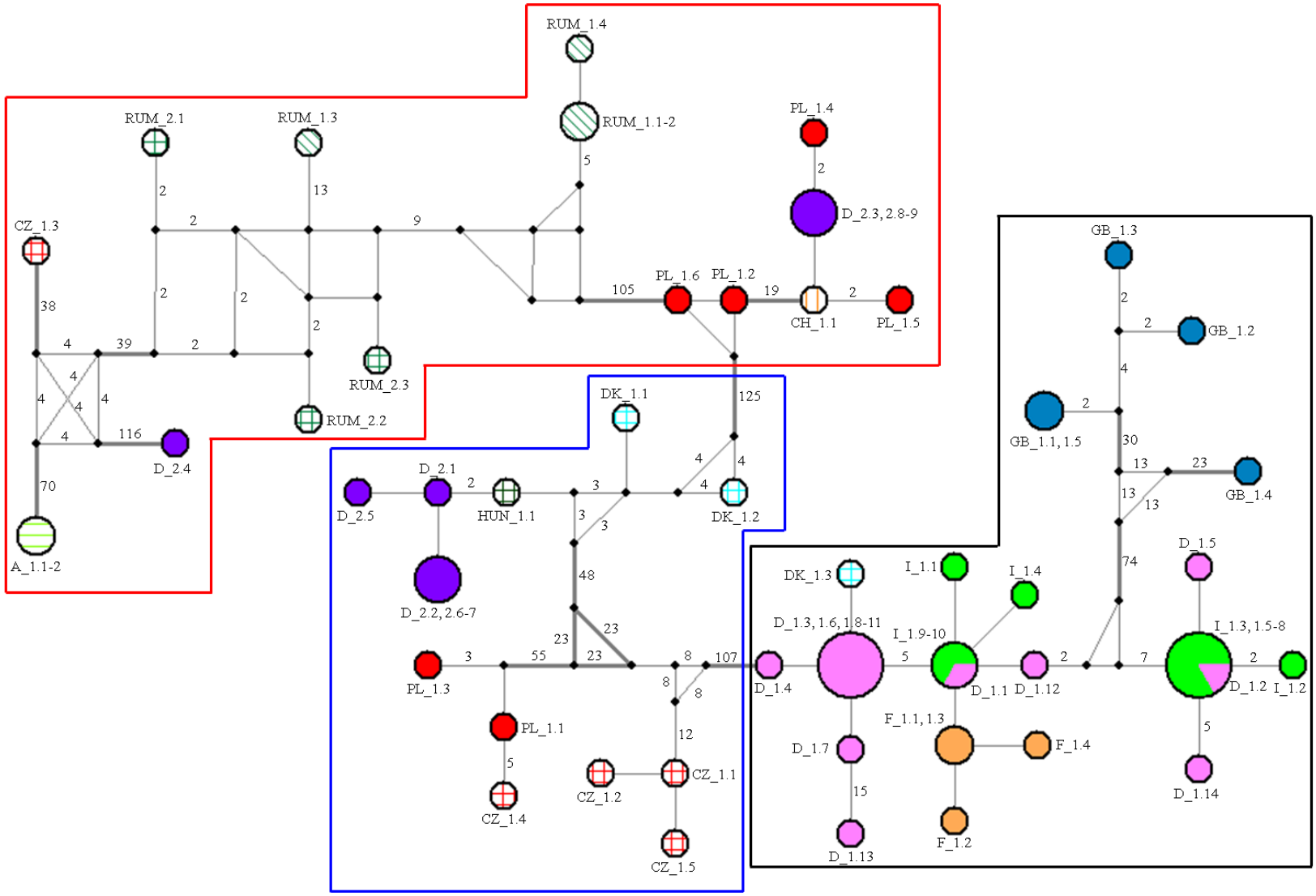

Figure 18: Median-joining haplotype network of 47 *COI* nucleotide haplotypes from *Steganacarus magnus*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one substitution step between haplotypes). Major subclades are marked by boxes.

The *COI* haplotype network based on the protein formed 29 haplotypes; several subclades recovered in the nucleotide network collapsed, as different nucleotide sequences coded for the same protein (Fig. 19).
The 20 nucleotide haplotypes that were connected in the black clade represented only ten haplotypes in the protein network. Similar to the nucleotide network, haplotypes from Great Britain were separated from all other haplotypes in the black clade by several substitution steps (eleven amino acid exchanges). All individuals from France shared the most common haplotype that also included individuals from Central Germany and Italy (I_1.9-10). The individual from Denmark (DK_1.3) shared the second most common haplotype in the black clade.
In the blue clade, the twelve nucleotide haplotypes formed nine amino acid haplotypes; four individuals from Germany (D_2.1-2, 2.6-7) and one individual of Hungary (HUN_1.1) shared the protein sequence of the most common haplotype in this clade. One haplotype was shared by one Polish (PL_1.1) and one Czech (CZ_1.4) individual. The other seven haplotypes were formed by single individuals from four locations [two haplotypes each from one location (D_2.5 and PL_1.3), two haplotypes from one Danish location (DK_1.1-2) and three haplotypes from one Czech location (CZ_1.1-2, 1.5)].
In the red clade, the 15 nucleotide haplotypes merged to ten. Three nucleotide haplotypes from one Romanian population shared the protein haplotype with two individuals from another Romanian population (RUM_1.1-2 and RUM_2.1-3). Further, the individual from Switzerland was identical to one haplotype from Northeast Germany. However, as in the nucleotide network protein haplotypes from individuals from Poland were distinct and were included in the blue (PL_1.1, 1.3) and the red clade (PL_1.2, 1.4-6).
Generally, the black clade was separated from the blue clade by at least 28 amino acid chances and the blue clade was separated from the red clade by at least 16 amino acid changes.

In the *ef 1α* nucleotide network the 37 sequences represented 35 haplotypes in the nucleotide merged to 33 haplotypes in the protein network (Fig. 20). In both datasets the same three main clades (black, blue and red) were recovered as in the *COI* network and the phylogenetic trees with one exception. Individual PL_1.2 of the red mitochondrial lineage in the *COI* dataset swapped position to the blue clade. In all three clades individuals that shared the same mitochondrial haplotype in the *COI* network, had isolated haplotypes in the *ef 1α* nucleotide network (black clade: I_1.5, I_1.6, I_1.7, GB_1.2 and GB_1.4; blue clade: D_2.6 and D_2.7; red clade: D_2.8, D_2.9, RUM_1.1 and RUM_1.2).

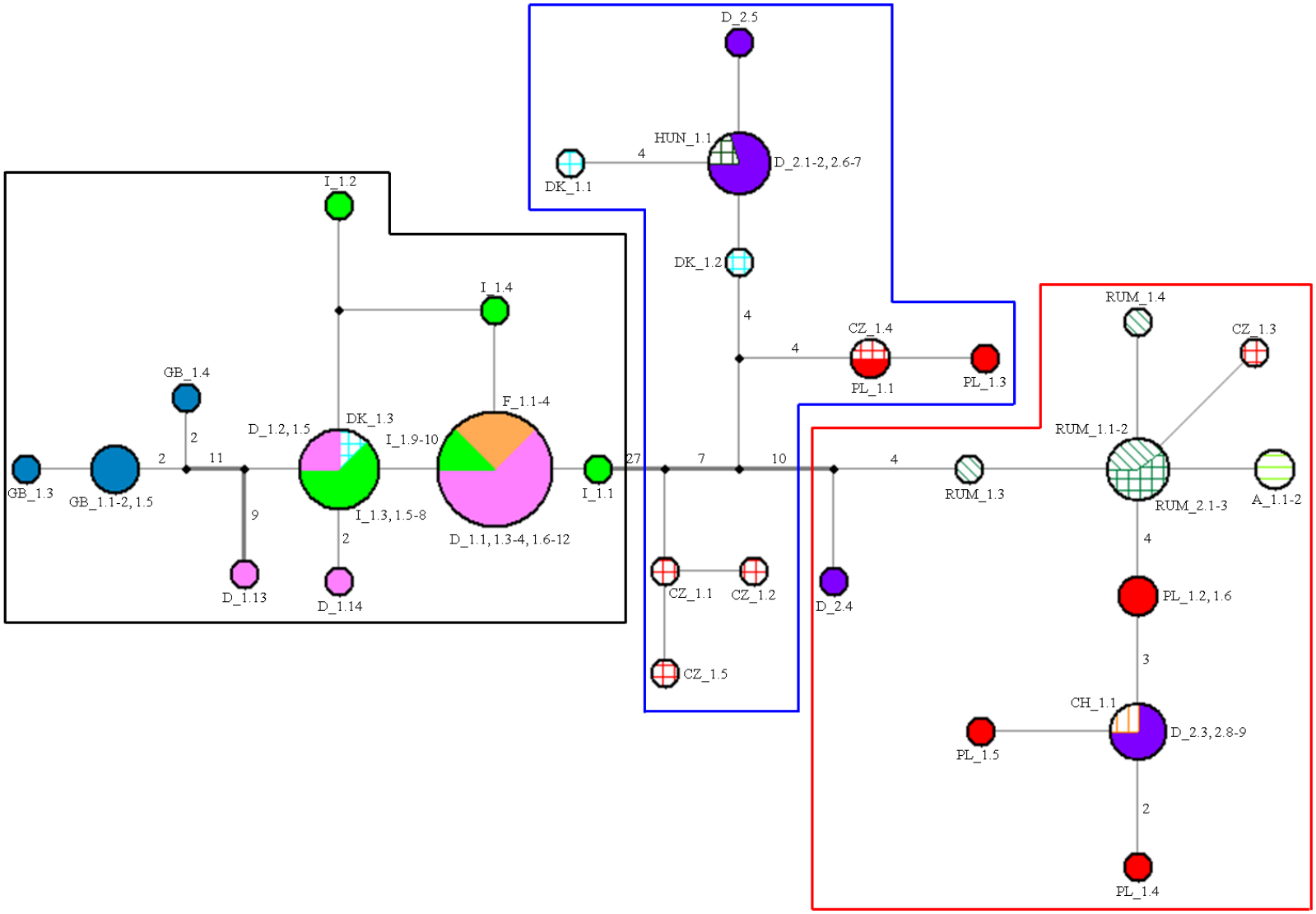

Figure 19: Median-joining haplotype network of 29 *COI* protein haplotypes from *Steganacarus magnus*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one amino acid change between haplotypes). Major subclades are marked by boxes.

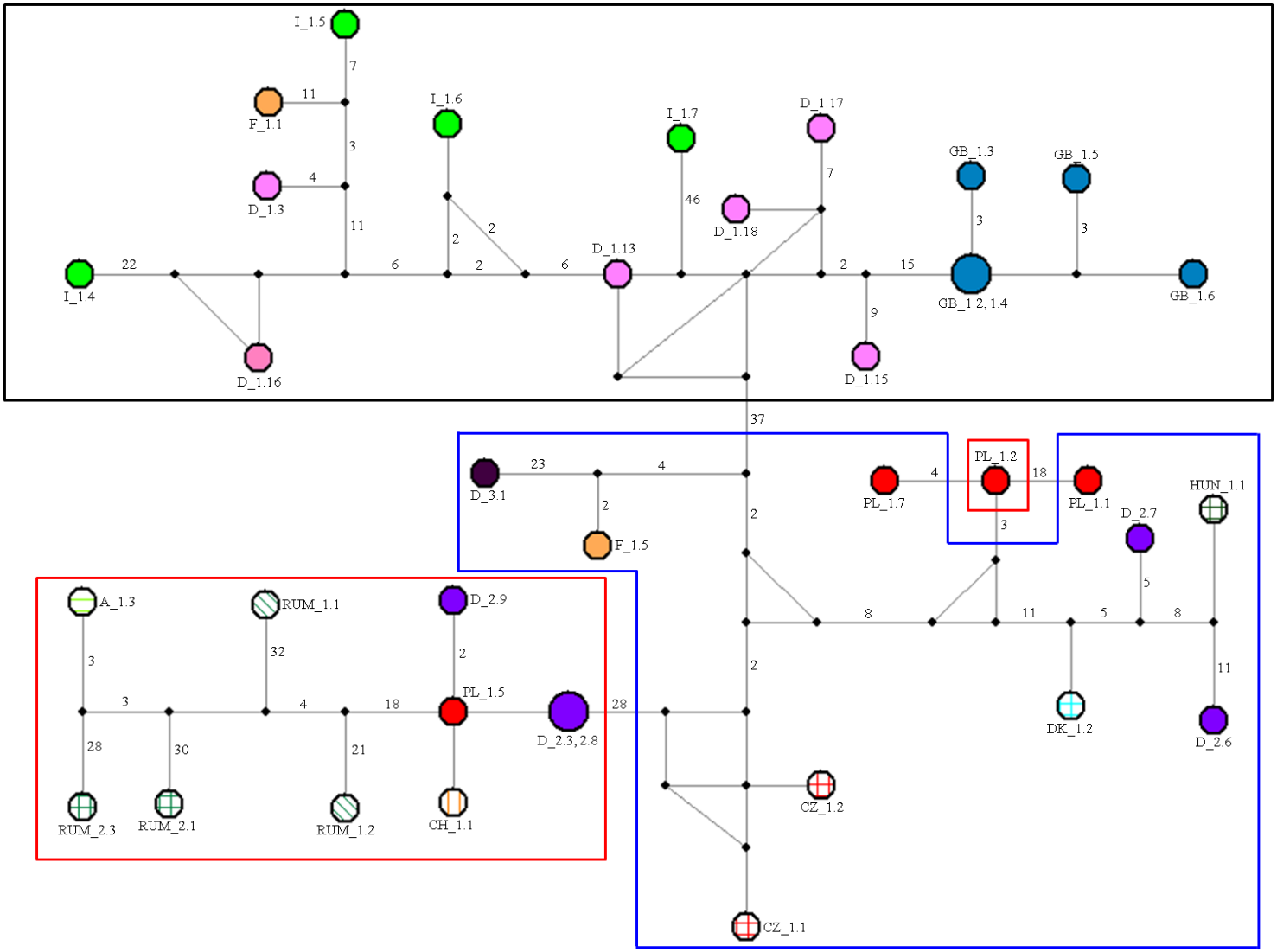

Figure 20: Median-joining haplotype network of the 35 *ef 1α* nucleotide haplotypes from *Steganacarus magnus*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one substitution step between haplotypes). Major subclades are marked by boxes.

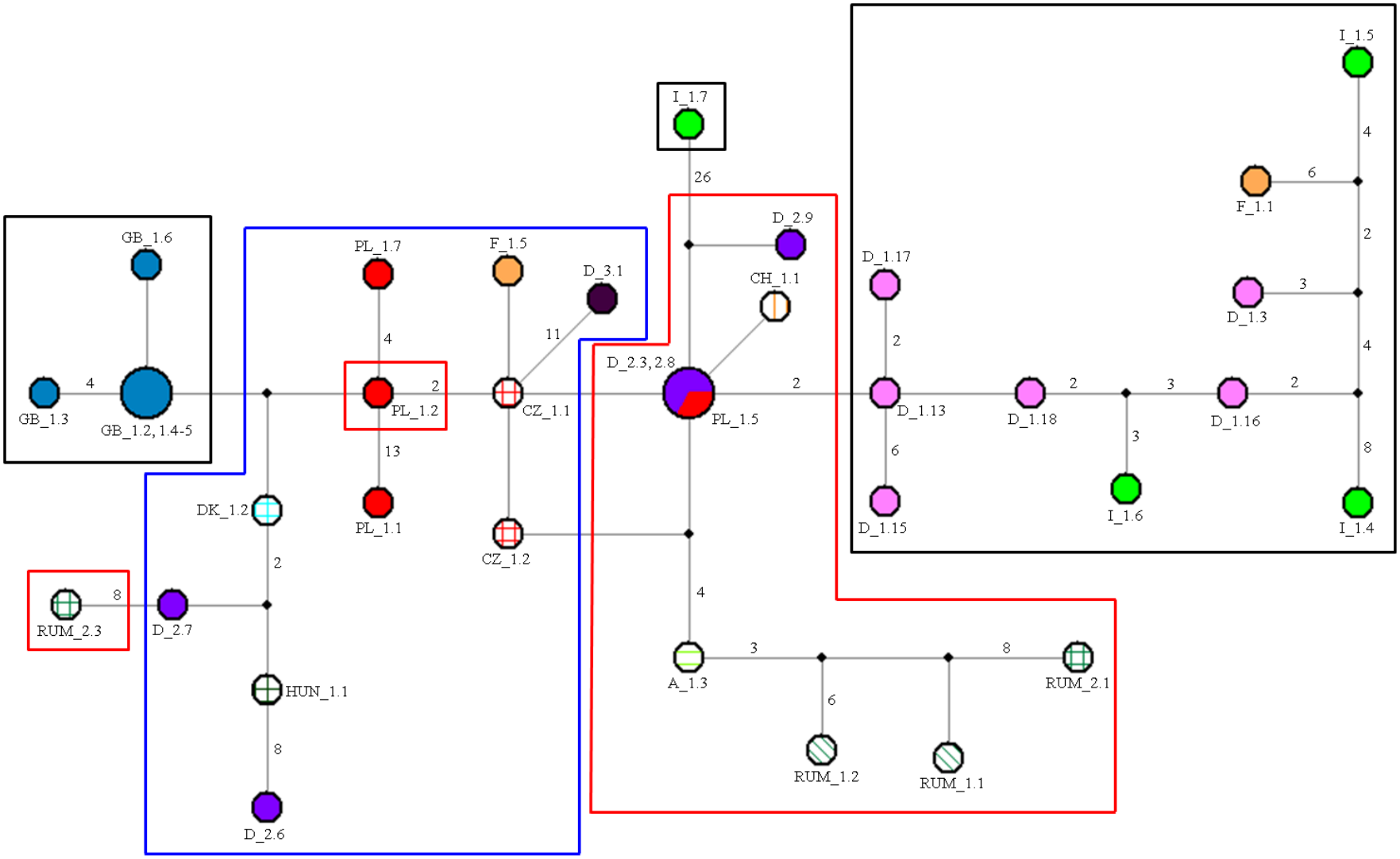

Figure 21: Median-joining haplotype network of the 33 *ef 1α* protein haplotypes from *Steganacarus magnus*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one amino acid change between haplotypes). Major subclades are marked by boxes.

3.3.3 Population genetic analyses

The minimum and maximum mean average pairwise uncorrected p-differences for the nucleotide sequences between populations were 1.4% (F_1/I_1) and 28.4% (A_1/DK_1) in *COI* and 0.6% (D_1/I_1) and 19.6% in *ef 1α* (I_1/RUM_2) (Table 8, 9). The minimum and maximum mean average pairwise uncorrected p-differences for the protein sequences among populations were in *COI* 0% (A_1/RUM_2) and 3.1% (A_1/D_1, D_1/RUM_1, D_1/RUM_2, I_1/RUM_1 and I_1/RUM_2) and for *ef 1α* 0.5% (CZ_1/D_2) and 3.7% (I_1/PL_1) (Table A9, A10).

Within populations the minimum and maximum mean average pairwise uncorrected p-differences of the nucleotide sequences were 0% (A_1) and 18.2% (DK_1) in *COI* and 0.7% (CZ_1) and 14% (F_1) in *ef 1α* (Table 8, 9). For the protein the minimum and maximum mean average pairwise uncorrected p-differences within populations were 0% (A_1, F_1, GB_1 and RUM_2) and 1.7% (DK_1) in COI and 0% (CZ_1, RUM_2) and 3.1% (I_1) in *ef 1α* (Table A9, A10).

The average uncorrected p-distances in the *COI* nucleotide within populations and among populations were 6.8% and 22.1%, respectively. Respective p distances in the protein were 0.5% and 1.9%. The average uncorrected p-distances in the *ef 1α* nucleotide were 6.4% within and 12.2% among populations and in the protein respective values were 1% and 1.6%.

Table 8: Mean percentage pairwise differences of uncorrected p-distances for the *COI* nucleotide of *Steganacarus magnus* from eleven locations. The diagonal is the within population differences (bold) and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Locations with less than two individuals were excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9	10	11
1	**A_1**	**0**										
2	**CZ_1**	24.7	**15.8**									
3	**D_1**	27.9	23.5	**1.5**								
4	**D_2**	25.1	22.5	24.6	**16**							
5	**DK_1**	**28.4**	22	17	16.8	**18.2**						
6	**F_1**	27.6	23.3	1.5	24.4	17	**0.2**					
7	**GB_1**	27.9	25.6	20.5	26.2	23.8	20	**5.3**				
8	**I_1**	28	23.6	1.7	24.4	17.1	**1.4**	20.4	**1.1**			
9	**PL_1**	23	21.5	23.9	18.5	23	23.6	27.2	23.8	**14.3**		
10	**RUM_1**	19.1	22.3	26.4	23.4	26.3	26.3	26.8	26.3	21.7	**1.7**	
11	**RUM_2**	19.2	22.2	26.9	23.1	26.1	26.7	27.2	26.9	22	2.8	**1.1**

Table 9: Mean percentage pairwise differences of uncorrected p-distances for the *ef 1α* nucleotide of *Steganacarus magnus* from nine locations. The diagonal is the within population differences (bold) and below the diagonal among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Locations with less than two individuals were excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9
1	**CZ_1**	**0.7**								
2	**D_1**	11.1	**3.7**							
3	**D_2**	7.2	13.7	**7.1**						
4	**F_1**	8.6	8.6	12.6	**14**					
5	**GB_1**	11.7	5.8	13.6	10.4	**0.7**				
6	**I_1**	13.8	**0.6**	16.1	10.9	9.3	**9.1**			
7	**PL_1**	5.7	12.9	7.8	11.3	12.2	15.3	**6.6**		
8	**RUM_1**	12.2	16.2	11.8	15.4	16	18.3	12.4	**7.6**	
9	**RUM_2**	13.5	17.4	12.2	16.4	16.6	**19.6**	13	8.2	**8.4**

The results of the AMOVA showed that the nucleotide variation among populations within countries (*COI* 59.9%, *ef 1α* 54.5%) and the variation within samples (*COI* 32.1%, *ef 1α* 46.8%) were significant and high (Table 10, 11). Also, the protein variation among populations within countries (*COI* 72.1%, *ef 1α* 42.4%) and the variation within samples (*COI* 27.3%, *ef 1α* 61.9%) were significant and high. The neutrality tests (Tajima's D and Fu's F_s) were not significant for the *COI* nucleotide (Table A11). Tajima's D was only significant for one population in the *COI* protein (Table A12) (D_1: D=-1.54, p-value=0.043) and *ef 1α* nucleotide (Table A13) (PL_1: D=-0.869, p-value=0.027), respectively. The neutrality test of Fu (Fu's F_s) was only significant for one population in the *ef 1α* protein dataset (Table A14) (D_2: FS=-1.79; FS p-value = 0.021).

Table 10: AMOVA table on variations among countries and within populations in nucleotide (black) and protein (red) sequences of *COI*. Each population was considered as a separate group. Populations with less than two individuals were excluded from the analysis. Significant level is p<0.05 (d.f.: degree of freedom).

Source of variance	d.f.	Sum of squares	Variance components	Percent of total variation	Fixation indices
Among countries	8 / 8	1986.28 / 62.29	4.88 Va / 0.01 Va	7.95 / 0.63	FCT: 0.08 / 0.01
Among populations within groups	2 / 2	569.03 / 20.44	36.81 Vb* / 1.35 Vb*	59.91 / 72.08	FSC: 0.65* / 0.73*
Within populations	54 / 54	1066.31 / 27.59	19.75 Vc* / 0.51 Vc*	32.14 / 27.28	FST: 0.68* / 0.73*
total	64 / 64	3621.62 / 110.31	61.44 / 1.87		

Table 11: AMOVA table on variations among countries and within populations in nucleotide (black) and protein (red) sequences in *ef 1α*. Each population was considered as a separate group. Populations with less than two individuals were excluded from the analysis. Significant level is p<0.05 (d.f.: degree of freedom).

Source of variance	d.f.	Sum of squares	Variance components	Percent of total variation	Fixation indices
Among countries	6 / 6	371.95 / 14.71	-0.35 Va / -0.05 Va	-1.28 / -4.11	FCT: -0.01 / -0.04
Among populations within groups	3 / 3	136.98 / 5.73	14.93 Vb* / 0.55 Vb*	54.49 / 42.41	FSC: 0.54* / 0.41*
Within populations	23 / 23	294.98 / 18.62	12.83 Vc* / 0.81 Vc*	46.79 / 61.9	FST: 0.53* / 0.38*
Total	31 / 31	803.91 / 39.06	61.44 / 1.31		

The McDonald-Kreitman test showed that the differences in the *COI* gene between seven of 55 populations (12.7%) were significant, between other two of 55 populations (3.6%) the differences

were highly significant and between seven of 55 populations (12.7%) they were very highly significant (Table 12, for more details see Table A15). All computed neutrality indices were 0 (Table A17).

The McDonald-Kreitman test showed that the differences in the *ef 1α* gene between two of 36 populations (5.6%) were significant, between other three of 36 populations (8.3%) the differences were highly significant and between 14 of 36 populations (38.9%) they were very highly significant (Table 13, Table A16). All computed neutrality indices were 0 (Table A18) indicating purifying selection.

Table 12: Results of the McDonald-Kreitman test of the *COI* gene of *Steganacarus magnus*. The differences between seven populations are significant (*0.01<P<0.05), between other nine population highly significant (**0.001<P<0.01, ***P<0.001).

Population	A 1	CZ 1	D 1	D 2	DK 1	F 1	GB 1	I 1	PL 1	RUM 1
CZ_1	*									
D_1	-	-								
D_2	-	-	**							
DK_1	-	-	-	-						
F_1	-	*	-	***	-					
GB_1	***	***	-	***	-	-				
I_1	-	*	-	***	-	-	-			
PL_1	-	-	-	-	-	-	**	-		
RUM_1	*	-	-	-	-	-	***	-	-	
RUM_2	-	*	-	-	-	*	***	-	-	-

Table 13: Results of the McDonald-Kreitman test of the *ef 1α* gene of *Steganacarus magnus*. The differences between two populations were significant (*0.01<P<0.05), between other 17 populations highly significant (**0.001<P<0.01, ***P<0.001).

Population	CZ 1	D 1	D 2	F 1	GB 1	I 1	PL 1	RUM 1
D_1	***							
D_2	-	***						
F_1	-	-	-					
GB_1	**	-	***	-				
I_1	***	-	***	-	-			
PL_1	-	***	-	-	***	***		
RUM_1	-	***	-	-	*	***	-	
RUM_2	***	***	**	**	***	***	*	-

3.4 Discussion

Commonly, a threshold of >3% genetic distance in the standard barcoding gene *COI* is taken as evidence for new species (Hebert *et al.* 2003a-b, 2004a-b), but this delineation often does not hold for invertebrates (Edmands 2001, Heethoff *et al.* 2007, Boyer *et al.* 2007, Rich *et al.* 2008, Torricelli *et al.* 2010).

In the oribatid mite *S. magnus*, high genetic differences among (31.8%) and within (25.8%) populations in Europe indicate the existence of cryptic species (Chapter 2). All *COI* and *ef 1α* sequences are translatable into proteins without stop codons. The high variance therefore did not result from *COI* pseudogenes (Bensasson *et al.* 2001, Williams and Knowlton 2001, Song *et al.* 2008, Buhay 2009). Gene duplications and heteroplasmy (Pohl *et al.* 2009) of the *COI* gene also can be excluded as the full mitochondrial genome of *S. magnus* has been sequenced (Domes *et al.* 2008, Domes-Wehner 2009) and no gene duplications and no heteroplasmy have been found. In contrast to Hurst and Jiggins (2005) the high genetic variance did not result from inherited symbionts such as *Wolbachia* or *Cardinium*, since none of these symbionts has been found in *S. magnus*. However, *Wolbachia* and *Cardinium* can trigger parthenogenesis and were found in an unidentified oribatid mite (Cordaux *et al.* 2001) and in some populations of *Oppiella nova* (Weeks *et al.* 2003).

Comparing phylogenetic relationships of the mitochondrial *COI* and the nuclear single copy gene *ef 1α* provides evidence for gene flow, i.e. sexual recombination, between individuals of different mitochondrial lineages. Individuals collected from Poland (PL_1) carry two mitochondrial and two nuclear lineages that cluster in two separate clades (red and blue) in all phylogenetic analyses. In contrast, one individual in these populations (PL_1.2) carries the red mitochondrial lineage but a nuclear haplotype of the blue lineage, a pattern that likely resulted from sexual reproduction between a female from the red mitochondrial lineage and a male from the blue nuclear lineage. Further evidence for recombination is provided by the protein dataset of the two genes and therefore is unlikely to be an artifact, e.g. due the fourfold degenerated mitochondrial code that tolerates high variance in the third codon position (Xia *et al.* 1996, Baker 2000). The phylogenetic signal from the protein supports the evidence for recombination, as the protein code is more conserved and reflects neutral and non-neutral substitutions. The linkage between the nucleus and the mitochondrion did not break and the communication between them is still functional; 40% of the amino acids of the *COI* barcoding region interact with the nuclear protein of the cytochrome oxidase complex IV (Ballard and Melvin 2010) and non-synonymous substitutions are likely to affect this interaction. Non-synonymous substitutions could be present in a small number since 60% of the amino acids of the *COI* barcoding region do not interact with another protein (Ballard and Melvin 2010).

Replication cycles and the genetic code of mitochondria are beyond the control of the nucleus. However, cooperation between nuclear and mitochondrial encoded genes is necessary for a fully functional respiratory chain. Nuclear and mitochondrial genes therefore are closely linked, as mutations in one of the two may disturb their interaction. The high genetic distances among lineages but the presence of gene flow among individuals of different mitochondrial lineages indicates that substitutions accumulate easily in the mitochondrial gene if they are neutral. Population sizes of *S. magnus* must be very large to maintain this high genetic variance at small and large geographic scales. *S. magnus* has poor dispersal abilities, phoresy and dispersal by wind or birds are unknown (Seyd 1962). However, long generation cycles of up to three years, relatively long life spans and pronounced cold tolerance are characteristic for this species (Webb 1977, 1989, Webb and Block 1993). The high genetic diversity at small scale supports the idea that European populations of *S. magnus* are very old and comprise several relict populations that survived the last ice age and other climatic changes in Central Europe (Chapter 2). The substitution rate for the *COI* gene of *S. magnus* is known (2.15% per million year; Salomone *et al.* 2002); taking this substitution rate the mitochondrial lineages from

Poland with an intraspecific variance of 14.3% (Table 8) separated >30 mya: Remarkably, they co-exist on very small scale allowing sexual reproduction and gene flow among these lineages. This, however, is only possible if the majority of variance is neutral ensuring that the interaction between nuclear and mitochondrial coded units of the cytochrome complex I remain functional and offspring therefore viable. The genus *Steganacarus* includes twelve European species but discrimination of species is difficult (Weigmann 2006). Each of the species varied in body size and colour and this variation changes with age and environment (R.A. Norton, pers. comm.). However, chaetotaxy, prodorsum and notogaster structure are distinct in *S. magnus* allowing unequivocal identification of this species. This morphological distinctness is contrasted by very high genetic variation in the *COI* gene where the third codon position which is in full saturation, suggesting that *COI* is unsuitable for barcoding in this oribatid mite species.

In contrast to Schaefer *et al.* (2006) and Laumann *et al.* (2007), the intraspecific divergence of the *ef 1α* sequences in *S. magnus* was very high (up to 20%). Both of these studies used individuals from one location and did not sample over a large scale as done in the present study.

High genetic differences in *COI* nucleotide sequences were also found in species of the genus *Steganacarus* from the Canary Islands (up to 28.6%; Salomone *et al.* 2002) and other arthropods such as the pan-Antarctic springtail *Friesea grisea* (17.7%; Torricelli *et al.* 2010), the harvestman *Aoraki denticulata* in New Zealand (up to 19.2%; Boyer *et al.* 2007) and the intertidal copepod *Tigriopus californicus* in North America (up to 23%; Edmands 2001). Potentially, soil and marine arthropods are more flexible in handling substitutions than aboveground organisms (0.2-2.5% in the pine shoot beetle *Tomicus piniperda*, Ritzerow *et al.* 2004, 0-2.6% in the snake *Vipera berus*, Ursenbacher *et al.* 2006, 1.5% in the snail *Trochulus villosus*, Dépraz *et al*. 2008).
The high local genetic variance in *S. magnus* may be due to sexual reproduction and recombination of different ecotypes (Meyers and Bull 2002). However, if sexual reproduction of different lineages combines different ecotypes and therefore provides selective advantage over other lineages remain to be tested.

3.5 Conclusions

High genetic distances characterize populations of the oribatid mite species *S. magnus* in Europe. This species probably survived the last ice ages in cryptic refugia in Central and Northern Europe. However, *S. magnus* does not represent a cryptic species complex as gene-flow between lineages that separated tens of millions of years ago still exists. Phylogenetic analyses of the mitochondrial *COI* and the nuclear *ef 1α* gene, both sequenced from the same individual, suggest recombination of the nuclear genome and the maternal inherited mitochondrial genome.
This is remarkable, as 40% of the amino acids of the *COI* barcoding region interact with the nuclear coded cytochrome oxidase complex IV (Ballard and Melvin 2010) and non-synonymous substitutions are likely to change this interaction. Sexual recombination may buffer variations in the mitochondria coded subunits of the cytochrome I protein complex by providing recombinant individuals with matching nuclear coded subunits ensuring functionality of the protein complex. To support this hypothesis, analyses of the mitochondrial genome of sexual and parthenogenetic species are needed. If true, sexual species should have more non-synonymous substitutions in the mitochondria encoded cytochrome oxidase subunits than parthenogenetic species.

Chapter Four

Differential colonization of Europe by sexual and asexual oribatid mite species: post- and pre- ice age events

Summary

Since the beginning of the Quaternary (~3 million years ago) ice ages shaped the biodiversity of Europe. The living space for aboveground species shrunk to areas south of the Alps, the Pyrenees and the Balkans. Whether this also applies to soil living animals, however, is unknown. Soil living animals include a high number of parthenogenetic species and parthenogenetic organisms have the advantage of faster colonization of new habitats than sexual species. I analyzed two parthenogenetic (*Nothrus silvestris* and *Platynothrus peltifer*) and two sexual (*Achipteria coleoptrata* and *Steganacarus magnus*) oribatid mite species and their colonization of Europe using the cytochrome c oxidase (*COI*) as molecular marker. I expected that species in Central Europe that were not affected by glaciation constitute isolated haplotypes that are distinct from populations of Southern Europe and Central Asia and that genetic diversity of these species is highest in refuge areas. The four oribatid mite species showed different colonization patterns. *N. silvestris* was highly affected by the last ice age. Only one clonal lineage exists and all European locations studied grouped in one haplotype. However, the origin of this clonal lineage remains unclear. Two possible options of re-colonisation of Central Europe exist, a Romanian or a Southern French refuge area. The genotype distribution of the other three species suggests that they were not affected by Quaternary glaciation. The second parthenogenetic oribatid mite species *P. peltifer* constitute several separated Scandinavian clusters with a maximum pairwise nucleotide difference of 20.4% but no differences in the protein. The two sexual oribatid mite species, *A. coleoptrata* and *S. magnus*, also showed very high nucleotide variation (up to 19.4% and 31.3% in *A. coleoptrata* and *S. magnus*, respectively) and also high protein variation (up to 2.6% and 4.2% in *A. coleoptrata* and *S. magnus*, respectively). Haplotype networks were dominated by single individual haplotypes and several clusters separated by a large number of substitutions. Phylogenetic trees of *P. peltifer, A. coleoptrata* and *S. magnus* were characterized by deep splits. The results suggest that these species radiated in the Miocene or earlier and survived in multiple refugia in Northern and Central Europe; potential Southern refugia (Iberian Peninsula, Italy and the Balkan) were not closely linked to the Northern or Central clusters. In *A. coleoptrata* haplotype clusters of Northern locations were separated by large numbers of substitution steps. The haplotype of *S. magnus* suggest that pre- and post-glacial splits exist. Generally, however, pre Quaternary splits dominated the haplotype networks of *A. coleoptrata*, *P. peltifer* and *S. magnus*. Cryptic refugia were detected in the Alps for *P. peltifer* and *A. coleoptrata*, and in Scandinavia, Northern, Central and South Germany, Poland, Czech Republic and Great Britain in *A. coleoptrata*, *P. peltifer* and *S. magnus*.

4.1 Introduction

For the past 2.6 million years (my), the climate in Europe oscillated between warm and cold periods (Hewitt 2000, Hewitt 2004, Mosbrugger *et al.* 2005) resulting in expansion - contraction scenarios for most living organisms (Hewitt 1999, Provan and Bennett 2008). During glacial periods, warm adapted animals and plants either went extinct or retreated to more favorable sites, whereas during interglacials they expanded from refugia. These oscillations shaped the genetic structure of the European fauna and flora which has been characterized as "Southern richness and Northern purity" (Hewitt 1999, Hewitt and Ibrahim 2001, Schmitt 2007). Genetic diversity is high in populations that live in former glacial refugia, whereas populations are genetically homogenous in areas which were re-colonised from refugial populations during interglacial periods. The Iberian Peninsula, Italy, Greece and the Balkans are well established refugia for many European species (Hewitt 1999, Steinfratz *et al*. 2000, Ursenbacher *et al.* 2006). Several phylogeographic studies on freshwater and terrestrial organisms confirmed the importance of Mediterranean refugia (Beheregaray 2008). Central Asia also contributed to the re-colonization of Central and Northern Europe in some species such as the brown bear (*Ursus arctos*; Hewitt 1999). The three most common patterns of re-colonization were delineated form the pattern in the common grasshopper (*Chorthippus parallelus*), the brown bear (*U. arctos*) and the hedgehog (*Erinaceus* spp.) (Hewitt and Ibrahim 2001).

Radiocarbon (Stewart and Lister 2001), pollen (Willis *et al.* 2000, Tollefsrud *et al.* 2008) and molecular (Pfenninger *et al.* 2003, Verovnik *et al.* 2005, Tollefsrud *et al.* 2008) data suggest that isolated areas in Central and Northern Europe also functioned as refugial areas. These cryptic refugia (Stewart and Lister 2001, Provan and Bennett 2008) are difficult to detect because presumably they were small, geographically isolated or located in wind protected valleys or ice free Nunataks (Stewart and Cooper 2008, Schmitt 2009).

Survival of populations in Central refugia implies that species expanding from Southern refugia met populations with different demographic and ecological history. Identifying Central and evaluating their contribution to the gene pool of present day populations is important for understanding the genetic structure of populations and their adaptation to local conditions, both important factors for speciation processes. Until now, phylogeographic studies on the postglacial colonization of Central and Northern Europe were restricted exclusively to aboveground species (Hewitt 1999, Hewitt and Ibrahim 2001, Ursenbacher *et al.* 2006), there is no information on belowground species and therefore it remains unknown if the identified patterns also apply to soil animals which typically are much smaller than those aboveground (Whitfield 2005).

Oribatid mites are soil-dwelling predominantly detritivorous and fungivourous microarthropods (Maraun and Scheu 2000, Schneider *et al.* 2004) and fossils of oribatid mites date back to Devonian sediments 380 million years ago (mya) (Shear *et al.*1984, Norton *et al.* 1988a), but the origin of this group is dated back to 440 mya (Lindquist 1984) and to even 570 mya using molecular clock analysis (Schaefer *et al.* 2010). They reach densities of up to 400,000 individuals per square meters in temperate and boreal forest soils (Maraun and Scheu 2000) and 80% of the individuals reproduce by parthenogenesis (Domes-Wehner 2009).

To investigate if sexual and asexual oribatid mite species colonized Europe differently after the last glaciations, a mt gene (*COI*) was sequenced from two sexual and two parthenogenetic oribatid mite species of Europe, since parthenogenetic species have a number of advantages compared to sexual species, e.g. the twofold advantage of not producing males (Williams 1975, Maynard Smith, 1978, 1998, Tagg *et al.* 2005), faster colonization of habitats and easier establishment of new populations (Williams 1975; Bell 1982; Scheu and Schulz 1996; Lindberg and Bengtsson 2005, Schön 2007) and they coexist in the same habitat with sexual species (Domes-Wehner 2009). In total 558 individuals (100 *N. silvestris*, 160 *P. peltifer*, 141 *A. coleoptrata* and 157 *S. magnus*) from 65 locations in Europe and two east Russian locations were analyzed. I assumed that the mt variance is similar in

parthenogenetic and sexual species since mitochondria are inherited maternally. Sampling in Central Europe was extensive in order to detect cryptic refugia which were proposed for Central and Northern Europe (Stewart and Lister 2001; Tollefsrud *et al.* 2008). I expected lineages which survived in Central refugia to contain isolated haplotypes that are distinct from populations from Southern Europe and Central Asia and that genetic diversity within this species' range is highest in these former refugial areas (Stewart *et al.* 2010).

4.2 Materials and methods

4.2.1 Taxon sampling

Oribatid mites were sampled from 65 locations in Europe and two locations in Russia (Fig. 22, described in more detail in Table 14). *A. coleoptrata* was sampled from 40 locations, *N. silvestris* from 26 locations, *P. peltifer* from 41 locations and *S. magnus* from 37 locations. Specimens were extracted from leaf litter using life extraction along a heat gradient (Macfadyen 1961, Kempson *et al.* 1963). *Carabodes femoralis*, *Carabodes marginatus*, *Hypochthonius rufulus*, *Nothrus palustris* and *Rhysotritia duplicata* were sampled as outgroups for phylogenetic analyses. Animals were identified under a stereomicroscope, determined after Weigmann (2006) and stored in 75% ethanol at -20°C until preparation.

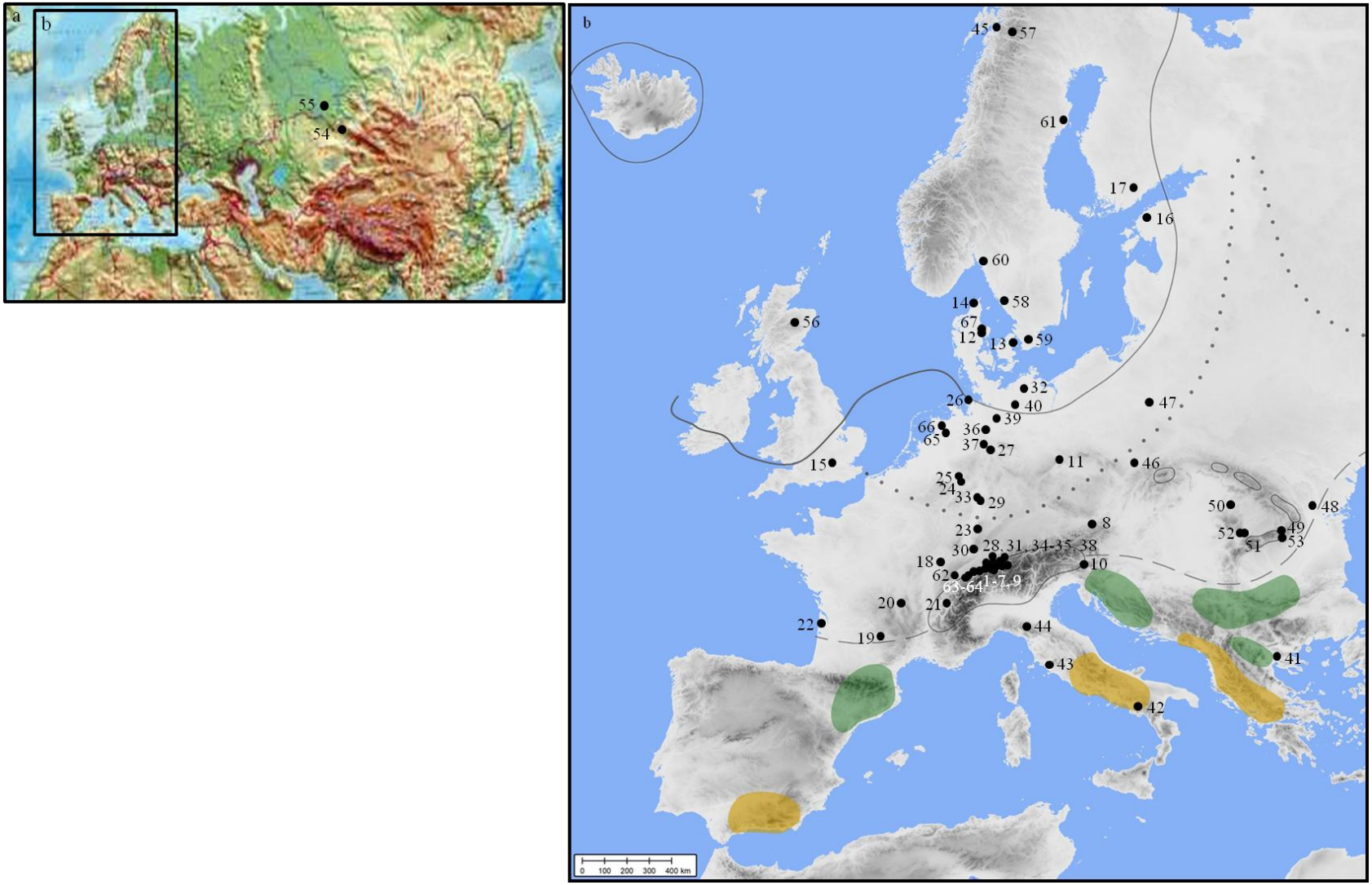

Figure 22: (a) Map of Europe and Russia and (b) map of Europe from the last glacial maximum (modified after Hewitt 1999). Black dots with numbers represent the sampling points of oribatid mites used in this study, green shading marks refugia of coniferous trees and yellow shading those of deciduous trees, grey lines mark the expansion of glaciers, the dotted line represents the polar desert climate border and the dashed line the tundra and permafrost border (Hewitt and Ibrahim 2001, Alexander Kartographie 2006).

Table 14: Localities where oribatid mites analyzed in this study were sampled [ln: location number, country, location, coordinates, abbreviations of sampling locations (code) and name of collector (for details see Acknowledgements)]. Each location is marked by a colour symbol (AC=*Achipteria coleoptrata*, NS=*Nothrus silvestris*, PP=*Platynothrus peltifer*, SM=*Steganacarus magnus*, outgroups: CF=*Carabodes femoralis*, CM=*Carabodes marginatus*, HR=*Hypochthonius rufulus*, NP=*Nothrus palustris* and RD=*Rhysotritia duplicata*).

ln	country	location	coordinates	code	species	colour	litter collector
1	Austria	Bregenz	47.49° 9.70°	A 2	PP		I. Schaefer
2		Glacier	47.17° 10.53°	A 9	PP		I. Schaefer
3		Hittisau	47.46° 9.95°	A 3	AC, PP		I. Schaefer
4		Holzgau	47.29° 10.33°	A 4	PP		I. Schaefer
5		Imst	47.24° 10.69°	A 8	AC		B. Eitzinger
6		Landeck	47.13° 10.57°	A 6	AC		I. Schaefer
7		Memminger Huette	47.21° 10.46°	A 5	AC, PP		I. Schaefer
8		Roggendorf	48.20° 15.37°	A 7	AC		E. Latz
9		Stuiben	47.52° 10.18°	A 10	PP		I. Schaefer
10		Villach	46.57° 13.85°	A 1	PP, SM		K. Domes-Wehner
11	Czech Republic	Decin	50.78° 14.23°	CZ 1	SM		M. Rosenberger
12	Denmark	Arhus	56.11° 10.21°	DK 3	AC, PP, SM		T. Bilde
13		Copenhagen	55.68° 12.58°	DK 1	SM		N. Eisenhauer
14		Hjørring	57.48° 9.96°	DK 2	AC, PP, SM		M. Rosenberger
15	England	Ascot	51.40° -0.68°	GB 1	AC, NS, PP, SM		A. Milcu
16	Estonia	Tallin	59.43° 24.69°	EST 1	AC		A. Micic
17	Finland	Lahti	60.99° 25.65°	FIN 1	AC, NS, PP, SM		H. Setela
18	France	Brunstatt	47.71° 7.32°	F 5	PP		B. Lerb
19		Haute Loire	44.99° 3.86°	F 4	AC, SM		A. Jousset
20		Loire	45.56° 4.79°	F 2	AC, SM		M .Maraun
21		Mont Blanc	45.82° 6.74°	F 1	AC, PP, SM		A. Jousset
22		Saint Isidore	45.27° -1.09°	F 3	NS, PP, SM		C. Digl
23	Germany	Black Forest	48.89° 8.43°	D 6	NS, PP, SM		K. Heidemann
24		Bonn	50.84° 7.14°	D 9	AC, NS, PP, SM		R. Koller
25		Colonge	50.83° 7.18°	D 10	AC, NS		M .Maraun
26		Cuxhaven	53.86° 8.65°	D 8	AC, NS, PP, SM		K. Heidemann
27		Goettingen	51.53° 9.96°	D 2	AC, NS, PP, SM		K. Schneider
28		Gunzesried	47.51° 10.23°	D 13	AC		I. Schaefer
29		Kranichstein	49.89° 8.69°	D 1	AC, NS, PP, SM		M. Rosenberger
30		Lake Constance	47.71° 9.37°	D 3	AC, NS, PP, SM		K. Heidemann
31		Langenwang	47.43° 10.28°	D 15	AC, PP		I. Schaefer
32		Mecklen. Seenplatte	53.57° 12.33°	D 4	NS, PP, SM		I. Schaefer
33		Moerfelden	49.96° 8.55°	D 5	AC, PP, SM		K. Domes-Wehner
34		Rubi	47.43° 10.28°	D 17	NS, PP		I. Schaefer
35		Steineberg	47.52° 10.19°	D 14	AC		I. Schaefer
36		Steinhuder Meer	52.48° 9.38°	D 18	NS, PP		E. Latz
37		Solling	51.76° 9.57°	D 16	NS		K. Schneider
38		Sonthofen	47.46° 10.22°	D 12	AC, PP		I. Schaefer
39		Uelzen	52.97° 10.52°	D 7	NS, PP, SM		H. Treptow
40		Wittmoor	53.19° 11.83°	D 11	AC, NS		K. Schneider
41	Greece	Thessaloniki	40.64° 22.97°	GR 1	AC		M. Tsiafaoli
42	Italy	Felitto	40.37° 15.24°	I 3	AC		G. Humpert
43		Grosseto	42.63° 11.11°	I 1	AC, PP, SM		M .Maraun
44		Parma	44.20° 10.34°	I 2	AC, SM		M .Maraun
45	Norway	Narvik	68.44° 17.40°	N 1	PP, SM		O. Butenschoen
46	Poland	Krakov	50.04° 19.84°	PL 1	AC, SM		S. Scheu
47		Warsaw	52.33° 20.76°	PL2	AC, NS, PP, SM		A. Uvarov
48	Romania	Bagau	46.55° 26.72°	RUM 3	SM		T. Pasca
49		Busteni	45.41° 25.55°	RUM 5	AC, NS, PP, SM		C. Ivanescu
50		Cluj	46.77° 23.52°	RUM 4	SM		T. Pasca
51		Sibiu 1	45.64° 23.74°	RUM 1	AC, NS, SM		S. Scheu
52		Sibiu 2	45.65° 23.70°	RUM 2	AC, NS, PP, SM		S. Scheu
53		Sinaia	45.35° 25.56°	RUM 6	AC, PP, SM		C. Ivanescu
54	Russia	Altai Mountains	51.73° 85.76°	RUS 1	SM		M. Ackermann
55		Novosibirsk	54.97° 83.39°	RUS 2	AC, SM		A. Uvarov
56	Scotland	Braemar	57.00° -3.40°	GB 2	AC, NS, PP, SM		A. Jousset
57	Sweden	Abisko	68.35° 18.82°	S 4	PP		O. Butenschoen
58		Gothenburg	57.67° 11.97°	S 5	PP		P. Pachl
59		Malmoe	56.00° 13.14°	S 3	NS		N. Lindberg
60		Stroemstad	58.87° 11.14°	S 2	AC, PP, SM		G. Kalinkat
61		Umeå	63.83° 20.29°	S 1	PP, SM		O. Butenschoen
62	Switzerland	Basel	47.50° 7.59°	CH 1	AC		M. Maraun
63		Buesingen	47.69° 8.69°	CH 2	NS		P. Pachl
64		Rorschach	47.47° 9.53°	CH 3	AC, NS, PP		I. Schaefer
65	The Netherlands	Hoge Veluwe	52.02° 5.87°	NL 2	NS, PP		M. Maraun
66		Wageningen	51.97° 5.70°	NL 1	NS, PP, SM		O. Butenschoen
67	Denmark	Arhus Stadion	56.13° 10.19°	DK 4	NP		T. Bilde
27	Germany	Goettingen	51.53° 9.96°	D 2	NP		M. Rosenberger
29		Kranichstein	49.89° 8.69°	D 1	CF, CM, HR, RD		M. Rosenberger
47	Poland	Warsaw	52.33° 20.76°	PL2	NP		A. Uvarov

4.2.2 DNA extraction and sequencing

Genomic DNA was extracted from single individuals using the DNeasy® Blood and Tissue Kit (Qiagen; Hilden, Germany) following the manufacturer's protocol for animal tissue. For *A. coleoptrata* a 512 bp, for *N. silvestris* a 581 bp and for *S. magnus* a 531 bp region of cytochrome c oxidase subunit I (*COI*) was amplified using the primers *COI*arch1 (5´-GGTCAACAAATCATAAAGAYATYGG-3´) and *COI*arch2 (5´-TAAACTTCAGGGTGACCAAAAAATCA-3´) (Heethoff *et al.* 2007) and the HotStarTaq® Master Mix Kit (1.25 units HotStarTaq® polymerase, 100 µM of each dNTP and 7.5 mM $MgCl_2$ buffer solution; Qiagen; Hilden, Germany) and the SuperHot Taq Mastermix [2.5 units SuperHot Taq polymerase, 10 µM of each dNTP and buffer solution (20 mM Tris-HCI (pH 8.3), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Nonidet P40 and 0.5% Tween 20), Genaxxon; Ulm, Germany], respectively. For *P. peltifer* a 558 bp region of cytochrome c oxidase subunit I (*COI*) was amplified using the primers *COI*arch1 (5´-GGTCAACAAATCATAAAGAYATYGG-3´) and Plat 52 (5´-ATAAATCCTAAGGATCATAGC-3´) (Domes-Wehner 2009). The polymerase chain reaction (PCR) contained 0.5 µl of each primer (100 pmol/µl), 1 µl $MgCl_2$ (25mM), 12.5 µl of HotStarTaq® Master Mix or SuperHot Taq Mastermix containing polymerase, 3 µl template DNA and filled up to 25 µl with RNase free water. PCR parameters included a 15 min step at 95°C for polymerase activation followed by 36 cycles of 30 s at 94°C for denaturation, 60 s at 51°C for primer annealing and 60 s at 72°C for elongation and a final 10 min step for elongation at 72°C. The PCR products were visualized by 1% agarose gel electrophoresis and purified using the QIAquick® PCR Purification Kit (Qiagen; Hilden, Germany) following the manufacturer's protocol. The purified PCR products were sequenced in both directions by Macrogen Inc. (Seoul, Korea) and the Department of Experimental Phycology and Culture Collection of Algae (Georg-August-University Göttingen, Germany).

4.2.3 Phylogeographic, population genetic and statistical analyses

Nucleotide sequences were edited and translated into amino acids using the invertebrate mitochondrial code implemented in SEQUENCHER v4.9 (Gene Codes) and aligned with ClustalX v1.81 (Thompson *et al.* 1997) using multiple alignment parameters: 10.0 for gap opening and 0.1 for gap extension for the nucleotide, default settings for the amino acids dataset. Phylogenetic trees were generated with Beast v1.5.4 (Drummond and Rambaut 2007), MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) and PAUP* (Swofford 1999) with the NJ analyses without and with model of sequence evolution to identify monophyletic clusters in the dataset. The best model of sequence evolution was estimated with Modeltest 3.6 (Posada and Crandall 1998) for PAUP* and MrModeltest (Nylander 2004) for the Bayesian analyses. The best fit model was GTR+I+G for both analyses. Model parameters were nst=6 rates=invgamma for nucleotide sequences of *S. magnus*. For *N. silvestris* the best fit model was HKY+G in both analyses and model parameters were nst=2 rates=gamma for the nucleotide. The best fit model for PAUP* was HKY+G and for MrBayes GTR+G for the nucleotide dataset of *P. peltifer*. Model parameters were nst = 6 rates = gamma for nucleotide sequences of *P. peltifer*. The best fit model for PAUP* was TVM+I+G for MrBayes GTR+I+ G for the nucleotide dataset of *A. coleoptrata*. Model parameters were nst=6 rates=invgamma for nucleotide sequences of *A. coleoptrata*. All protein alignments were analyzed with aamodel=equalin in MrBayes and mtrev in Beast. One individual of *H. rufulus* (C.L. Koch, 1835) (Oribatida, Enarthronota) and *R. duplicata* (Grandjean, 1953) (Oribatida, Mixonomata) were used as outgroups for *P. peltifer* and *S. magnus*. For *A. coleoptrata* outgroups were one individual of *C. femoralis* (Nicolet, 1855) (Oribatida, Brachypylina) and *C. marginatus* (Michael, 1884) (Oribatida, Brachypylina); for *N. silvestris* four individuals of *N. palustris* (C.L. Koch, 1839) (Oribatida, Desmonomata) were used as outgroup. The Markov Chain Monte Carlo was run for ten million generations and sampled every 1000th generation,

the 50% majority consensus tree excluded the first 2,500,000 trees (burnin of 25%) in MrBayes and 10% burnin in Beast.

Standard diversity indices for the nucleotide sequences [haplotype number (N_h), haplotype diversity (H_d,) nucleotide diversity (Π_n), number of variable (N_{vs}) and invariable sites (N_{is}), parsimony informative sites (N_{pars}), the number of singletons (N_s) and the total number of substitutions (N_m)] and the McDonald-Kreitman (MK) test, to detect selection, were calculated with DNASP v5.0 (Rozas *et al.* 2003). In the McDonald-Kreitman test selection is detected by examining the distribution of synonymous and non-synonymous substitutions among populations; the test is robust against demographic and recombination events (McDonald and Kreitman 1991). The geographical structure of genetic diversity among and within populations and geographical clades were calculated with ARLEQUIN v3.01 (Excoffier *et al.* 2005) using analysis of molecular variance (AMOVA, 16.000 permutations); populations with less than two sampled individuals were excluded from the AMOVA. Estimates for demographic expansion (Tajima's D and Fu's F_s neutrality tests) and pairwise differences (F_{ST} 10.000 permutations) were also calculated in ARLEQUIN. I calculated significance with 10.000 permutations.

The dataset exceeded the connection limit of TCS (Clement *et al.* 2000); therefore a parsimony based median-joining haplotype network (Bandelt *et al.* 1999) was generated in NETWORK v4.5 (Fluxus-Technology; Suffolk, UK) with default settings for nucleotide sequences and amino acids.

To investigate the effect of the number of sample locations and the richness of haplotypes Jackknife rarefaction curves were calculated using the program EstimateSWin820 with default settings for the four nucleotide and protein alignments (Colwell, 1994-2004. EstimateS: statistical estimation of species richness and shared species from samples (http://viceroy.eeb.uconn.edu/estimates).

Geographical distances of the populations were generated by GenAlEx6 (Peakal and Smouse 2006). I analyzed the associations of genetic differentiation at mitochondrial markers with geographical distances using the Mantel test implemented in the program Isolation by Distances version 1.52 (IBD 1.52, Bohonak 2002) or with Isolation by Distances Web Service version 3.16 (http://ibdws.sdsu.edu/~ibdws/) (Jensen *et al.* 2005) with default settings for the four oribatid mites. Graphical constructions were generated with Statistica 7 (Stat Soft Inc, 2002).

Delimitation of mtDNA clusters on the *COI* trees with the generalized mixed Yule coalescent method (GMYC) (Pons *et al.* 2006, Fontaneto *et al.* 2007, Papadopoulou *et al.* 2009) were done with the R package 'splits' (SPecies LImits by Threshold Statistics, http://r-forge.r-project.org/projects/splits/) in R 2.11.1 (R Development Core Team 2010, http://www.R-project.org) for the four oribatid mite species. Ultrametric trees were generated with the strict clock method in Beast.

4.3 Results

4.3.1 Nothrus silvestris

A total of 104 individuals (100 individuals of *N. silvestris* and four individuals of *N. palustris* as outgroup) from 26 locations in ten countries were sequenced. The sequences contained 29.1% A, 25% C, 17.6% G and 28.4% T. The 100 *COI* nucleotide sequences consisted of 25 haplotypes for the nucleotide (25%) (Fig. 23) with 45 variable sites (7.75%); 21 of these were parsimony informative (Table A19). The protein consisted of eight haplotypes (8%) in the network (Fig. 24). The haplotype diversity (H_d) was high with 0.67 in the nucleotide.

Haplotype networks

The nucleotide haplotype network formed four subclades and two isolated one individual haplotypes which were separated each by seven or more substitution steps (Fig. 23). The isolated haplotypes comprised only one individual from Germany and one from France (D_4.5, F_3.4). Subclade 1 comprised nine haplotypes with a starlike arrangement. Six haplotypes comprised one individual from different locations (one English GB_1.3, one Romanian RUM_1.3, one Swedish S_3.2, one Swiss CH_3.2 and two German D_6.2 and D_16.1). Two individuals from different locations in Germany (D_4.2/D_17.2) and four individuals from one location in Romania also were of the same haplotype (RUM_5.1-4). The last haplotype comprised 57 individuals from 19 locations of South-east, Central and Northern Europe [twelve individuals are one Polish location (PL_2.1-5, 2.7-13); four individuals each from one Finish (FIN_1.1-4), one Romanian (RUM_2.1-4) and three German locations (D_1.1-4, D_9.1-4 and D_18.1-4); three individuals each from one Dutch (NL_1.1-3) and two German locations (D_8.1-3 and D_16.2-4); two individuals each from one English (GB_1.2, 1.4), one German (D_4.4, 4.7), one Scottish (GB_2.1, 2.4), one Romanian (RUM_1.1-2), one Swedish (S_3.1, 3.4) and one Swiss (CH_2.1-2) location; the other four individuals were from one Dutch (NL_2.2), one Swiss (CH_3.1) and two German locations(D_3.1 and D_17.3)]. Subclade 2 comprised four haplotypes with a starlike arrangement (one big haplotype with three surrounding haplotypes). All three satellite haplotypes were from the same German location (D_4). Two of the three satellite haplotypes comprised one individual (D_4.6 and D_4.8). The third satellite haplotype comprised two individuals (D_4.1 and D_4.3). The fourth haplotype comprised ten individuals from seven locations (CH_2, D_6, D_7, D_10, GB_1, NL_1 and S_3) [four individuals were from the same German location (D_7.1-4); the other six were from six different locations from Central and Northern Europe (one Dutch NL_2.1, one English GB_1.1, one Swedish S_3.3 one Swiss CH_2.3 and two German D_6.1 and D_10.1)]. Subclade 3 comprised seven haplotypes from four locations, two German (D_2 and D_11), one French (F_3) and one Polish (PL_2). One haplotype comprised four individuals from two German locations (D_2.2/D_11.1/D_11.3-4). Another haplotype comprised two individuals from the same location (F_3.1-2). The other five haplotypes comprised one individual [two comprised individuals from one German location (D_2.1 and D_2.4); one comprised one individual from another German location (D_11.2); one comprised one Polish individual (PL_2.6) and one comprised one French individual (F_3.3)]. Subclade 4 comprised three haplotypes from two locations in Germany (D_2 and D_17) and one location in Scotland (GB_2) [one comprised two individuals of the same haplotype, one from a German (D_2.3) and one from a Scottish location (GB_2.3); two comprised one individual each from a German and Scottish location (D_17.1, GB_2.2)].

The protein haplotype network formed eight haplotypes with a starlike arrangement (Fig. 24). Five haplotypes comprised one individual from four locations [one English (GB_1.3), one Scottish /GB_2.2) and two German (D_4.6, 4.8 and D_17.1)]. One comprised four individuals from three German locations (D_2.1, 2.4, D_11.2 and D_16.1). One comprised twelve individuals from eight locations [four individuals from one German location (D_7.1-4); two individuals from another German location (D_4.1 and 4.3); six individuals from six different locations (one Dutch NL_2.1, one English GB_1.1, one Swedish S_3.3, one Swiss CH_2.3 and two German D_6.1 and D_10.1)]. The Central haplotype comprised 79 individuals from 24 locations [all 13 Polish individuals (PL_2.1-13), all eleven Romanian individuals from the three locations (RUM_1.1-3, RUM_2.1-4 and RUM_5.1-4), all four French individuals (F_3.1-4), all four Finish individuals (FIN_1.1-4), 31 individuals from eleven German locations (D_1.1-4, D_2.2-3, D_3.1, D_4.2, 4.4-5, 4.7, D_6.2, D_8.1-3, D_9.1-4, D_11.1, 11.3-4, D_16.2-4. D_17.2-3 and D_19.1-4), five individuals from two locations in Great Britain (GB_1.2, 1.4 and GB_2.1 and 2.3-4), four individuals from two Dutch locations (NL_1.1-3 and NL_2.2), four individuals from two Swiss locations (CH_2.1-2 and CH_3.1-2) and three

individuals from one Swedish location (S_3.1-2, 3.4)]. The other haplotypes were connected by one or two amino acid changes to the Central haplotype.

Phylogenetic and population genetic analyses

All phylogenetic analyses (NJ with and without model of sequence evolution, MrBayes and Beast) showed a maximum supported monophyletic group of *N. silvestris* with four subclades and two isolated individuals in the nucleotide (Fig. 25, A22-25). The four subclades were supported by high bootstrap values and posterior probabilities. Only the arrangement of the four subclades was variable in the analyses. The individuals which built these clades are presented in Table 15. In the phylogenetic trees of the protein all sequences were grouped in one highly supported monophyletic clade (Fig. 26, A26-28).

The minimum and maximum mean average pairwise differences for the nucleotide sequences between populations were 0% (D_1/D_8, D_1/D_9, D_1/D_16, D_1/D_18, D_1/FIN_1, D_1/NL_1, D_1/RUM_2, D_8/D_9, D_8/D_16, D_8/D_18, D_8/FIN_1, D_8/NL_1, D_8/RUM_2, D_9/D_16, D_9/D_18, D_9/FIN_1, D_9/NL_1, D_9/RUM_2, D_16/D_18, D_16/FIN1, D_16/NL_1, D_16/RUM_2, D_18/FIN_1, D_18/NL_1, D_18/RUM_2, FIN_1/NL_1, FIN_1/RUM_2 and NL_1/RUM_2) and 1.8% (CH_3/D_2). Within populations the minimum and maximum mean average pairwise differences were 0% (D_1, D_7, D_8, D_9, D_18, FIN_1, NL_1, RUM_2 and RUM_5) and 1.7% (D_6) (Table 16). For the protein the minimum and maximum mean average pairwise differences between populations were 0% (all populations except for GB_2) and 0.13% (each population to population GB_2). The minimum and maximum mean average pairwise differences within population were 0% (all populations except for GB_2) and 0.26% (GB_2) for the protein (Table A20).

The results of the AMOVA showed that the nucleotide variation among samples within countries (53.9%) and the variation within samples (57.2%) were significant and high. In contrast, variation among countries was not significant (Table 17).

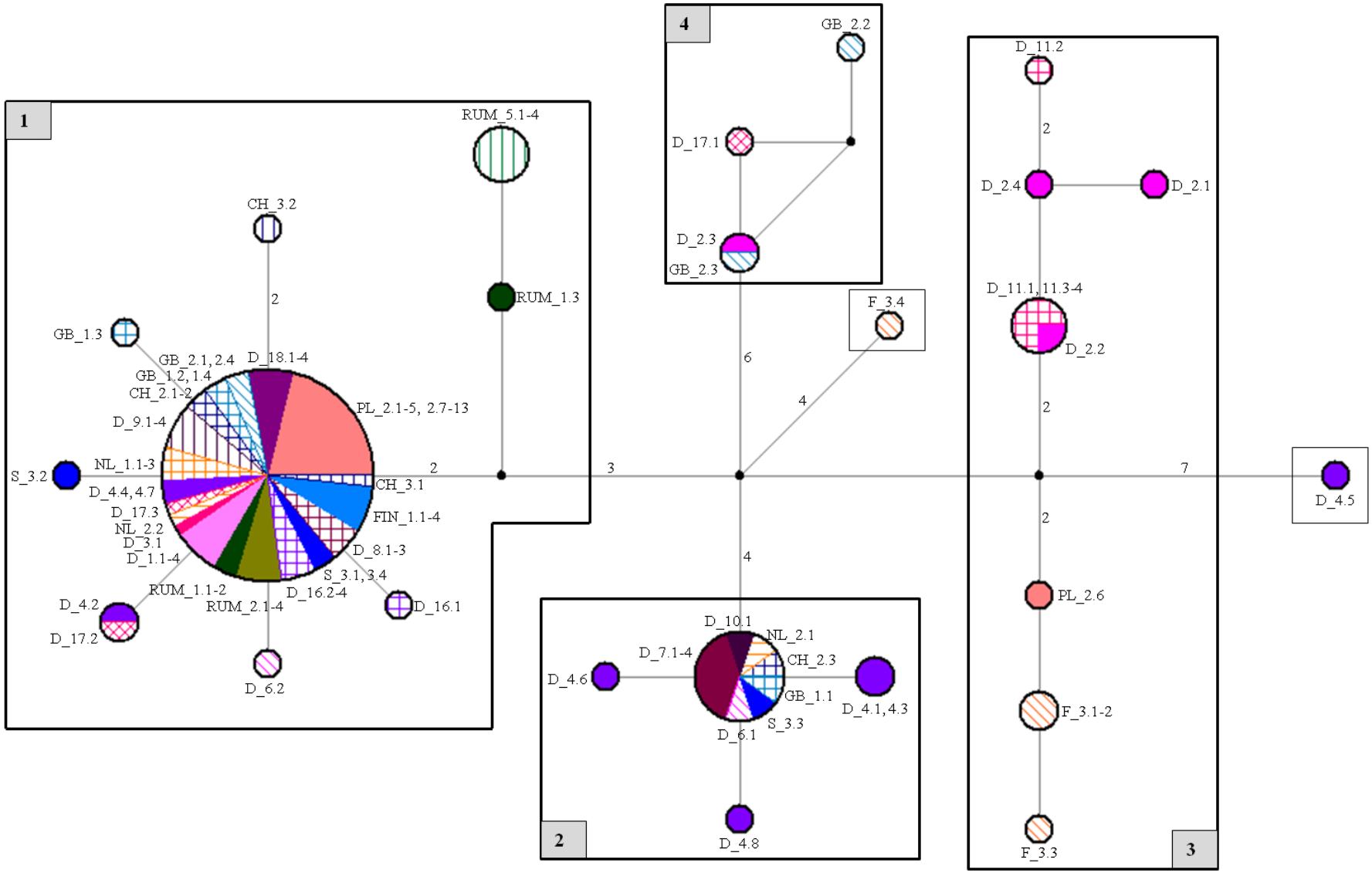

Figure 23: Median-joining haplotype network for the *COI* nucleotide of 25 haplotypes from *Nothrus silvestris*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one substitution step between haplotypes). Major subclades are marked by boxes.

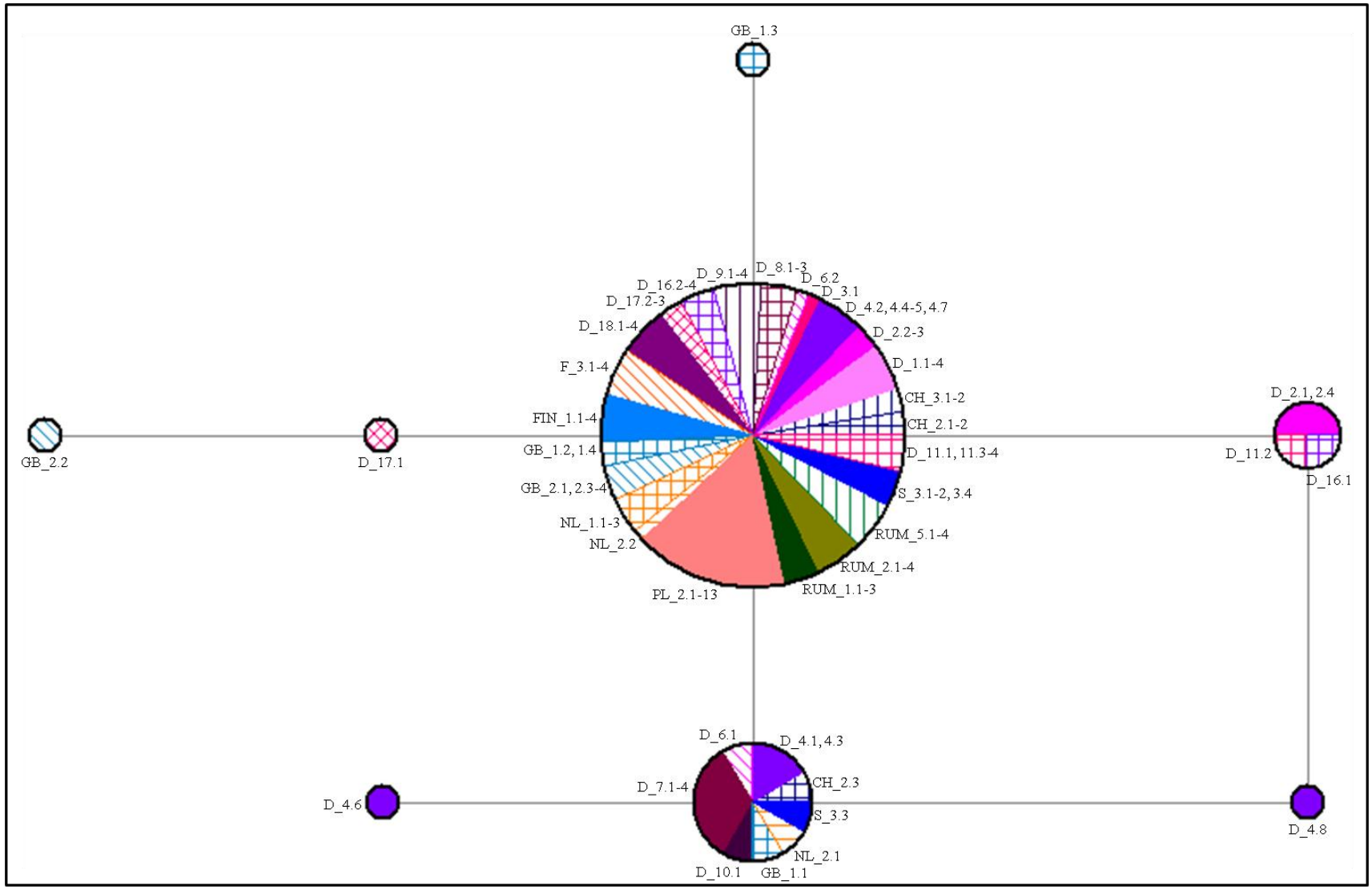

Figure 24: Median-joining haplotype network for the *COI* protein of eight haplotypes from *Nothrus silvestris*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one amino acid change between haplotypes).

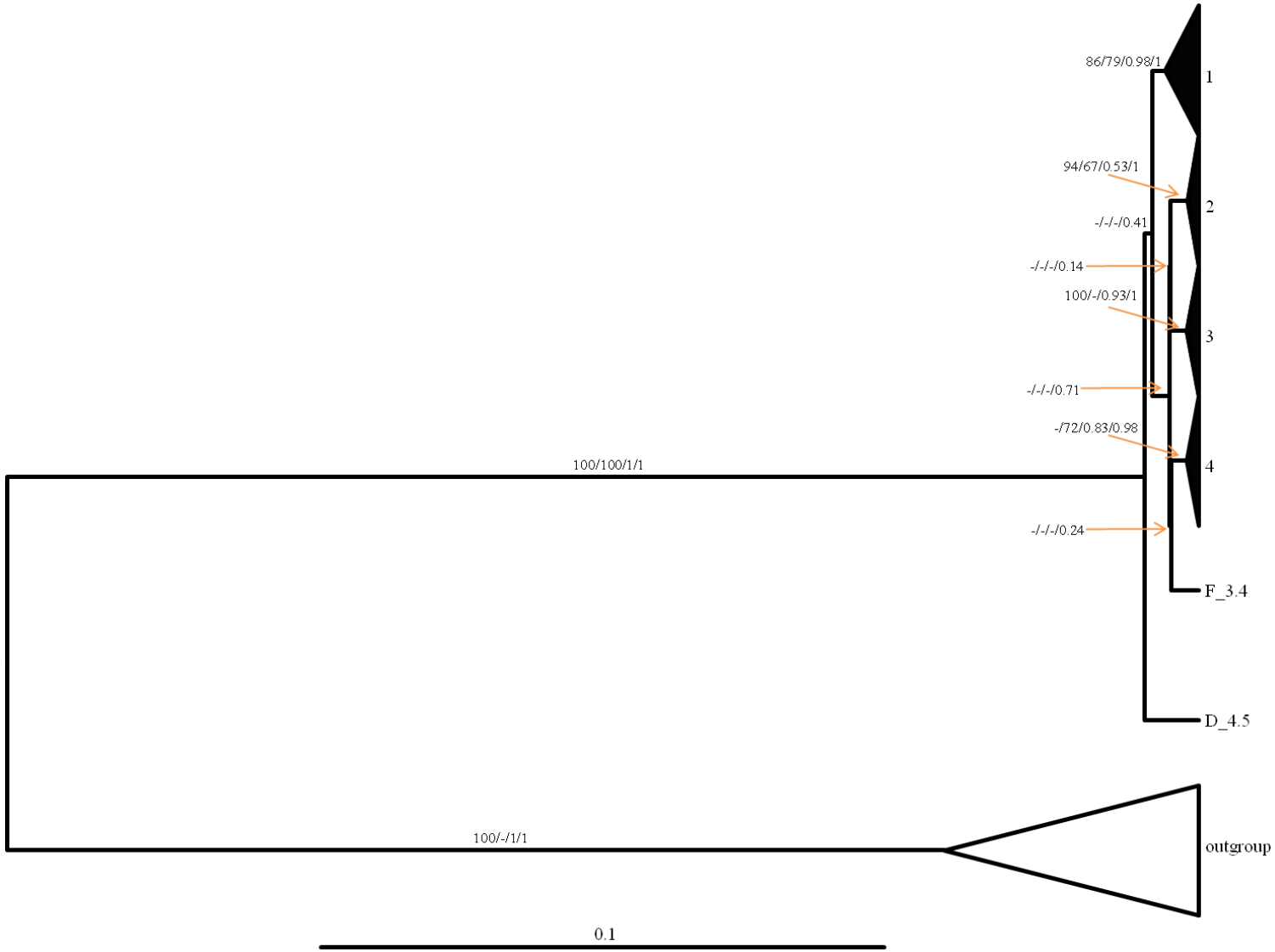

Figure 25: Bayesian phylogeny after $10x10^6$ generations from the 100 *COI* nucleotide sequences of *Nothrus silvestris* with Beast v1.5.4. Outgroups are four individuals of *N. palustris* from different locations. Numbers on the branches are bootstrap values from NJ without and with evolution model (HKY+G) analysis and posterior probabilities from MrBayes and Beast. Tip numbers are the different subclades and explained in Table 15.

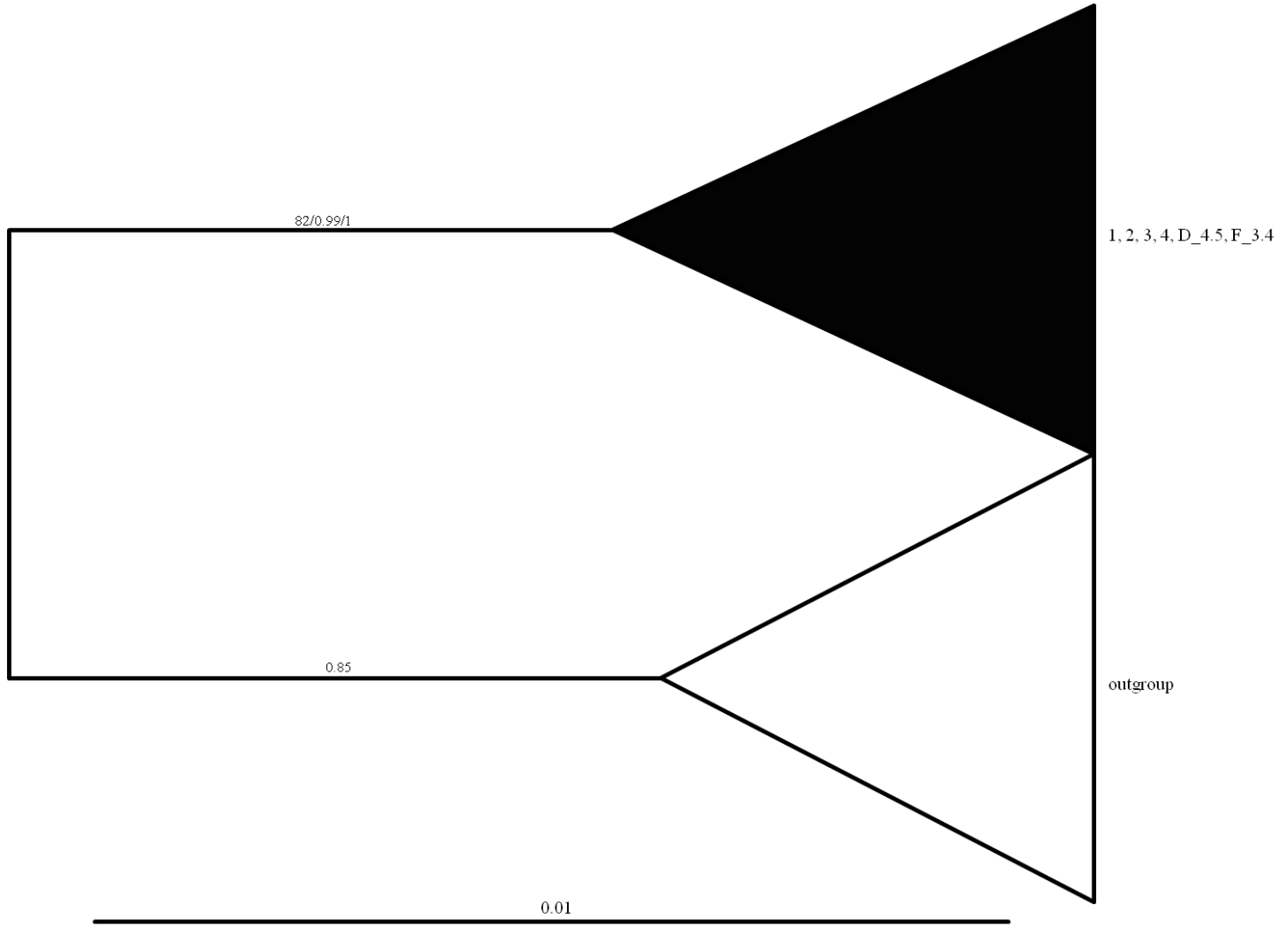

Figure 26: Bayesian phylogeny after $10x10^6$ generations from the 100 *COI* protein sequences of *Nothrus silvestris* with Beast v1.5.4. Outgroups are four individuals of *N. palustris* from different locations. Numbers on the branches are bootstrap values from NJ and posterior probabilities from MrBayes and Beast. Tip numbers are the different subclades and explained in Table 15.

Table 15: Subclades of Bayesian phylogenetic trees of *Nothrus silvestris* based on *COI* nucleotide sequences (#ind=number or individuals, pp=posterior probabilities, sampling sites with abbreviations, individuals and ind. pop.=quantity of individuals from the population).

Subclade	# ind	pp	sampling sites		individuals	ind. pop.
1	**69**	**1**	Buesingen	CH_2	1-2	2/3
			Rorschach	CH_3	1-2	2/2
			KW	D_1	1-4	4/4
			Lake Constance	D_3	1	1/1
			Meckl. Seenplatte	D_4	2, 4, 7	3/8
			Black Forrest	D_6	2	1/2
			Cuxhaven	D_8	1-3	3/3
			Bonn	D_9	1-4	4/4
			Solling	D_16	1-4	4/4
			Rubi	D_17	2-3	2/3
			Steinhuder Meer	D_18	1-4	4/4
			Lahti	FIN_1	1-4	4/4
			Ascot	GB_1	2-4	3/4
			Braemar	GB_2	1, 4	2/4
			Wageningen	NL_1	3	3/3
			Hoge Veluwe	NL_2	2	1/2
			Warsaw	PL_2	1-5, 7-13	12/13
			Sibiu_1	RUM_1	1-3	3/3
			Sibiu_2	RUM_2	1-4	4/4
			Busteni	RUM_5	1-4	4/4
			Malmoe	S_3	1-2, 4	3/4
2	**14**	**1**	Buesingen	CH_2	3	1/3
			Meckl. Seenplatte	D_4	1, 3, 6, 8	4/8
			Black Forrest	D_6	1	1/2
			Uelzen	D_7	1-4	4/4
			Cologne	D_10	1	1/1
			Ascot	GB_1	2-4	3/4
			Hoge Veluwe	NL_2	1	1/2
3	**4**	**1**	Goettingen	D_2	3	1/4
			Rubi	D_17	1	1/3
			Braemar	GB_2	2-3	2/4
4	**11**	**0.98**	Goettingen	D_2	1-2, 4	3/4
			Wittmoor	D_11	1-4	4/4
			Saint Isidore	F_3	1-3	3/4
			Warsaw	PL_2	6	1/13
isol. ind.	**1**		Saint Isidore	F_3	4	1/4
isol. ind.	**1**		Meckl. Seenplatte	D_4	5	1/8

Table 17: AMOVA table on variations among countries and within populations in nucleotide (black) and protein (red) sequences of *COI* of *Nothrus silvestris*. Each population was considered as separate groups. Populations with less than two individuals were excluded from the analysis. Significance level is $p<0.05$ (d.f.: degree of freedom).

Source of variance	d.f.	sum of squares	variance components	percent of total variation	fixation indices
among countries	8 / 8	41.18 / 0.12	-0.28 Va / 0 Va	-11.15 / 9.41	FCT: -0.11 / 0.09
among populations	15 / 15	96.49 / 0.11	1.36 Vb* / 0 Vb	53.93 / -8.21	FSC: 0.49* / -0.09
within population	73 / 73	105.37 / 0.75	1.44 Vc* / 0.01 Vc	57.22 / 98.74	FST: 0.43* / 0.01
total	96 / 96	243.04 / 0.98	2.52 / 0.01041		

Table 16: Mean percentage pairwise differences of uncorrected p-distances of the *COI* nucleotide sequences of *Nothrus silvestris* from 24 locations. The diagonal is the within population differences (bold) and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Locations with less than two individuals were excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	**CH 2**	**1**																							
2	**CH 3**	0.7	**0.3**																						
3	**D 1**	0.5	0.2	**0**																					
4	**D 2**	1.6	**1.8**	1.6	**1**																				
5	**D 4**	1.1	1.3	1.1	1.7	**1.4**																			
6	**D 6**	0.9	1	0.9	1.6	1.1	**1.7**																		
7	**D 7**	1	1.7	1.6	1.5	1	0.9	**0**																	
8	**D 8**	0.5	0.2	**0**	1.6	1.1	0.9	1.6	**0**																
9	**D 9**	0.5	0.2	**0**	1.6	1.1	0.9	1.6	**0**	**0**															
10	**D 11**	1.5	1.7	1.5	0.6	1.5	1.5	1.3	1.5	1.5	**0.3**														
11	**D 16**	0.6	0.2	**0**	1.6	1.2	0.9	1.6	**0**	**0**	1.5	**0.1**													
12	**D 17**	1.1	0.9	0.8	1.6	1.5	1.3	1.7	0.8	0.8	1.7	0.8	**1.5**												
13	**D 18**	0.5	0.2	**0**	1.6	1.1	0.9	1.6	**0**	**0**	1.5	**0**	0.8	**0**											
14	**F 3**	1.4	1.5	1.3	1.1	1.6	1.5	1.4	1.3	1.3	0.9	1.4	1.6	1.3	0.8										
15	**FIN 1**	0.5	0.2	**0**	1.6	1.1	0.9	1.6	**0**	**0**	1.5	**0**	0.8	**0**	1.3	**0**									
16	**GB 1**	0.8	0.8	0.6	1.6	1.1	0.9	1.1	0.6	0.6	1.5	0.6	1.1	0.6	1.5	0.6	**1.2**								
17	**GB 2**	1.3	1.2	1	1.6	1.6	1.5	1.7	1	1	1.7	1.1	1.1	1	1.6	1	1.3	**1.4**							
18	**NL 1**	0.5	0.2	**0**	1.6	1.1	0.9	1.6	**0**	**0**	1.5	**0**	0.8	**0**	1.3	**0**	0.6	1	**0**						
19	**NL 2**	0.8	1	0.8	1.6	1	0.9	0.8	0.8	0.8	1.4	0.8	1.2	0.8	1.4	0.8	0.8	1.4	0.8	**1.6**					
20	**PL 2**	0.6	0.3	0.1	1.6	1.1	0.9	1.5	0.1	0.1	1.5	0.1	0.2	0.1	1.3	0.1	0.6	1.1	0.1	0.8	**0.2**				
21	**RUM 1**	0.6	0.3	0.2	1.6	1.2	0.9	1.5	0.2	0.2	1.5	0.2	0.8	0.2	1.4	0.2	0.7	1.1	0.2	0.8	0.2	**0.3**			
22	**RUM 2**	0.5	0.2	**0**	1.6	1.1	0.9	1.6	**0**	**0**	1.5	**0**	0.8	**0**	1.3	**0**	0.6	1	**0**	0.8	0.1	0.2	**0**		
23	**RUM 5**	1	0.9	0.7	1.6	1.4	1.2	1.6	0.7	0.5	1.5	0.7	1.2	0.7	1.6	0.7	1	1.4	0.7	1.1	0.7	0.5	0.7	**0**	
24	**S 3**	0.7	0.6	0.4	1.6	1.1	0.9	1.2	0.4	0.4	1.5	0.5	1	0.4	1.4	0.4	0.8	1.3	0.4	0.8	0.5	0.6	0.4	1	**0.9**

The neutrality test of Tajima's D and Fu's FS neither were significant for the nucleotide nor for the protein (Table A21 and A22). The McDonald-Kreitman test was not significant for the whole dataset of *N. silvestris* (Table 19, for more detail Table A23). All neutrality indices which were computed were ≥0 (Table A24) and indicated purifying selection.

None of the estimated rarefaction curves of the nucleotide and the protein levelled off (Fig. 27 and 28). The Jackknife rarefaction curves ended at 60 haplotypes for the nucleotide and 17 haplotypes for the protein. The unique rarefaction curves ended at 17 haplotypes for the nucleotide and five haplotypes for the protein. The observed rarefaction curves (Sobs Mao Tau) ended at 25 haplotypes for the nucleotide and eight haplotypes for the protein.

The results of the Mantel test showed no evidence for isolation by distance in *N. silvestris* [R^2=0.0066, p=0.27 (Fig. A29) and R^2=0.0005, p=0.411 for log10 transformed geographical distance (Fig. 29) using 1000 randomizations].

The 'Generalized Mixed Yule Coalescent Model' (gmyc) had a significantly better fit in the single and the multiple analysis than the null model (Table 28). The model identified seven distinct mtDNA (*COI*) clusters for the single analysis and 19 for the multiple analysis. The clusters of the single and multiple analyses differed from the clusters which were delimited by the phylogenetic analyses (Fig. A30 and 31). The phylogenetic clade I was split in three distinct clusters and the phylogenetic clade IV was split in two distinct clusters in the single method. In the multiple method clade I was split into 14 distinct clusters, clade III shrunk to a two individual cluster and clade IV was split into three distinct clusters.

Table 18: Results of the generalized mixed Yule coalescent analyses of the four oribatid mite species (*Nothrus silvestris*, *Platynothrus peltifer*, *Achipteria coleoptrata*, *Steganacarus magnus*; log L null=likelihood of the null model, log L GMYC=likelihood of the GMYC model, No. of clusters=number of GMYC clusters corresponding to the optimized threshold with confidence limits. Asterisks indicate the significance as assessed by the likelihood ratio test ***$P\leq0.001$, **$P\leq0.005$, *$P<0.01$).

Species	method	*log* L (null)	*log* L (GMYC)	No. of clusters
N. silvestris	single	950.0625	959.1814	7 (2-19)***
	multiple	950.0625	960.703	19 (7-22)**
P. peltifer	single	1481.659	1497.187	6 (4-12)***
	multiple	1481.659	1497.978	6 (4-6)***
A. coleoptrata	single	1071.225	1074.535	5 (1-13)ns
	multiple	1071.225	1076.376	30 (5-31)ns
S. magnus	single	1123.333	1175.131	21 (21-23)***
	multiple	1123.333	1177.836	26 (22-26)***

Table 19: Results of the McDonald-Kreitman test of *Nothrus silvestris*. The differences between the populations were not significant. Locations with less than two individuals were excluded.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1 CH_2																							
2 CH 3	-																						
3 D 1	-	-																					
4 D 2	-	-	-																				
5 D 4	-	-	-	-																			
6 D 6	-	-	-	-	-																		
7 D 7	-	-	-	-	-	-																	
8 D 8	-	-	-	-	-	-	-																
9 D 9	-	-	-	-	-	-	-	-															
10 D 11	-	-	-	-	-	-	-	-	-														
11 D 16	-	-	-	-	-	-	-	-	-	-													
12 D 17	-	-	-	-	-	-	-	-	-	-	-												
13 D 18	-	-	-	-	-	-	-	-	-	-	-	-											
14 F 3	-	-	-	-	-	-	-	-	-	-	-	-	-										
15 FIN 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-									
16 GB 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
17 GB 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
18 NL 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
19 NL 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
20 PL 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
21 RUM 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
22 RUM 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
23 RUM 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
24 S 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

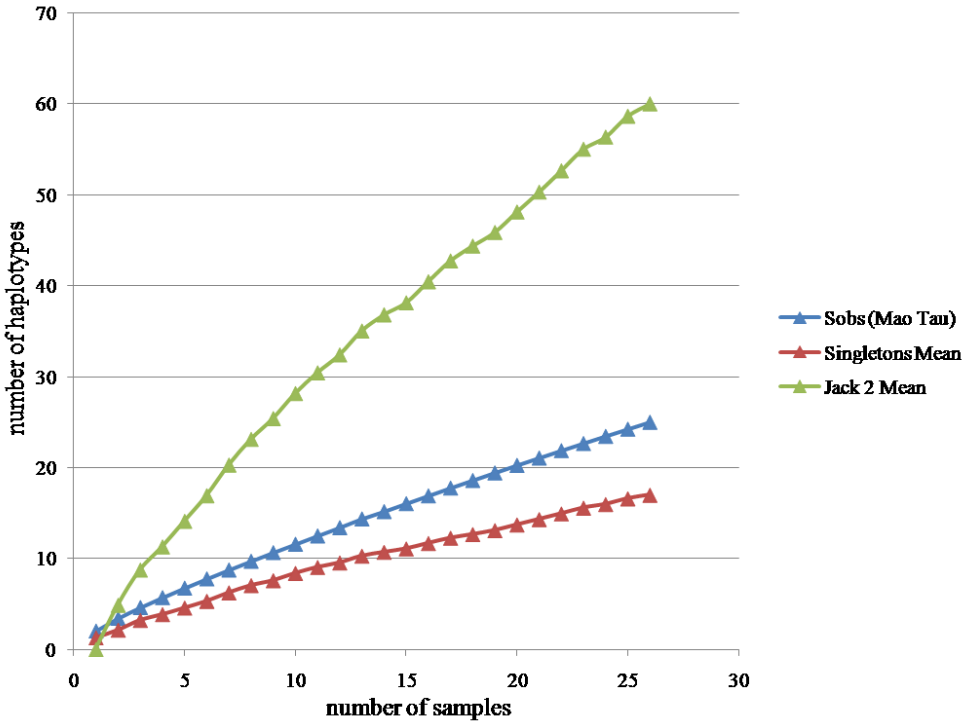

Figure 27: Sample based rarefaction analysis of haplotypes of the *COI* gene of *Nothrus silvestris*. Jack 2 Mean, second-order Jackknife richness estimator, Singletons, numbers of haplotypes each present in only one sample, Sob (Mao Tau) is the observed haplotype richness.

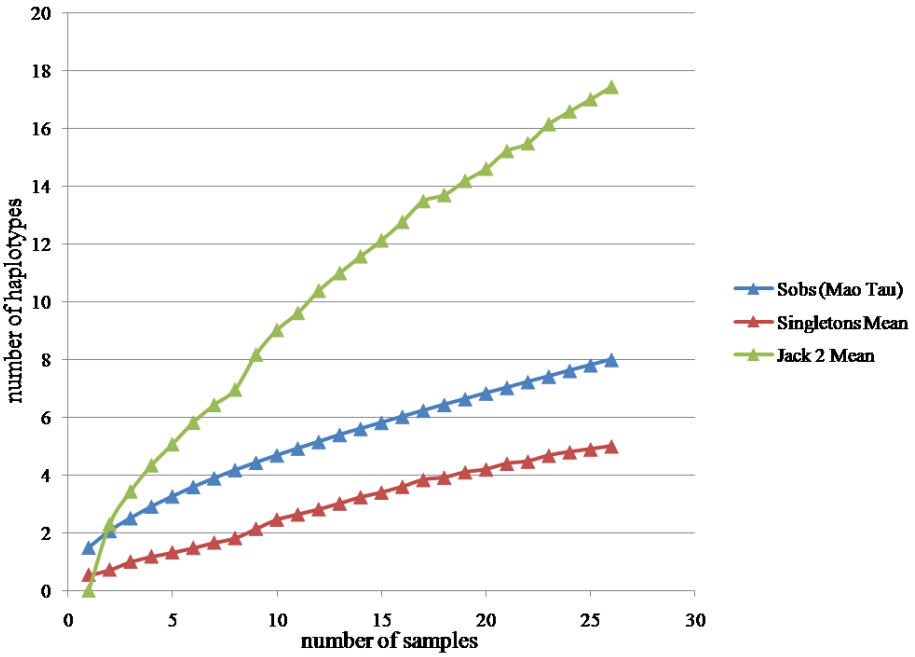

Figure 28: Sample based rarefaction analysis of haplotypes of the *COI* protein of *Nothrus silvestris*. Jack 2 Mean, second-order Jackknife richness estimator, Singletons, numbers of haplotypes each present in only one sample, Sob (Mao Tau) is the observed haplotype richness.

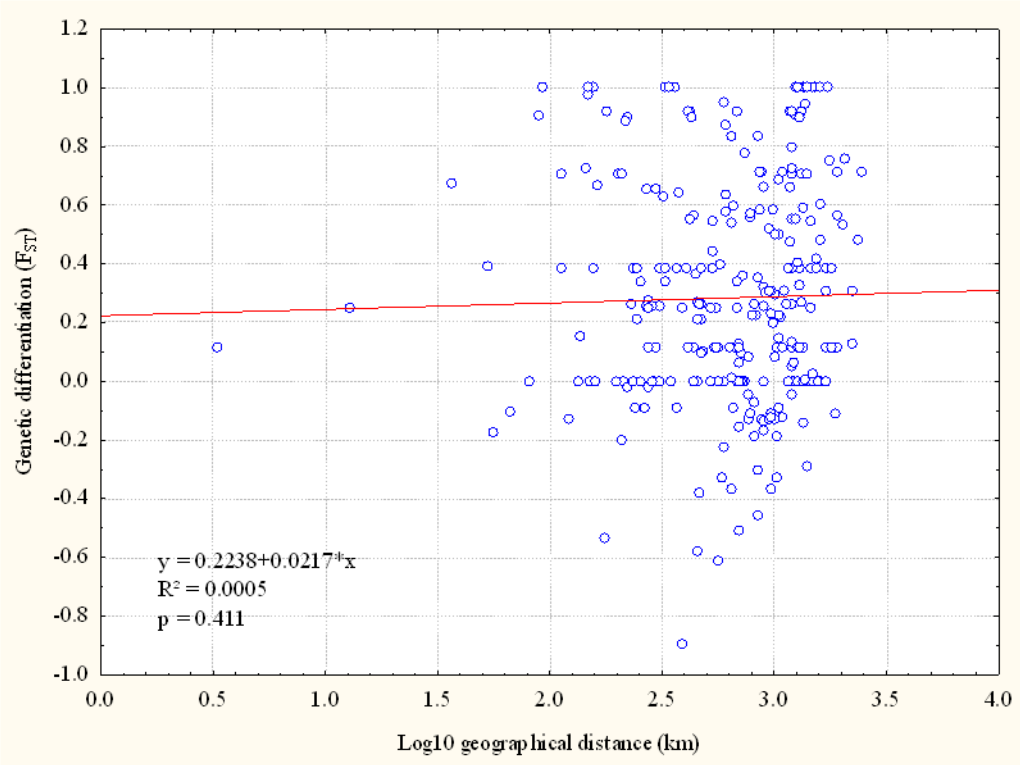

Figure 29: Linear regression of log10 geographical distances (kilometers) vs. genetic differentiations (F_{ST}) based on *COI* nucleotide sequences of *Nothrus silvestris*. The regression is not significant using 1000 randomizations.

4.3.2 Platynothrus peltifer

A total of 162 individuals (160 individuals of *P. peltifer*, one individual of *H. rufulus* and one individual of *R. duplicata* as outgroup) from 41 locations in 14 countries were sequenced. The sequences contained 24.7% A, 25.6% C, 18.5% G and 31.2% T. The 160 *COI* sequences consisted of 75 haplotypes for the nucleotide (46.9%) (Fig. 30) with 196 variable sites (35.3%) and 172 parsimony informative sites (Table A25). The 160 sequences of the protein consisted of 18 haplotypes (11.3%) (Fig. 31). Haplotypes diversity (H_d) was very high with 0.98 in the nucleotide.

Haplotype networks

The nucleotide haplotype network formed six subclades and one isolated individual haplotype from a Swedish location (S_4.2) which were separated each by 16 or more substitution steps (Fig. 30). Subclade 1a comprised 20 single individual haplotypes and five haplotypes which comprised two to nine individuals from the same or different locations [single individual haplotypes: five from one Polish location (PL_2.1, PL_2.4, Pl_2.6, PL_2.8 and PL_2.9), two each from one Austrian (A_1.1-2) and French location (F_5.1 and F_5.3); one from a Danish (DK_2.5) and a Romanian (RUM_2.2) location, nine from six locations in Germany (D_1.3, D_1.4, D_4.1, D_5.1, D_5.2, D_5.4, D_6.5, D_11.1 and D_18.1). Haplotypes of more than one individual: two with two individuals each one Danish (DK_2.3-4) and one Polish (PL_2.5 and PL_2.7) site, one haplotype of four individuals from the one German location (D_6.1-4), one haplotype of seven individuals each one location in Italy (I_1.1), one location in Scotland (GB_2.2-4) and two locations in Germany (D_1.1 and D_2.1-2), one haplotype of nine individuals from one French (F_5.2) and four German locations (D_4.2-4, 4.6, 4.8, D_5.3, D_9.3 and D_18.2)]. Subclade 1b comprised 17 haplotypes from 15 locations [nine single individual haplotypes from seven different locations (one Dutch NL_2.1, one Finish FIN_1.4, one Scottish GB_2.1, one Swedish S_2.5, two Polish PL_2.2 and Pl_2.3 and three German D_8.1, D_8.3 and D_18.3), three haplotypes of two individuals each from one location (F_1.3-4, F_3.1-2 and

NL_2.2-3) and one haplotype of two individuals from two locations (NL_2.4/S_2.6), one haplotype of three Finish individuals (FIN_1.1-3) and another of three individuals from three different locations (one Austrian A_5.3 and two German D_15.3 and D_17.1), one haplotype of four individuals from the same German location (D_18.4-7), one haplotype of seven individuals from four locations (one Danish DK_2.1-2 and 2.6, one English GB_1.1 and one German D_7.1-3), all other haplotypes of the subclade were connected starlike to the most abundant haplotype]. Subclade 1c comprised five single individual haplotypes from one Swedish location (S_2.1, S_2.2, S_2.3, S_2.4 and S_2.8).
Subclade 2 comprised four single individual haplotypes from four locations [one Austrian (A_5.2), one Norwegian (N_1.2) and two Swedish (S_1.2 and S_4.1)], one haplotype of three Swedish individuals from two locations (S_1.1 and S_2.7, 2.9) and one haplotype of five individuals from four locations [one Austrian (A_5.1), one Norwegian (N_1.1) and two Swedish (S_1.3 and S_4.3-4)].
Subclade 3 differed from the other subclades by the high number of identical haplotypes shared by different individuals. Ten of 19 haplotypes were shared by 2-14 individuals and the haplotypes had a starlike arrangement with the haplotype shared by most individuals in the centre. The nine single individual haplotypes were from three German (D_3.1, 3.3, 3.4, D_11.2 and D_12.2), one Austrian (A_9.1 and A_9.5), one Romanian (RUM_6.1) and one Swedish (S_5.3) location. Two haplotypes were shared by two individuals [one by two individuals from the one location in the Netherlands (NL_1.1 and NL_1.3), one by two individuals from two German locations (D_1.2 and D_15.1)]. Three haplotypes were shared by four individuals from two to four locations [one was shared by three individuals from one German location (D_12.3-5) and one individual from an Austrian location (A_3.1), one shared by individuals from three locations, one Austrian (A_4.1-2), one German (D_15.2) and one Swiss (CH_3.3), one was shared by individuals from four locations, one Austrian (A_9.4), one Swedish (S_5.4) and two German (D_4.7 and D_17.3)]. Two haplotypes were shared by five individuals [one from one Austrian location (A_10.1-5), one three locations (two Austrian A_2.1-2 and A_3.2-3 and one German D_8.4)]. One haplotype was shared by six individuals from five locations [one Romanian (RUM_2.1), one Swedish (S_5.1-2), one Swiss (CH_3.5) and two German (D_3.2 and D_8.2)]. One haplotype was shared by seven individuals from two locations [one Danish (DK_3.1-6), one French (F_1.2)]. One haplotype comprised 14 individuals from nine locations [one English (GB_1.2-3), one Romanian (RUM_5.1), one Swiss (CH_3.1-2, 3.4), two Austrian (A_5.4, A_9.2-3), four German (D_4.5, D_9.1-2, D_12.1, D_17.2)].
Subclade 4 comprised two single individual haplotypes from two locations [one French (F_1.1), one Dutch (NL_1.2)]. Subclade 1a was connected to two other subclades by 24 (subclade 1b) and 95 substitution steps (subclade 3). Subclade 1b was connected to two other subclades by 16 (subclade 1c) and 24 substitution steps (subclade 1a). Subclade 1c was connected to two other subclades by 16 (subclade 1b) and 71 substitution steps (subclade 2). Subclade 2 was connected to subclade 1c by 71 and to the single individual subclade (S_4.2) by 16 substitution steps. Subclade 3 was connected to two other subclades by 81 (subclade 4) and 95 substitution steps (subclade 1a). Subclade 4 was connected to subclade 3 by 81 substitution steps.

The protein haplotypes network comprised 18 haplotypes with a starlike arrangement (Fig. 31). Four of the 17 satellite haplotypes differed by two and one satelilite haplotype by three amnio acid changes from the Central haplotype. The other twelve satellite haplotype differed by one amino acid change from the Central haplotype. Four satellite haplotypes were shared by two individuals [three from one location, one Austrian (A_1.1-2), one Danish (DK_2.3-4) and one French (F_3.1-2), one from two locations, one German (D_12.2) and one Romanian (RUM_2.2)]. The other 13 satellite haplotypes were single individual haplotypes from nine locations [one Dutch location (NL_2.1), one Norwegian location (N_1.2), one Polish location (PL_2.2, PL_2.9), two Swedish locations (S_2.1, S_2.2, S_2.4,

S_2.8 and S_4.1) and four German locations (D_3.3, D_6.5, D_8.1 and D_11.1)]. All other 139 individuals from the 40 locations were in the Central haplotype.

Phylogenetic and population genetic analyses

The phylogenetic analyses (NJ without and with model of sequence evolution, MrBayes and Beast) showed a highly supported monophyletic group of *P. peltifer* with six subclades and one isolated individual in the nucleotide (Fig. 32, A32-35). The clade 1a was paraphyletic in the Bayesian phylogeny (Fig. A34), but the complete clade 1 was monophyletic and highly supported. The six subclades were supported by high bootstrap values and posterior probabilities. The individuals which built these clades are presented in Table 20. In the phylogenetic trees of the protein all sequences were grouped in one highly supported monophyletic clade (Fig. 33, A36-38).

The minimum and maximum mean average pairwise differences for the nucleotide sequences between populations were 0.2% (A_2/A_3, A_4/CH_3, A_9/CH_3, D_2/F_5, D_7/F_3 and D_7/NL_2) and 20.4% (DK_3/F_3). Within populations the minimum and maximum mean average pairwise differences were 0% (A_2, A_4, A_10, D_2, D_7, DK_3 and F_3) and 18.5% (D_11) (Table 21). For the protein the minimum and maximum mean average pairwise differences between populations were 0% (all populations except for D_12, NL_2, PL_2 and RUM_2) and 0.41% (NL_2/RUM_2). The minimum and maximum mean average pairwise differences within population were 0% (all populations except for D_12, NL_2, PL_2 and RUM_2) and 0.54% for the protein (RUM_2) (Table A26).

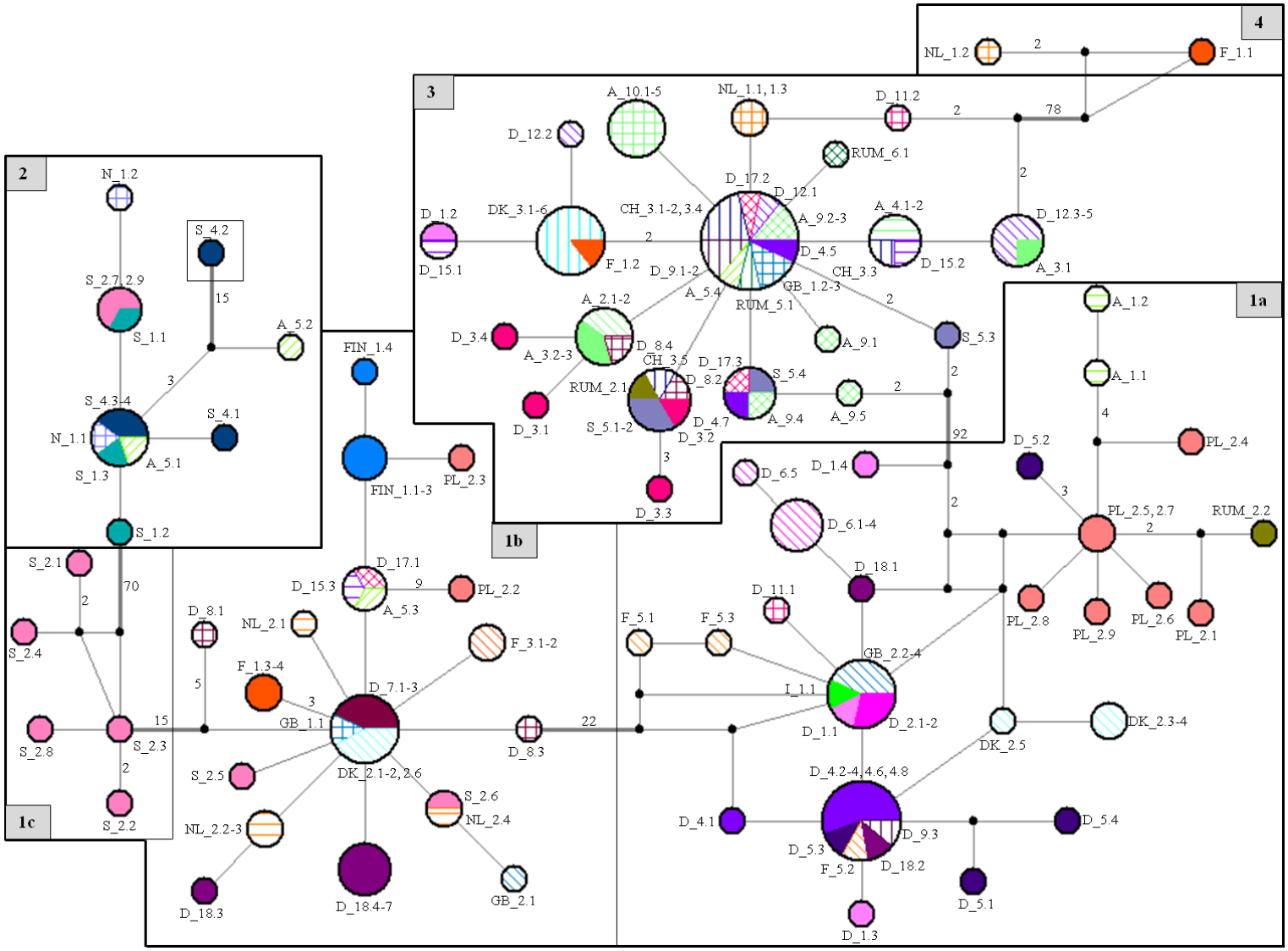

Figure 30: Median-joining haplotype network for the *COI* nucleotide of 75 haplotypes from *Platynothrus peltifer*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one substitution step between haplotypes). Major subclades are marked by boxes.

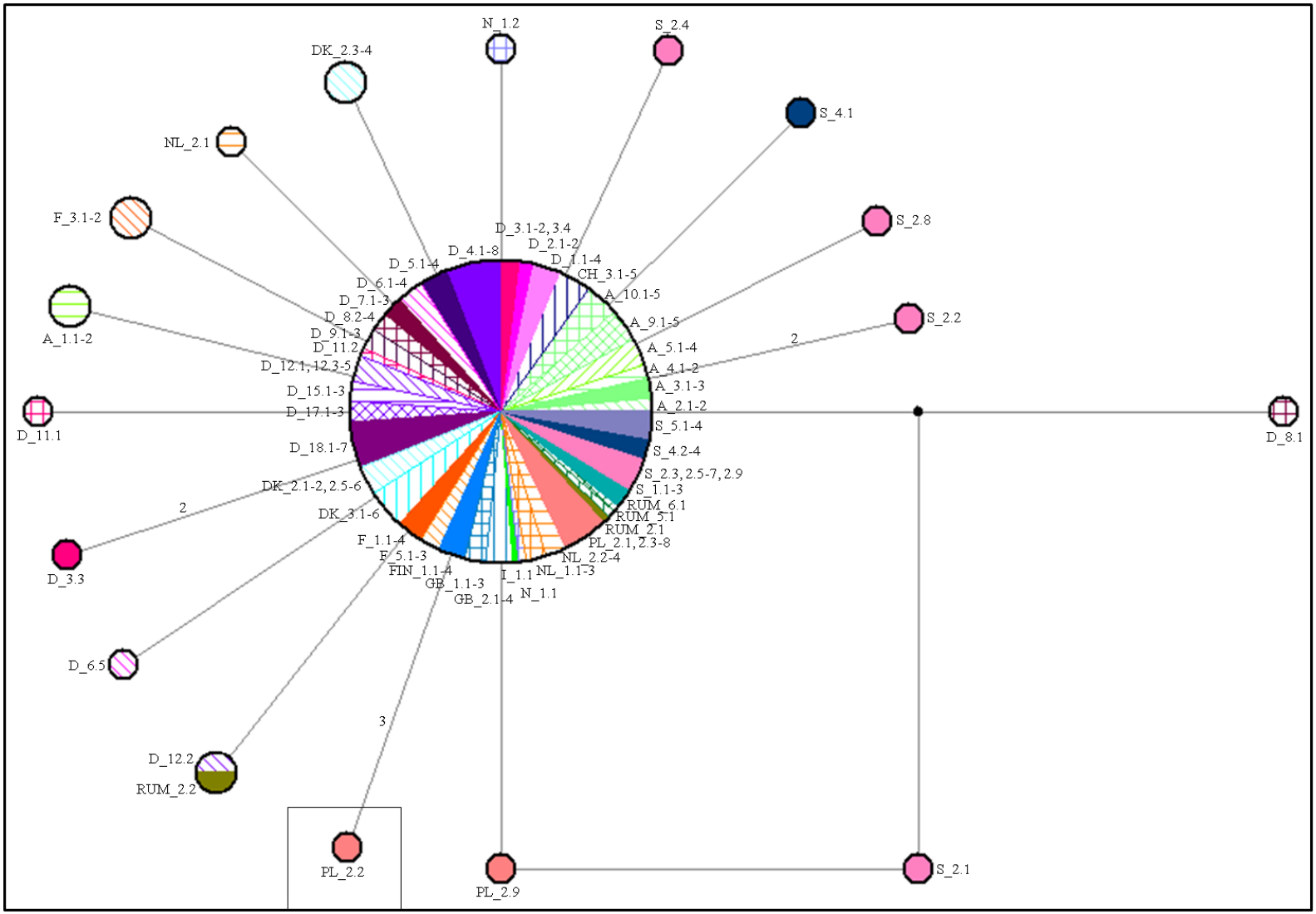

Figure 31: Median-joining haplotype network for the *COI* protein of 18 haplotypes from *Platynothrus peltifer*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one amino acid change between haplotypes).

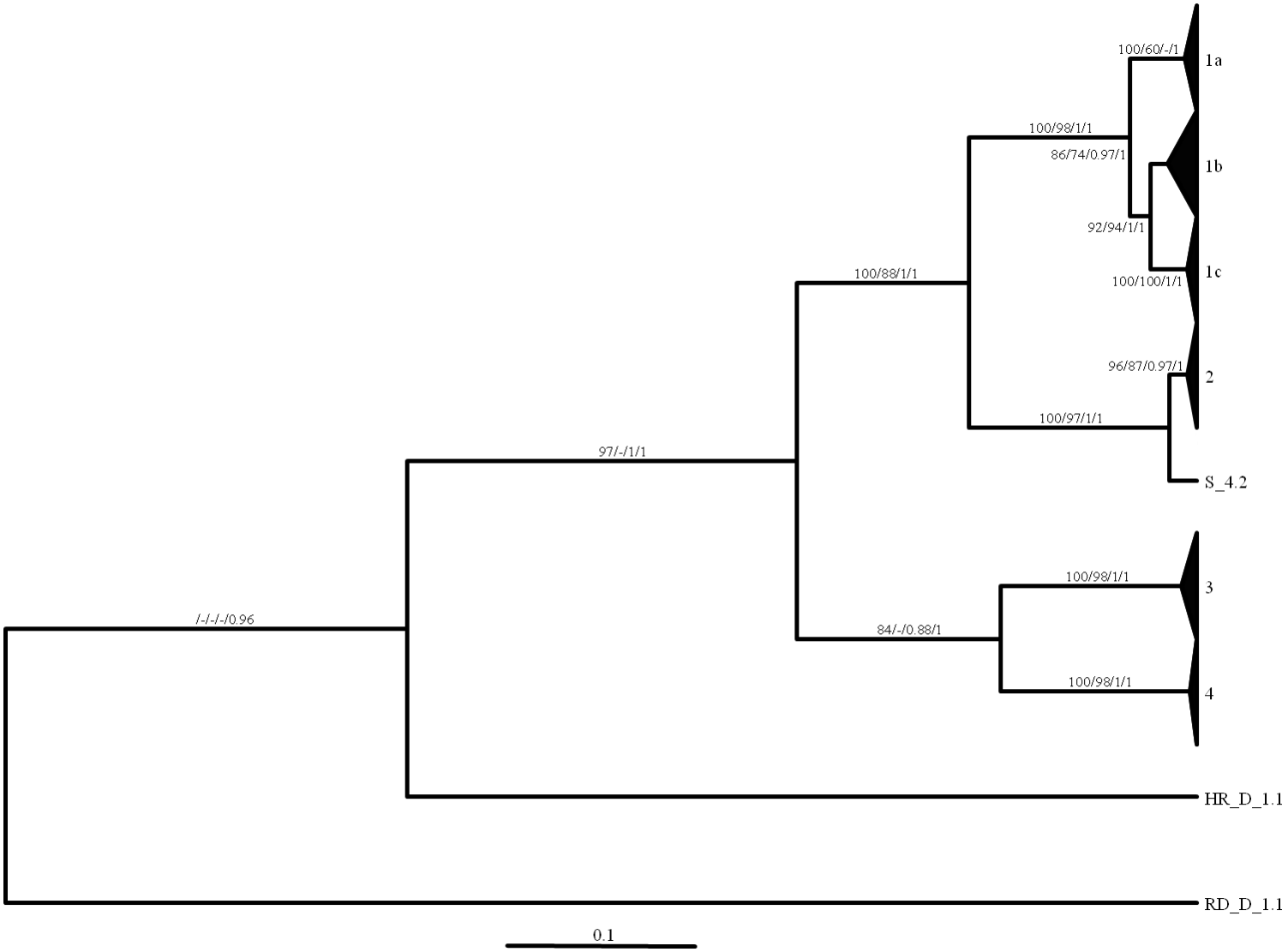

Figure 32: Bayesian phylogeny after 10 x 10^6 generations from the 160 *COI* nucleotide sequences of *Platynothrus peltifer* with Beast v1.5.4. Outgroups are one individual of *Hypochthonius rufulus* and *Rhysotritia duplicata*, respectively. Numbers on the branches are bootstrap values from NJ without and with evolution model (HKY+G) analysis and posterior probabilities from MrBayes and Beast. Tip numbers are the different subclades and explained in Table 20.

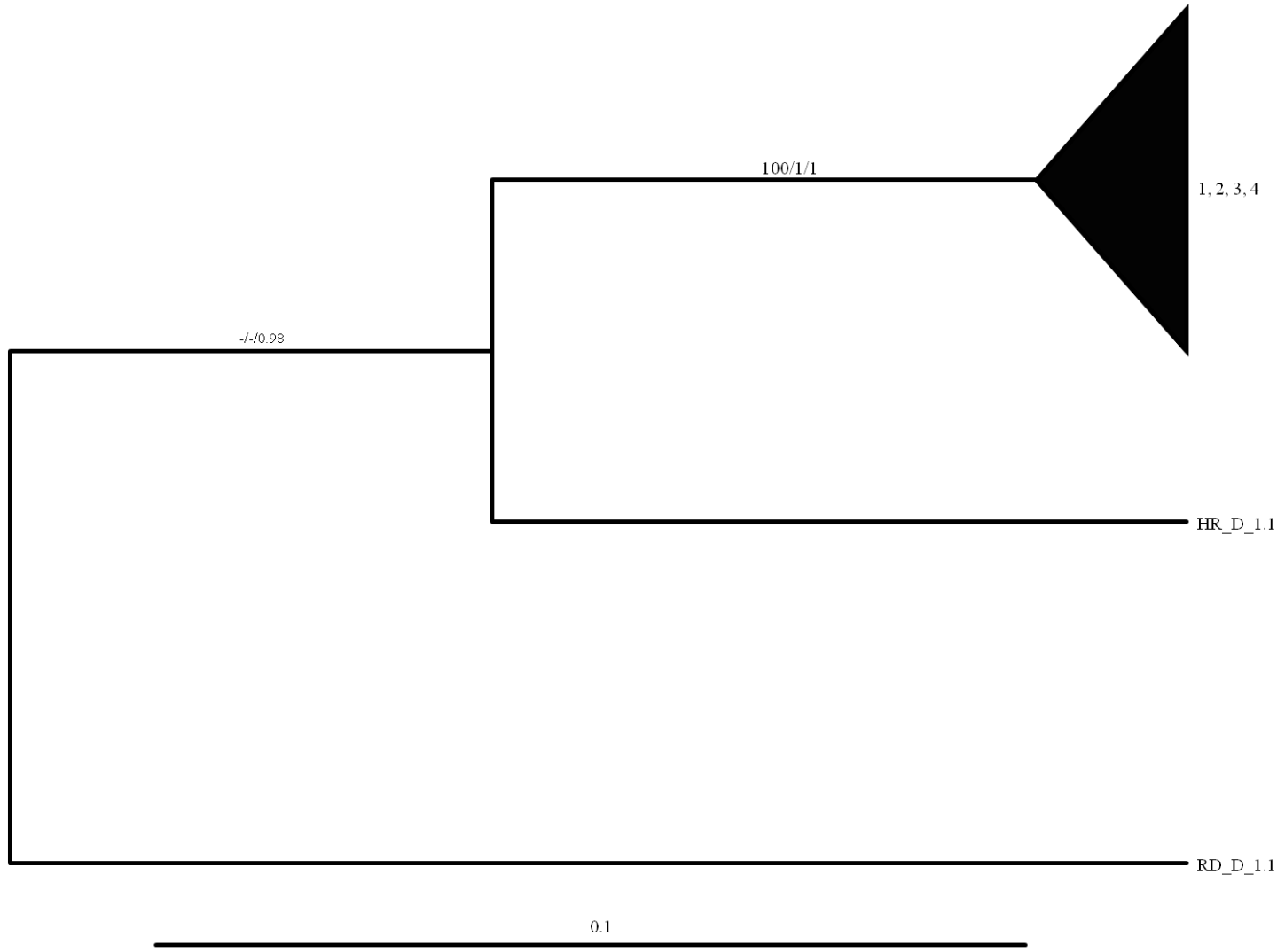

Figure 33: Bayesian phylogeny after 10 x 10^6 generations from the 160 *COI* protein sequences of *Peltifer peltifer* with Beast v1.5.4. Outgroups are one individual of *Hypochthonius rufulus* and *Rhysotritia duplicata*, respectively. Numbers on the branches are bootstrap values from NJ and posterior probabilities from MrBayes and Beast. Tip numbers are the different subclades and explained in Table 20.

Table 20: Subclades of Bayesian phylogenetic trees of *Platynothrus peltifer* based on *COI* nucleotide sequences (#ind=number or individuals, pp=posterior probabilities, sampling sites with abbreviations, individuals and ind. pop.=quantity of individuals from the population).

Subclade	# ind	pp	sampling sites		individuals	ind. pop
1	**83**	**1**	**26**			
1a	**44**	**1**	Villach	A_1	1-2	2/2
			Kranichstein	D_1	1, 3-4	3/4
			Goettingen	D_2	1-2	2/2
			Meckl. Seenplatte	D_4	1-4, 6, 8	6/8
			Moerfelden	D_5	1-4	4/4
			Black Forrest	D_6	1-5	5/5
			Bonn	D_9	3	1/3
			Wittmoor	D_11	1	1/2
			Steinhuder Meer	D_18	1-2	2/7
			Hjørring	DK_2	3-5	3/5
			Brunstatt	F_5	1-3	3/3
			Braemar	GB_2	2-4	3/4
			Grosseto	I_1	1	1/1
			Warsaw	PL_2	1, 4-9	7/9
			Sibiu_2	RUM_2	2	1/2
1b	**34**	**1**	Memminger Huette	A_5	3	1/4
			Uelzen	D_7	1-3	3/3
			Cuxhaven	D_8	1, 3	2/4
			Langenwang	D_15	3	1/3
			Rubi	D_17	1	1/3
			Steinhuder Meer	D_18	3-7	5/7
			Hjørring	DK_2	1-2, 6	3/6
			Mont Blanc	F_1	3-4	2/4
			Saint Isidore	F_3	1-2	2/2
			Lahti	FIN_1	1-4	4/4
			Ascot	GB_1	1	1/3
			Braemar	GB_2	1	1/4
			Hoge Veluwe	NL_2	1-4	4/4
			Warsaw	PL_2	2-3	2/9
			Stroemstad	S_2	5-6	2/9
1c	**5**	**1**	Stroemstad	S_2	1-4, 8	5/9
2	**12**	**1**	Memminger Huette	A_5	1-2	2/4
			Narvik	N_1	1-2	2/2
			Umea	S_1	1-3	3/3
			Stroemstad	S_2	7, 9	2/9
			Abisko	S_4	1, 3-4	3/4
isol. ind.	**1**		Abisko	S_4	2	1/4

Table 20 continue:

Subclade	# ind	pp	sampling sites		individuals	ind. pop.
3	**62**	**1**	Bregenz	A_2	1-2	2/2
			Hittisau	A_3	1-3	3/3
			Holzgau	A_4	1-2	2/2
			Memminger Huette	A_5	4	1/4
			Glacier	A_9	1-5	5/5
			Stuiben	A_10	1-5	5/5
			Rorschach	CH_3	1-5	5/5
			Kranichstein	D_1	2	1/4
			Lake Constance	D_3	1-4	4/4
			Meckl. Seenplatte	D_4	5, 7	2/8
			Cuxhaven	D_8	2, 4	2/4
			Bonn	D_9	1-2	2/3
			Wittmoor	D_11	2	1/2
			Sonthofen	D_12	1-5	5/5
			Langenwang	D_15	1-2	2/3
			Rubi	D_17	2-3	2/3
			Arhus_Morvadsvej	DK_3	1-6	6/6
			Mont Blanc	F_1	2	1/4
			Ascot	GB_1	2-3	2/3
			Wageningen	NL_1	1, 3	2/3
			Sibiu_2	RUM_2	1	1/2
			Busteni	RUM_5	1	1/1
			Sinaia	RUM_6	1	1/1
			Gothenburg	S_5	1-4	4/4
4	**2**	**1**	Mont Blanc	F_1	1	1/4
			Wageningen	NL_1	2	1/3

Table 21: Mean percent pairwise differences of uncorrected p-distances of the *COI* nucleotide sequences of *Platynothrus peltifer* from 39 locations. The diagonal is the within population differences (bold) and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Locations with less than two individuals are excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	A 1	**0.2**																											
2	A 2	18.2	**0**																										
3	A 3	18.3	**0.2**	**0.4**																									
4	A 4	18.2	0.4	0.3	**0**																								
5	A 5	12.8	14.3	14.3	14.3	**14.4**																							
6	A 9	18.3	0.3	0.4	0.3	14.4	**0.3**																						
7	A 10	18.6	0.4	0.4	0.4	14.4	0.3	**0**																					
8	CH 3	18.3	0.3	0.3	**0.2**	14.3	**0.2**	0.3	**0.1**																				
9	D 1	5.9	13.4	13.5	13.4	13.1	13.4	13.7	13.5	**9.3**																			
10	D 2	1.5	17.9	18	17.9	12.5	18	18.3	18	4.8	**0**																		
11	D 3	18.3	0.4	0.5	0.6	14.4	0.6	0.6	0.4	13.5	18.0	**0.7**																	
12	D 4	5.9	13.5	13.6	13.5	13	13.6	13.8	13.6	7.1	4.7	13.7	**7.8**																
13	D 5	18.1	18.4	18	18	18.1	12.7	0.6	18.1	5.2	0.6	18.1	4.9	**0.9**															
14	D 6	1.9	17.6	17.7	17.6	12.4	17.7	18	17.7	5	0.4	17.7	4.9	1	**0.1**														
15	D 7	5.7	19.7	19.8	19.7	12	19.8	20.1	19.8	8.7	4.5	19.8	8.4	5.1	4.9	**0**													
16	D 8	12.1	9.8	9.9	9.9	13.2	9.9	10.1	9.9	11.1	11.3	10	11	11.7	11.4	10.2	**13.2**												
17	D 9	12.8	6.1	6.2	6.1	13.7	6.1	6.2	6.1	10.6	12.1	6.3	10.6	12.3	12	14.8	10.4	**12.1**											
18	D 11	10.1	9.3	9.4	9.3	13.6	9.3	9.5	9.3	9.4	9.2	9.5	9.3	9.6	9.3	12.4	10.8	9.3	**18.5**										
19	D 12	18.4	0.5	0.4	0.3	14.5	0.5	0.5	0.4	13.6	18.1	0.7	13.7	18.2	17.8	20	10.1	6.3	9.5	**0.4**									
20	D 15	14.1	6.9	6.9	6.8	13.6	6.9	7	6.8	11.8	13.5	7.1	11.9	13.8	13.4	13.3	10.1	9.1	10.4	6.9	**13.4**								
21	D 17	14.1	6.7	6.8	6.7	13.6	6.7	6.8	6.7	11.9	13.6	6.9	11.9	13.8	13.5	13.3	10	9	10.3	6.9	8.9	**13.1**							
22	D 18	4.7	19.1	19.1	19.1	12.2	19.2	19.4	19.2	7.7	3.4	19.2	7.4	4	3.8	1.5	10.5	14	11.5	19.3	13.4	13.3	**2.4**						
23	DK 2	3.6	18.8	18.9	18.8	12.3	18.9	19.2	18.9	6.9	2.5	18.9	6.6	18.2	2.9	2.5	10.9	13.5	10.9	19	13.5	13.5	2.7	**3**					
24	DK 3	18.4	0.5	0.6	0.5	14.5	0.5	0.5	0.4	13.4	18.1	0.8	13.7	13.4	17.8	20.3	10.3	6.3	9.5	0.5	6.9	7	19.5	19.2	**0**				
25	F 1	12.5	13.7	13.7	13.6	14	13.7	13.8	13.6	12.5	12	13.8	12.4	2.9	12	10.3	12.1	13.1	13	13.7	12.5	12.5	10.8	11.2	13.8	**15.7**		
26	F 3	5.8	19.9	20	19.9	12.2	20	20.3	20	8.9	4.7	20	8.6	12.3	5.1	**0.2**	10.4	15	12.5	20.2	13.5	13.4	1.7	2.7	**20.4**	10.4	**0**		
27	F 5	1.8	18	18	18	12.6	18.1	18.3	18.1	5	**0.2**	18.1	4.8	5.2	0.6	4.6	11.4	12.2	9.4	18.2	13.6	13.6	3.5	2.6	18.3	12.1	4.8	**0.4**	
28	FIN 1	6	19.7	19.7	19.7	12	19.8	20	19.8	9	4.8	19.8	8.7	5.4	5.2	0.4	10.4	14.9	12.5	20	13.3	13.3	1.9	2.8	20.2	10.4	0.6	4.9	**0.1**
29	GB 1	14.1	6.7	6.8	6.7	13.6	6.7	6.8	6.7	11.9	13.6	6.9	11.9	1.8	13.5	13.3	10	9	10.3	6.9	9.0	8.8	13.3	13.5	7	12.5	13.4	13.6	13.7
30	GB 2	2.6	18.5	18.5	18.5	12.4	18.5	18.8	18.6	5.9	1.2	18.6	5.7	2.2	1.6	3.5	11.1	12.9	10.1	18.7	13.6	13.6	3	2.6	18.7	11.6	3.7	1.4	3.8
31	N 1	13.4	18.6	18.6	18.6	8.6	18.8	18.7	18.7	15	13.7	18.6	14.9	1.8	13.4	14	16.3	17	16.2	18.8	17.2	17.3	14.1	13.8	18.7	16.1	14.3	13.7	14
32	NL 1	19.1	5.4	5.4	5.3	15.5	5.3	5.3	5.2	15.3	18.8	5.5	15.4	18.8	18.4	20	12.6	9.7	12	5.4	10.2	10.1	19.5	19.4	5.4	13.8	20.1	18.8	19.8
33	NL 2	5.8	19.7	19.8	19.7	12.1	19.8	20.1	19.8	8.9	4.7	19.8	8.6	5.2	5.1	**0.2**	10.3	14.9	12.5	20	13.4	13.3	1.6	2.7	20.3	10.4	0.4	4.8	0.6
34	PL 2	2.4	18.3	18.3	18.3	12.6	18.3	18.6	18.4	6.2	1.8	18.4	6.1	13.6	2.2	4.3	11.4	13	10.3	18.5	13.7	13.7	3.8	3.1	18.5	12	4.5	2	4.5
35	RUM 2	9.9	9.1	9.1	9.1	13.5	9.1	9.2	9	9.4	9.5	9.1	9.5	9.7	9.5	12.6	10.8	9.2	9.5	9.2	10.3	10.2	11.8	11.1	9.2	13	12.8	9.7	12.8
36	S 1	13.3	18.5	18.5	18.5	8.4	18.7	18.6	18.6	14.8	13.6	18.6	14.7	7.7	13.2	13.8	16.1	16.9	16.1	18.7	17	17.1	13.8	13.6	18.6	15.9	14	13.6	13.7
37	S 2	8.2	18.9	18.9	18.9	11.4	19.0	19.2	19	10.6	7.3	19	10.3	18	7.5	4.9	12.1	15.2	13.3	19.1	14.4	14.4	5.8	6.3	19.3	12.3	5.1	7.4	5.2
38	S 4	13.3	18.7	18.7	18.7	8.8	18.9	18.9	18.8	14.9	13.5	18.8	14.7	13.5	13.2	13.7	16.2	16.2	17	18.9	17.1	17.3	13.8	13.5	18.9	15.9	13.9	13.5	13.7
39	S 5	18.2	0.4	0.5	0.4	14.3	0.3	0.4	0.3	13.4	17.9	0.5	13.5	13.8	17.6	19.7	9.9	6.1	9.3	0.6	6.9	6.7	19	18.7	0.6	13.6	19.9	17.9	19.6

Table 21continue:

Population		29	30	31	32	33	34	35	36	37	38	39
29	**GB 1**	**13.3**										
30	**GB 2**	13.6	**2.4**									
31	**N 1**	17.2	13.7	**0.5**								
32	**NL 1**	10.1	19.1	18.4	**10**							
33	**NL 2**	13.3	3.6	4.5	20	**0.3**						
34	**PL 2**	13.7	2.5	13.9	19	4.5	**2.7**					
35	**RUM 2**	10.2	10.4	16.0	11.9	12.7	10.1	**17.7**				
36	**S 1**	17	13.5	0.3	18.3	13.9	13.7	15.9	**0.2**			
37	**S 2**	14.3	6.8	10.6	19.3	5.1	7.4	13.4	10.4	**6.3**		
38	**S 4**	17.1	13.5	1.1	18.5	13.8	13.6	16	1	10.7	**1.7**	
39	**S 5**	6.7	18.4	18.7	5.3	19.7	18.2	9	18.6	18.9	18.8	**0.4**

The results of the AMOVA showed that the nucleotide variation among samples within countries (57.2%) and the variation within samples (36.5%) were significant and high. In contrast variation among countries was not significant (Table 22).

Table 22: AMOVA table on variations among countries and within populations in nucleotide (black) and protein (red) sequences of *COI* of *Platynothrus peltifer*. Each population was considered as separate groups. Populations with less than two individuals were excluded from the analysis. Significance level is $p<0.05$ (d.f.: degree of freedom).

Source of variance	d.f.	sum of squares	variance components	percent of total variation	fixation indices
among countries	11 / 11	1280.79 / 0.73	2.04 Va / 0.01 Va*	6.3 / 19.04	FCT: 0.06 / 0.19
among populations	27 / 27	2243.04 / 0.29	18.5 Vb* / 0 Vb	57.17 / -14.04	FSC: 0.61* / -0.17
within population	118 / 118	1394.94 / 2.94	11.82 Vc* / 0.03 Vc	36.53 / 95	FST: 0.63* / 0.05
total	156 / 156	4918.76 / 3.96	32.36 / 0.03		

The neutrality test of Tajima's D was only significant for one population in the nucleotide (D_3 Tajima's D=0, p-value=0.0359, Table A27) and was not significant for the protein (Table A28). Fu's FS neutrality test was not significant for both datasets (Table A27 and Table A28).

The results of the McDonald-Kreitman test showed that the differences between 64 of 741 populations were significant (8.64%), between other 17 of 741 were high significant (2.29%) and between three other of 741 populations were highly significant (0.41%) (Table 23 and for detail Table A29). All computed neutrality indices in the McDonald-Kreitman test were ≥ 0 (Table A30).

None of the calculated rarefaction curves of the nucleotide and the protein reached saturation (Fig. 34 and 35). The Jackknife rarefaction curves ended at 183 haplotypes for the nucleotide and 45 haplotypes for the protein. The unique rarefaction curves ended at 49 haplotypes for the nucleotide and twelve haplotypes for the protein. The observed rarefaction curves (Sobs Mao Tau) ended at 75 haplotypes for the nucleotide and 17 haplotypes for the protein.

The results of the Mantel test indicate isolation by distance in *P. peltifer* [R^2=0.0629, p=0.006; (Fig. A39) and R^2=0.0524, p=0.003**; for log transformed geographic distances (Fig. 36) using 1000 randomizations], but this explained only 5% of the variation in the data.

The gmyc model had a significantly better fit in the single and the multiple analysis than the null model (Table 18). For both the single and the multiple analysis the model identified six distinct mtDNA (*COI*) clusters. The clusters of the single analysis differed from the clusters which were delimited by the phylogenetic analyses in one point; in clade 1c the individual PL_2.2 was excluded from the generated *COI* cluster (Fig. A40 and A41).

Table 23: Results of the McDonald-Kreitman test of *Platynothrus peltifer*. The differences between 64 populations were significant (*0.01<P<0.05), between other 17 populations high significant (**0.001<P<0.01) and between three populations highly significant (***P<0.001). Locations with less than two individuals were excluded.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	A 1																												
2	A 2	-																											
3	A 3	-	-																										
4	A 4	-	-	-																									
5	A 5	-	-	-	-																								
6	A 9	-	-	-	-	-																							
7	A 10	-	-	-	-	-	-																						
8	CH 3	-	-	-	-	-	-	-																					
9	D 1	*	-	-	-	-	-	-	-																				
10	D 2	-	-	-	-	-	-	-	-	**																			
11	D 3	*	-	-	-	-	-	-	-	-	-																		
12	D 4	-	-	-	-	-	-	-	-	-	-	-																	
13	D 5	-	-	-	-	-	-	-	-	-	-	*	-																
14	D 6	-	*	*	*	-	*	*	*	-	-	***	-	-															
15	D 7	-	-	-	-	-	-	-	-	-	-	**	-	-	*														
16	D 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
17	D 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-												
18	D 11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-											
19	D 12	-	-	-	-	-	-	-	-	-	*	-	-	-	**	*	-	-	-										
20	D 15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-									
21	D 17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
22	D 18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
23	DK 2	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-						
24	DK 3	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-					
25	F 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
26	F 3	-	-	-	-	*	-	-	-	-	-	**	-	-	-	-	*	-	-	-	*	*	*	-	-	*			
27	F 5	-	-	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
28	FIN 1	-	-	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
29	GB 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**	**	-	-
30	GB 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-
31	N 1	-	*	*	*	-	*	*	*	-	*	***	-	-	**	*	-	-	-	**	-	-	-	-	*	-	-	-	*
32	NL 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	NL 2	-	*	-	*	-	*	*	*	-	-	***	-	-	*	-	-	-	-	**	-	-	-	-	*	-	-	-	-
34	PL 2	-	*	*	*	-	*	*	*	-	-	**	-	-	-	-	-	-	-	**	-	-	-	-	*	-	-	-	-
35	RUM 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	S 1	-	-	-	-	-	-	-	-	-	-	**	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	S 2	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-
38	S 4	-	*	*	*	-	*	*	*	-	*	**	-	-	*	*	-	-	-	**	-	-	-	-	*	-	-	-	-
39	S 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 23 continue:

Population		29	30	31	32	33	34	35	36	37	38
30	GB 2	-									
31	N 1	-	-								
32	NL 1	-	-	-							
33	NL 2	-	-	**	-						
34	PL 2	-	-	*	-	-					
35	RUM 2	-	-	-	-	-	-				
36	S 1	-	-	-	-	-	*	-			
37	S 2	-	-	-	-	-	-	-	-		
38	S 4	-	-	-	-	*	*	-	-	-	
39	S 5	-	-	-	-	-	*	-	-	-	*

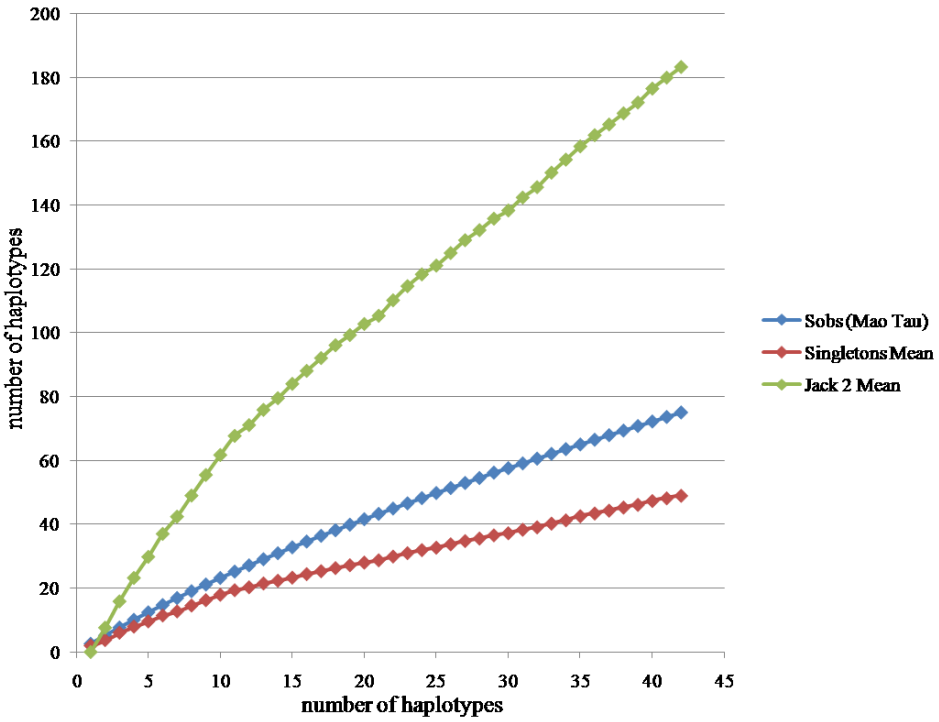

Figure 34: Sample based rarefaction analysis of haplotypes of the *COI* nucleotide of *Platynothrus peltifer*. Jack 2 Mean, second-order Jackknife richness estimator, Singletons, numbers of haplotypes each present in only one sample, Sob (Mao Tau) is the observed haplotype richness.

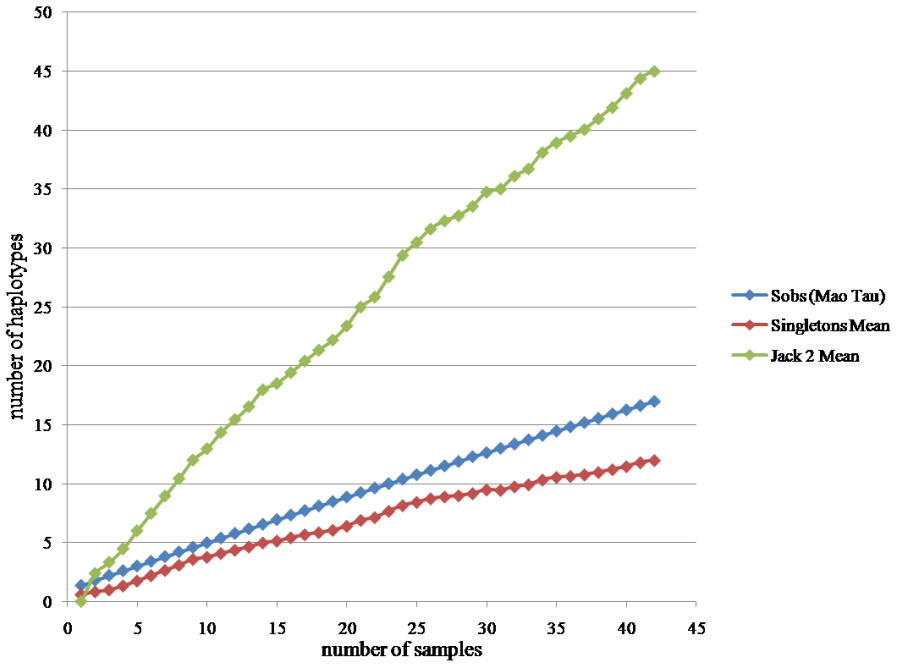

Figure 35: Sample based rarefaction analysis of haplotypes of the *COI* protein of *Platynothrus peltifer*. Jack 2 Mean, second-order Jackknife richness estimator, Singletons, numbers of haplotypes each present in only one sample, Sob (Mao Tau) is the observed haplotype richness.

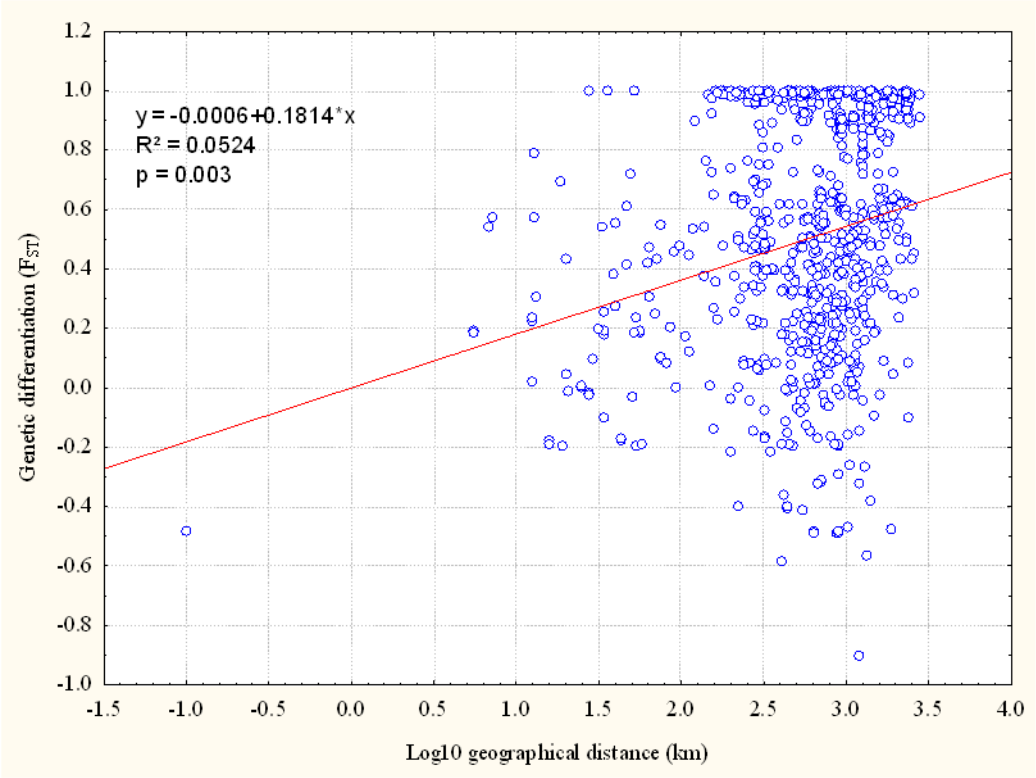

Figure 36: Linear regression of log10 geographical distances (kilometers) vs. genetic differentiations (F_{ST}) based on *COI* nucleotide sequences of *Platynothrus peltifer*. The regression is not significant using 1000 randomizations.

4.3.3 Achipteria coleoptrata

A total of 143 individuals (141 individuals of *A. coleoptrata*, one individual of *C. femoralis* and one individual of *C. marginatus* as outgroup) from 40 locations in 15 countries were sequenced. The sequences contained 25.2% A, 17.5% C, 16.4% G and 40.9% T. The 141 *COI* sequences consisted of 93 haplotypes for the nucleotide (66%) (Fig. 37) with 219 variable sites (43.6%) and 197 parsimony informative sites (Table A31). The protein consisted of 39 haplotypes (27.7%) in the network (Fig. 38). Haplotype diversity (H_d) was very high with 0.98 in the nucleotide.

Haplotype networks

The nucleotide haplotype network formed 14 subclades and five isolated single individual haplotypes which were separated each by 21 to over 100 substitution steps to the next subclade (Fig. 37). Six subclades were Southern subclades [one Greek (subclade 4a), two Romanian (subclade 1and 5c) and three Italian (subclade 4b-c and haplotype I_3.1)], absolutely. Subclades 4a-c comprised individuals from one location each, [GR_1.1-6 (4a), I_1.1-8 (4c) and I_2.1-4 (4b)]. Subclades 1 and 5c comprised individuals from two Romanian locations [RUM_1/RUM_2 (subclade 5c) and RUM_5/RUM_6 (subclade 1)]. One East-Russian subclade (subclade 3b) and five Central subclades [subclade 2, subclade 5g, seven alpine subclades (subclade 3a, subclade 5a-b, d and three isolated individuals D_12.2, A_7.2, D_15.1)] existed in the network in addition to the six Southern subclades. Five single individual haplotypes each from one location [one Austrian (A_7.1), one Italian (I_3.1), one Polish (PL_1.2) and two German (D_12.2, D_15.1)] were present in the network. Subclades 5a-b comprised two haplotypes [5a comprised two single individual haplotypes from the one German location (D_15.2 and D_15.3), 5b comprised two single individual haplotypes from another German location (D_14.1 and D_14.2)].

Subclade 3a-b comprised two haplotypes with three individuals [subclade 3a comprised individuals from three Austrian locations (A_3.1, A_5.3, A_8.3), subclade 3b comprised three individuals from one Russian location (RUS_2.1-3)]. Subclades 4a-c and 5e comprised three haplotypes [subclade 4a: two single individual haplotypes and one shared by four individuals from one Greek location (GR_1.1, GR_1.5 and GR_1.2-4/1.6), subclade 4b: two single individual haplotypes and one haplotype shared by individuals from one Italian location (I_2.1, I_2.2 and I_2.3-4), subclade 4c: a single individual haplotype, one haplotype shared by two individuals and one haplotype shared by five individuals from on Italian location (I_1.6, I_1.3/1.7, I_1.1-2/1.4-5/1.8), subclade 5e: two single individual haplotypes from one location in Poland (PL_2.7-2.8) and one haplotype shared by two Finish individuals (FIN_1.1, FIN_1.4)]. Subclade 5c comprised four haplotypes from two Romanian locations (RUM_1, RUM_2) [one haplotype shared by two individuals from both locations (RUM_1.4 and RUM_2.3), one haplotype with two individuals from one location (RUM_1.1-2), one shared by four individuals from two locations (RUM_1.3, RUM_2.1-2, RUM_2.4)].

Two subclades existed in the network with five haplotypes [subclade 1 comprised five single individual haplotypes from two Romanian locations (RUM_5.1-3, RUM_6.1-2), subclade 2 comprised four single individual haplotypes from three locations {two German locations (D_1.1, D_1.3 and D_5.3), one Polish location (PL_2.1)} and one haplotype shared by five individuals from two German locations (D_1.2/D_1.4/D_5.1-2/D_5.4)]. Subclade 5f comprised ten haplotypes from six locations [single individual haplotypes: one English (GB_1.1), three Estonian (EST_1.1, EST_1.2, EST_1.3), three Polish (Pl_1.4, PL_2.4, PL_2.6); haplotypes of more than one individual: two haplotypes of two individuals each from one location (one German D_11.1-2, one Polish (PL_1.1, PL1.3), one haplotype of six individuals from three locations, three individuals from one Polish location (PL_2.2-3, PL_2.5), two individuals from the one Finish location (FIN_1.2-3), one individual from an Estonian location (EST_1.4)]. Alpine subclade 5d comprised 16 haplotypes from eight locations [two German (D_12 and D_13), two Swiss (CH_1 and CH_3), four Austrian (A_5, A_6, A_7 and A_8)]. One haplotype

comprised two individuals from two locations [one Austrian (A_8.2), one Swiss (CH_1.3)] and the other 15 haplotypes were single individual haplotypes from seven locations [four from one Swiss location (CH_1.1, CH_1.2, CH_1.4, CH_1.5), two from another Swiss location (CH_3.1, CH_3.2), three two German locations (D_12.1, D_12.3, D_14.1), six from four Austrian locations (A_5.1, A_5.2, A_5.4, A_6.1, A_7.1 and A_8.1)]. Subclade 5g was the largest subclade and comprised 21 single individual haplotypes from twelve locations [one Danish (DK_3.2, DK_3.3, DK_3.4), one English (GB_1.2, GB_1.4), one Scottish (GB_2.2), one Swedish (S_2.2), three French (F_1.1, F_1.2, F_2.3, F_2.4, F_2.5, F_2.6, F_4.1), five German (D_2.1, D_3.2, D_3.3, D_8.3, D_9.2, D_10.2, D_10.5)], five haplotypes shared by two individuals each from one location [two French (F_2.1-2, F_4.2-3), three German (D_3.1/3.4, D_8.4/8.6, D_10.1/10.3)], one haplotype shared by three individuals from two locations [one Danish (DK_2.1), one Scottish (GB_2.1/2.3)] and one haplotype of 14 individuals from seven locations [one Danish (DK_3.1), one English (GB_1.3), one Swedish (S_2.1/2.3-6), four German (D_2.2, D_8.1-2/8.5, D_9.1/9.3, D_10.4)].

The protein haplotype network comprised 39 haplotypes with three major starlike arrangements (Fig. 38). The network comprised 27 single individual haplotypes from 21 locations [one Danish (DK_3.2, DK_3.4), one English (GB_1.2), one French (F_2.4), one Greek (GR_1.1), one Italian (I_2.1, I_2.2), one Russian (RUS_2.3), one Swedish (S_2.2), one Swiss (CH_3.2), two Austrian (A_3.1,A_5.1), two Polish (PL_1.2, PL_2.6), two Romanian (RUM_1.4, RUM_5.1, RUM_5.2), seven German locations (D_1.3, D_2.1, D_5.3, D_10.5, D_13.1, D_14.1, D_14.2, D_15.1, D_15.2, D_15.3)], five haplotypes of two individuals from five locations [one French (F_1.1-2), one German (D_8.4/8.6), one Polish (PL_1.1/1.3), one Romanian (RUM_1.1-2), one Russian (RUS_2.1-2)] and two haplotypes of three individuals from two locations [one from two Italian locations (I_2.3-4/I_3.1), one from two Romanian locations (RUM_5.3/RUM_6.1-2)].

Phylogenetic and population genetic analyses

All phylogenetic analyses (NJ without and with model of sequence evolution, MrBayes and Beast) showed a highly supported monophyletic group of *A. coleoptrata* with 14 subclades and five isolated individuals (four in MrBayes) in the nucleotide (Fig. 39, A42-45). The 14 subclades were supported by high bootstrap values and posterior probabilities. Only the arrangement of the 14 subclades was variable in the analyses. The individuals which built these clades are presented in Table 24. In the phylogenetic trees of the protein all sequences are grouped in one highly supported monophyletic clade (Fig. 40, A46-48).

The minimum and maximum mean average pairwise differences for the nucleotide sequences between populations were 0.2% (D_8/S_2) and 19.4% (D_14/RUS_2). Excluding Russia the maximum mean average pairwise differences was 18.7% (D_5/RUM_1 and D_5/RUM_2). Within populations the minimum and maximum mean average pairwise differences were 0% (D_11) and 12% (A_8) (Table 25). For the protein the minimum and maximum mean average pairwise differences between populations were 0% (all populations except for A_5, CH_3, D_2, D_5, D_8, D_10, D_14, D_15, DK_3, GB_1, I_2, PL_1, RUM_1, RUM_2, RUM_5 and RUS_2) and 2.6% (D_2/D_15). The minimum and maximum mean average pairwise differences within population were 0% (A_7, A_8, CH_1, D_1, D_3, D_9, D_11, D_12, EST_1, F_1, F_2, F_4, FIN_1, GB_2, GR_1, I_1, PL_2, RUM_6 and S_2) and 2.4% for the protein (D_15) (Table A32).

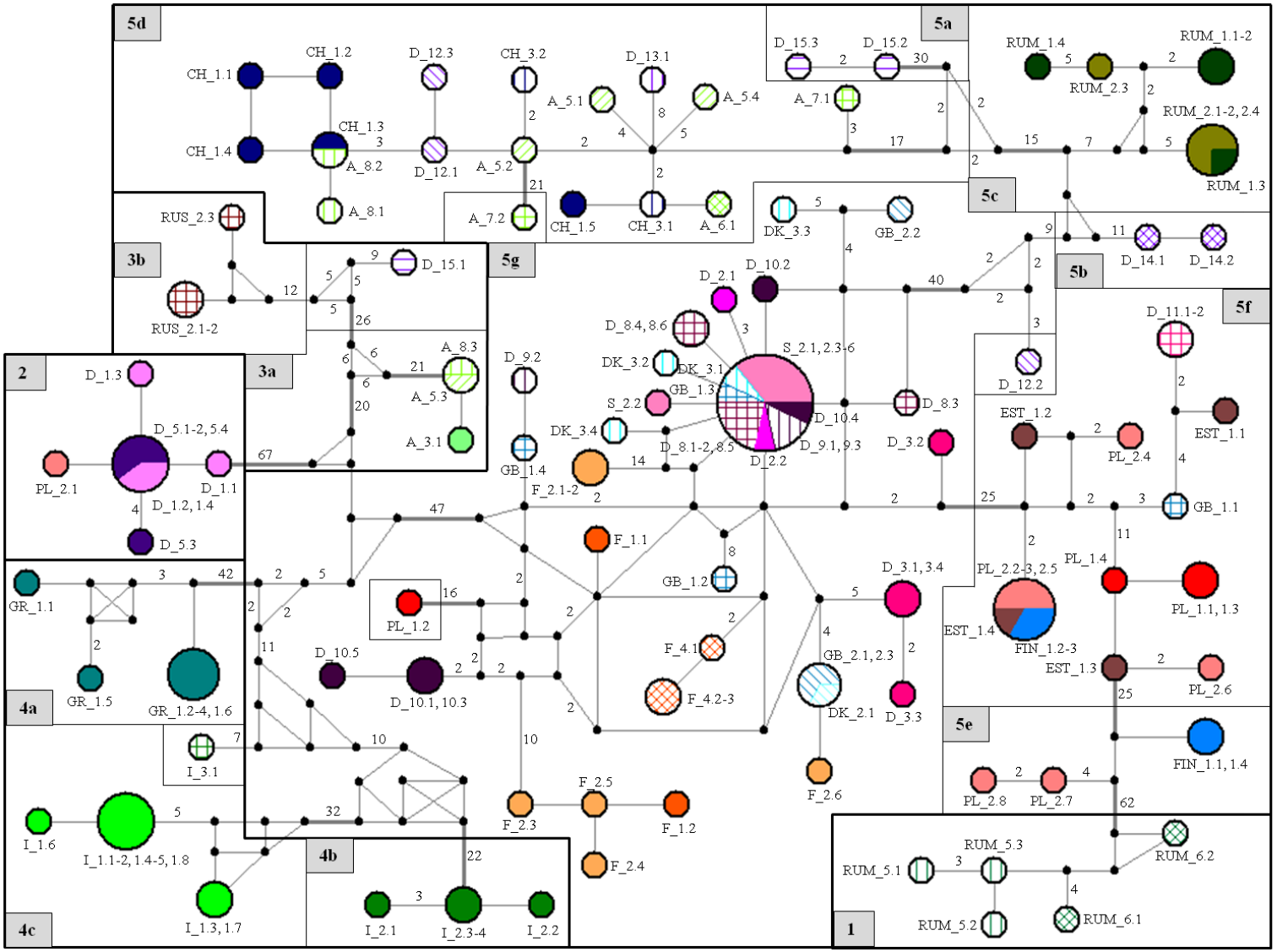

Figure 37: Median-joining haplotype network for the *COI* nucleotide of 93 haplotypes from *Achipteria coleoptrata*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one substitution step between haplotypes). Major subclades are marked by boxes.

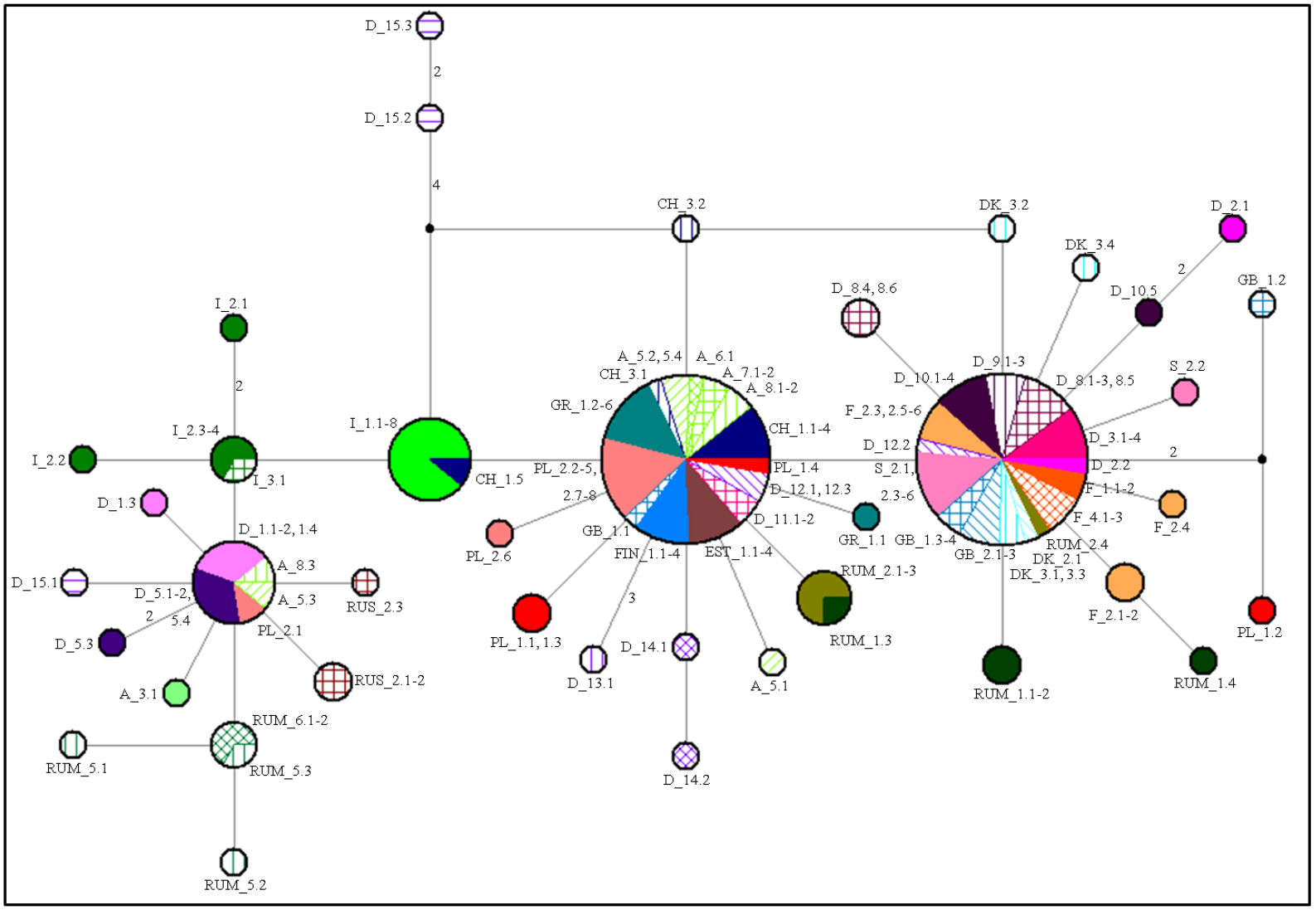

Figure 38: Median-joining haplotype network for the *COI* protein of 39 haplotypes from *Achipteria coleoptrata*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one amino acid change between haplotypes).

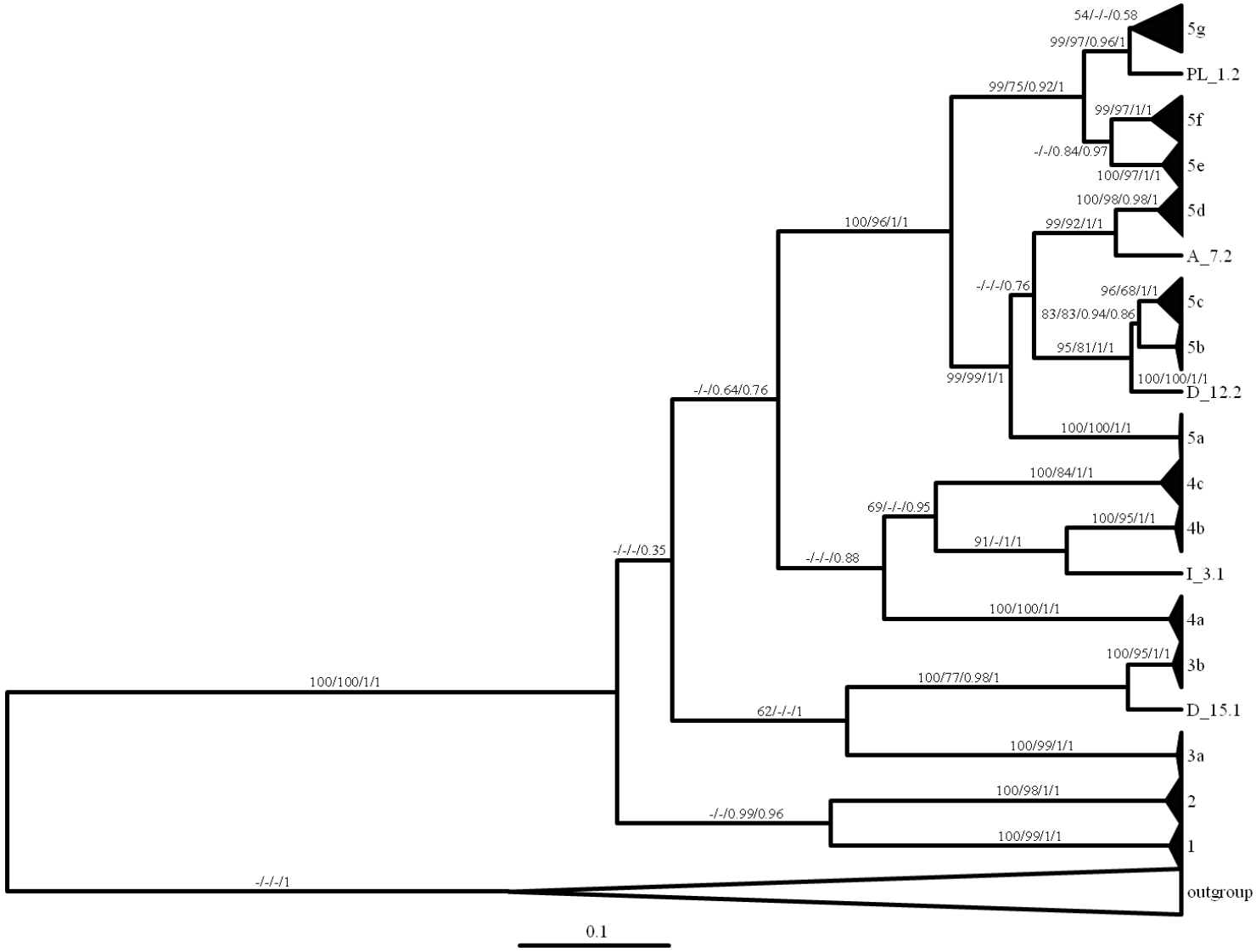

Figure 39: Bayesian phylogeny after 10 x 10^6 generations from the 141 *COI* nucleotide sequences of *Achipteria coleoptrata* with Beast v1.5.4. Outgroups are one individual of *Carabodes femoralis* and *C. marginatus*, respectively. Numbers on the branches are bootstrap values from NJ without and with evolution model (GTR+I+G) analysis and posterior probabilities from MrBayes and Beast. Tip numbers are the different subclades and explained in Table 24.

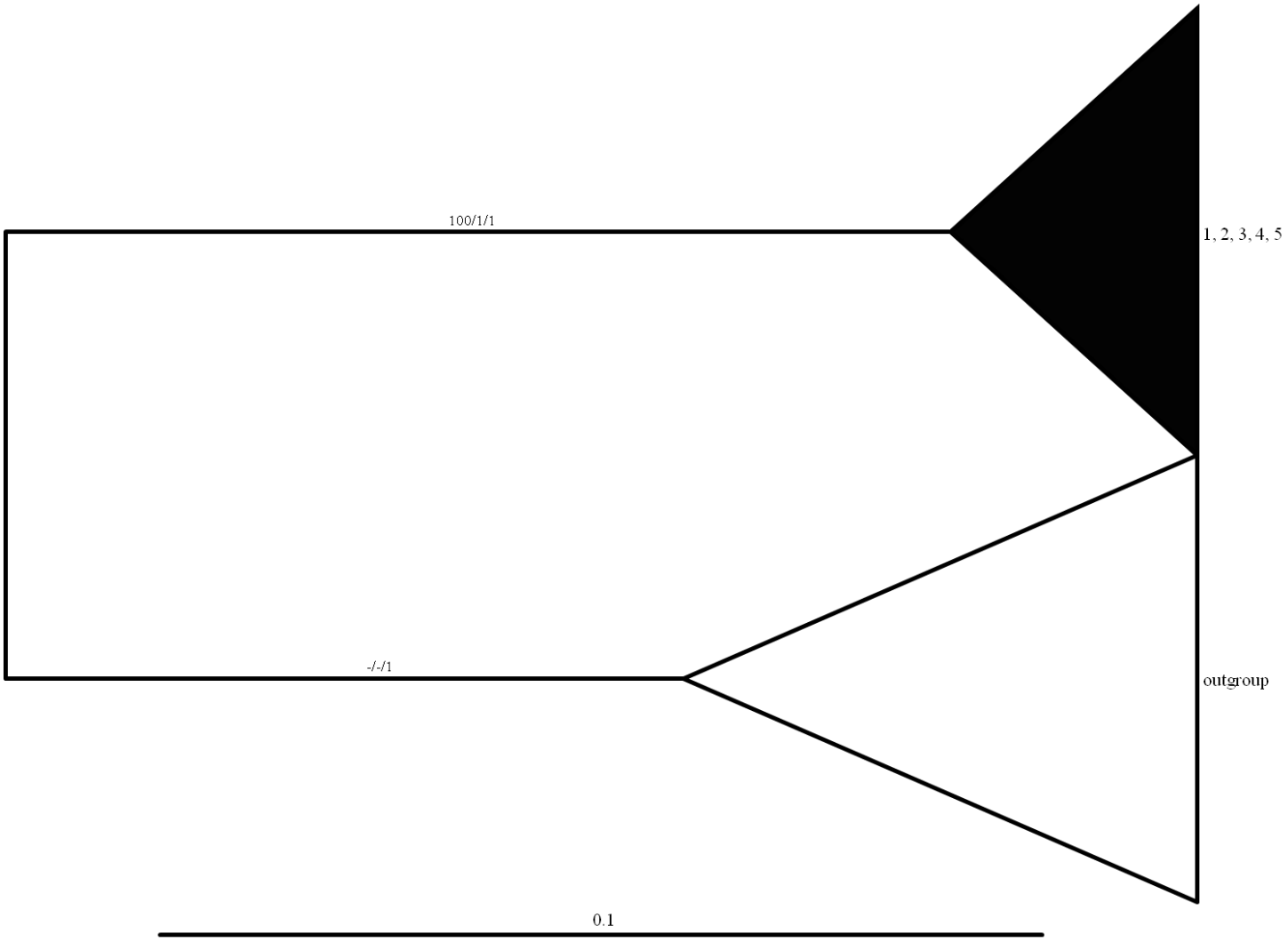

Figure 40: Bayesian phylogeny after $10x10^6$ generations from the 141 *COI* protein sequences of *Achipteria coleoptrata* with Beast v1.5.4. Outgroups are one individual of *Carabodes femoralis* and *C. marginatus*, respectively. Numbers on the branches are bootstrap values from NJ and posterior probabilities from MrBayes and Beast. Tip numbers are the different subclades and explained in Table 24.

Table 24: Subclades of Bayesian phylogenetic trees of *Achipteria coleoptrata* based on *COI* nucleotide sequences (#ind=number or individuals, pp=posterior probabilities, sampling sites with abbreviations, individuals and ind. pop.=quantity of individuals from the population).

Subclade	# ind	pp	sampling sites		individuals	ind. pop.
1	**5**	**1**	Busteni	RUM_5	1-3	3/3
			Sinaia	RUM_6	1-2	2/2
2	**9**	**1**	Kranichstein	D_1	1-4	4/4
			Moerfelden	D_5	1-4	4/4
			Warsaw	PL_2	1	1/8
3	**7**	1	**5**			
3a	**3**	**1**	Hittisau	A_3	1	1/1
			Memminger Huette	A_5	3	1/4
			Imst	A_8	3	1/3
isol. ind.	**1**		Langenwang	D_15	1	1/3
3b	**3**	**1**	Novosibirsk	RUS_2	1-3	3/3
4	**19**	**0.88**	**4**			
4a	**6**	**1**	Thessaloniki	GR_1	1-6	6/6
isol. ind.	**1**		Felitto	I_3	1	1/1
4b	**4**	**1**	Parma	I_2	1-4	4/4
4c	**8**	**1**	Grosseto	I_1	1-8	8/8
5	**101**	**1**	**30**			
5a	**2**	**1**	Langenwang	D_15	2-3	2/3
isol. ind.	**1**		Sonthofen	D_12	2	1/3
5b	**2**	**1**	Steineberg	D_14	1-2	2/2
5c	**8**	**1**	Sibiu_1	RUM_1	1-4	4/4
			Sibiu_2	RUM_2	1-4	4/4
isol. ind.	**1**		Roggendorf	A_7	2	1/2
5d	**17**	**1**	Memminger Huette	A_5	1-2, 4	3/4
			Landeck	A_6	1	1/1
			Roggendorf	A_7	1	1/2
			Imst	A_8	1-2	2/3
			Basel	CH_1	1-5	5/5
			Rorschach	CH_3	1-2	2/2
			Sonthofen	D_12	1, 3	2/3
			Gunzesried	D_13	1	1/1
5e	**4**	**1**	Lahti	FIN_1	1, 4	2/4
			Warsaw	PL_2	7-8	2/8
5f	**17**	**1**	Wittmoor	D_11	1-2	2/2
			Tallin	EST_1	1-4	4/4
			Lahti	FIN_1	2-3	2/4
			Ascot	GB_1	1	1/4
			Krakow	PL_1	1, 3-4	3/4
			Warsaw	PL_2	2-6	5/8
isol. ind.	1		Krakow	PL_1	2	1/4

Table 24 continue:

Subclade	# ind	pp	sampling sites		individuals	ind. pop.
5g	**48**	**0.58**	Goettingen	D_2	1-2	2/2
			Lake Constance	D_3	1-4	4/4
			Cuxhaven	D_8	1-6	6/6
			Bonn	D_9	1-3	3/3
			Colonge	D_10	1-5	5/5
			Hjørring	DK_2	1	1/1
			Arhus_Morvadsvej	DK_3	1-4	4/4
			Mont Blanc	F_1	1-2	2/2
			Loire	F_2	1-6	6/6
			Haute Loire	F_4	1-3	3/3
			Ascot	GB_1	2-4	3/4
			Braemar	GB_2	1-3	3/3
			Stroemstad	S_2	1-6	6/6

Table 25: Mean percentage pairwise differences of uncorrected p-distances of the *COI* nucleotide sequences of *Achipteria coleoptrata* from 35 locations. The diagonal is the within population differences (bold) and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Bold letters in pink are the maximum differences, if Russia is excluded. Locations with less than two individuals were excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	A 5	**9.7**																											
2	A 7	6.8	**4.9**																										
3	A 8	8.2	8.1	**12**																									
4	CH 1	5.6	3.4	6.2	**0.8**																								
5	CH 3	5.4	3	6.8	1.3	**1.2**																							
6	D 1	17.8	17.4	17.4	17.4	18	**0.2**																						
7	D 2	13	11.8	13.1	12.2	12.4	17.1	**0.6**																					
8	D 3	13.2	11.8	13.1	12.4	12.5	17.1	1.7	**1.2**																				
9	D 5	17.9	17.5	17.5	17.5	18.1	0.3	17.2	17.1	**0.4**																			
10	D 8	12.7	11.5	12.8	11.9	12.1	16.9	0.4	1.5	17	**0.2**																		
11	D 9	12.8	11.6	12.8	12.1	12.3	16.9	0.7	1.6	17	0.5	**0.8**																	
12	D 10	13	11.6	13	12.2	12.5	17	1.6	2.3	17.2	1.5	1.5	**1.5**																
13	D 11	12.4	10.7	13	11.6	11.3	17.2	6.4	6.6	17.3	6.1	6.1	6.4	**0**															
14	D 12	7.1	4.8	8.1	3.5	3.5	17.1	11	11.3	17.2	10.7	11	11.2	10.9	**5.5**														
15	D 14	11.5	9	12.4	9	8.9	18.6	12.5	12.8	18.6	12.2	12.3	12.8	11.8	7.7	**0.2**													
16	D 15	13.1	12	13.3	11.9	12	17.5	14.4	14.9	17.7	14.1	14.2	14.7	13	12.1	13.3	**11**												
17	DK 3	12.9	11.8	12.9	12	12.3	16.8	0.8	1.8	16.9	0.6	0.8	1.7	6.4	10.9	12.3	14.3	**0.9**											
18	EST 1	12.9	11	13.3	11.9	11.7	17.6	6.5	6.6	17.7	6.2	6.2	6.4	2	11.2	12	13.6	6.4	**2.4**										
19	F 1	13.6	12.2	13.5	12.9	13.1	17.4	3.2	3.7	17.5	3	3	2.9	7	11.7	13	15	3	7.3	**3.9**									
20	F 2	13.3	12.1	13.3	12.4	12.7	17.6	3.3	3.9	17.8	3.1	3.2	3.1	7.5	11.4	12.9	15	3.2	7.5	3.1	**3.4**								
21	F 4	12.9	11.5	13	11.9	12.2	16.2	0.9	1.9	16.4	0.8	1	1.7	5.8	10.7	12	14.1	1.1	5.9	2.9	3.2	**0.1**							
22	FIN 1	12.4	10.5	12.6	11.1	11.1	16.5	6.3	6.5	16.7	11.9	11.9	6.3	3.9	10.7	12	13.4	6.3	3.6	7.4	7.3	5.8	**3.9**						
23	GB 1	13.1	11.7	13.2	12.4	12.5	17.3	2.6	3.2	17.4	2.5	2.4	3.2	5	11.3	12.5	14.2	2.7	5.2	4.2	4.6	2.7	5.6	**4.2**					
24	GB 2	12.6	11.3	12.5	11.8	12	16.8	1.5	2.1	16.9	1.4	1.5	2.3	5.9	10.9	12.7	14.2	1.5	5.9	3.6	3.6	1.8	5.8	3	**1.7**				
25	GR 1	15.2	15.2	14.8	14.6	15.1	16	14.3	14.3	16.1	14	14	13.9	13.4	14.7	16.3	15.9	13.9	13.5	14.4	14.4	13.9	13.3	13.9	13.9	**0.7**			
26	I 1	16.5	16.4	16	16.5	17.1	17.1	15.6	15.9	17.2	15.3	15.1	15.1	14	17.1	17.8	16.6	15.3	14.2	15.8	15.7	15	14.4	15.3	15.5	12.8	**0.6**		
27	I 2	16.4	16.3	15.8	16.4	16.6	16	15.5	15.3	16.1	15.3	15	15.2	14.3	16.5	16.7	16.2	15.1	14.5	15.4	15.8	15.6	15	15.1	14.7	13.8	11.7	**0.4**	
28	PL 1	13.3	11.6	13.5	12.3	12.4	18	6.1	6.4	18.1	5.9	5.9	5.8	4.2	11.4	12.7	14.3	6.1	3.8	6.8	7	5.6	5.1	5.6	5.8	14.1	14.4	15.2	**4.1**
29	PL 2	13.4	11.7	13.6	12.4	12.4	15.1	8	8.1	15.3	7.7	7.7	7.9	5.4	11.9	12.8	14.3	7.9	5	8.8	8.9	7.5	5.2	7.2	7.5	13.9	14.7	15	6.4
30	RUM 1	11.8	9.3	12.7	9.8	9.6	18.6	11.1	11.4	**18.7**	10.8	11.1	11.4	11.4	8.2	5.2	13.5	10.9	11.7	11.8	11.7	11	11.7	11.4	11.2	16.5	18.4	17.9	12.6
31	RUM 2	11.5	9.1	12.4	9.4	9.4	18.6	10.8	11.2	**18.7**	10.6	10.8	11.1	10.9	8	5.1	13.4	10.7	11.2	11.6	11.6	10.7	11.3	11.1	10.9	16.3	18.2	17.8	12.2
32	RUM 5	16.3	16	15.9	15.8	16.1	14.9	15.9	16	15	15.7	15.6	15.1	14.2	15.7	16.8	17.1	15.7	14.8	16.1	16.2	15.4	14.2	15.6	15.6	16.9	15	16.1	15.3
33	RUM 6	16.1	15.7	15.9	15.8	15.8	14.5	15.4	15.5	14.6	15.2	15.2	14.7	13.9	15.6	16.5	17	15.3	14.4	15.7	15.8	15	14	15.2	15.4	16.6	14.9	15.7	14.7
34	RUS 2	16.8	17.4	16.2	14.4	17.6	18.6	17.1	17.7	18.7	16.9	16.8	17.2	16.6	17.3	**19.4**	14	16.9	17	17.4	17.7	16.8	16.9	16.9	17.1	16.3	16.5	17.7	17.4
35	S 2	12.8	11.6	12.8	11.9	12.1	16.8	0.3	1.4	16.9	**0.2**	0.4	1.4	6.1	10.8	12.2	14.1	0.5	6.2	3	3.1	0.7	6	2.4	1.3	14	15.3	15.3	5.8

Table 25 continue:

Population		29	30	31	32	33	34	35
29	**PL 2**	**7.4**						
30	**RUM 1**	12.6	**1.6**					
31	**RUM 2**	12.2	1.5	**0.9**				
32	**RUM 5**	14.7	17.9	17.4	**0.5**			
33	**RUM 6**	14.3	17.5	17.1	1	**1.2**		
34	**RUS 2**	17.5	18.8	18.6	16.1	16.4	**0.4**	
35	**S 2**	7.7	10.8	10.6	15.5	15.1	16.8	**0.1**

The results of the AMOVA showed that the nucleotide variation among countries (24.5%), among samples within countries (55.4%) and the variation within samples (20.1%) were significant and high (Table 26).

Table 26: AMOVA table on variations among countries and within populations in nucleotide (black) and protein (red) sequences of *COI* of *Achipteria coleoptrata*. Each population was considered as separate groups. Populations with less than two individuals were excluded from the analysis. Significance level is $p<0.05$ (d.f.: degree of freedom).

Source of variance	d.f.	sum of squares	variance components	percent of total variation	fixation indices
among countries	12 / 12	1891.5 / 4.5	7.71 Va* / -0.04 Va	24.51 / -13.31	FCT: 0.26* / -0.13
among populations	20 / 20	1337.35 / 11.75	17.43 Vb* / 0.11 Vb*	55.41 / 41.22	FSC: 0.73* / 0.36*
within population	96 / 96	606.18 / 18.88	6.31 Vc* / 0.2 Vc*	20.08 / 72.09	FST: 0.8* / 0.28*
total	128 / 128	3835.03 / 35.13	31.45 / 0.27		

The neutrality tests of Tajima's D and Fu's FS were neither significant for the nucleotide nor for the protein in *A. coleoptrata* (Table A33 and A34). The results of the McDonald-Kreitman test showed that the differences between 58 of 595 (9.8%) populations were significant, between other 41 of 595 (6.9%) were high significant and between 37 of 595 (6.2%) other populations were highly significant (Table 27 and for detail Table A35). All computed Neutrality indices were ≥0 in the *COI* nucleotide sequences of *A. coleoptrata* (Table A36).

None of the calculated rarefaction curves of the nucleotide and the protein reached saturation (Fig. 41 and 42). The Jackknife rarefaction curves ended at 245 haplotypes for the nucleotide and 97 haplotypes for the protein. The unique rarefaction curves ended at 69 haplotypes for the nucleotide and 27 haplotypes for the protein. The observed rarefaction curves (Sobs Mao Tau) ended at 91 haplotypes for the nucleotide and 39 haplotypes for the protein.

The results of the Mantel test indicate isolation by distance in *A. coleoptrata* [R^2=0.0713, p=0.004 (Fig. A49) and R^2=0.109, p=0.001 for log transformed geographic distances (Fig. 43) using 1000 randomizations], but this explained only 10% of the variation in the data.

The gmyc model had a better fit but not a significant better fit in the single and the multiple analyses than the null model (Table 18). For the single analysis the model identified five distinct *COI* clusters and for the multiple analysis 30. The clusters of the single analysis were identical with the phylogenetic main clusters, but differed in their grouping (Fig. A50). The clades 4a-c and clades 5a-g were grouped to one cluster, respectively.

The clusters of the multiple analysis differed from the clusters which were delimited by the phylogenetic analyses (Fig. A50). Phylogenetic clade 1 was not identified in the gmyc model, clade 2

was split into two distinct clades and the clade 3a included the isolated individual D_15.1. In clade 3b individual A_3.1 was excluded. Clade 4a shrunk to four individuals (GR_1.2-4 and GR_1.6), clade 4b to two individuals (I_2.2-3) and clade 4c was split into three distinct clades with two individuals (I_1.1 and I_1.6, I_1.3 and I_1.7, I_1.5 and I_1.8), respectively. The clade 5b was not identified by the gmyc model, clade 5a was identically in the phylogenetic and the gmyc analysis. The clades 5c-g were split in multiple distinct clusters, (clade 5g had nine, clade 5d four, clade 5f three and clades 5c and 5e two distinct clusters).

Table 27: Results of the McDonald-Kreitman test of *Achipteria coleoptrata*. The differences between 58 populations were significant (*0.01<P<0.05), between other 41 populations high significant (**0.001<P<0.01) and between 37 populations highly significant (***P<0.001). Locations with less than two individuals were excluded.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	A 5																																		
2	A 7	-																																	
3	A 8	-	-																																
4	CH 1	-	-	-																															
5	CH 3	-	-	-	-																														
6	D 1	-	-	-	-	-																													
7	D 2	-	-	-	**	***	***																												
8	D 3	-	-	-	-	-	-	-																											
9	D 5	-	-	-	*	*	-	***	-																										
10	D 8	-	-	-	-	*	*	-	-	-																									
11	D 9	-	-	-	-	-	-	-	-	-	-																								
12	D 10	-	-	-	-	-	-	-	-	-	-	-																							
13	D 11	-	-	-	-	-	-	***	-	*	-	-	-																						
14	D 12	-	-	-	-	-	-	-	-	-	*	-	-	-																					
15	D 14	-	-	-	-	*	**	***	-	**	-	-	-	*	-																				
16	D 15	-	-	-	-	-	**	-	-	**	-	-	-	-	-	-																				
17	DK 3	-	-	-	*	-	*	-	-	*	-	-	-	-	-	*	-																		
18	EST 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																	
19	F 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																
20	F 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-															
21	F 4	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-														
22	FIN 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
23	GB 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-												
24	GB 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-											
25	GR 1	-	-	-	*	*	-	**	-	*	-	-	-	-	-	*	-	*	-	-	-	-	-	*	-										
26	I 1	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-									
27	I 2	-	-	-	**	**	***	***	-	***	***	*	*	***	-	***	**	**	-	-	-	**	-	*	-	**	*								
28	PL 1	-	-	-	*	*	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	*							
29	PL 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
30	RUM 1	-	-	-	**	***	**	***	**	***	***	**	**	***	-	*	-	***	*	*	*	***	-	*	**	***	**	***	**	-					
31	RUM 2	-	-	-	*	*	*	***	-	**	**	*	*	*	-	-	-	**	-	-	-	*	-	-	-	**	-	***	*	-	-				
32	RUM 5	-	-	-	*	**	***	***	-	***	**	-	-	*	-	**	*	**	-	-	-	*	-	-	-	*	-	***	*	-	***	**			
33	RUM 6	-	-	-	-	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-	*	-	-		
34	RUS 2	-	-	-	*	*	***	***	-	***	**	-	-	**	-	**	-	**	-	-	-	*	-	-	-	*	-	***	-	-	***	**	***	*	
35	S 2	-	-	-	-	*	**	-	-	**	-	-	-	-	-	**	-	-	-	-	-	-	-	-	-	*	-	***	-	-	***	**	**	-	***

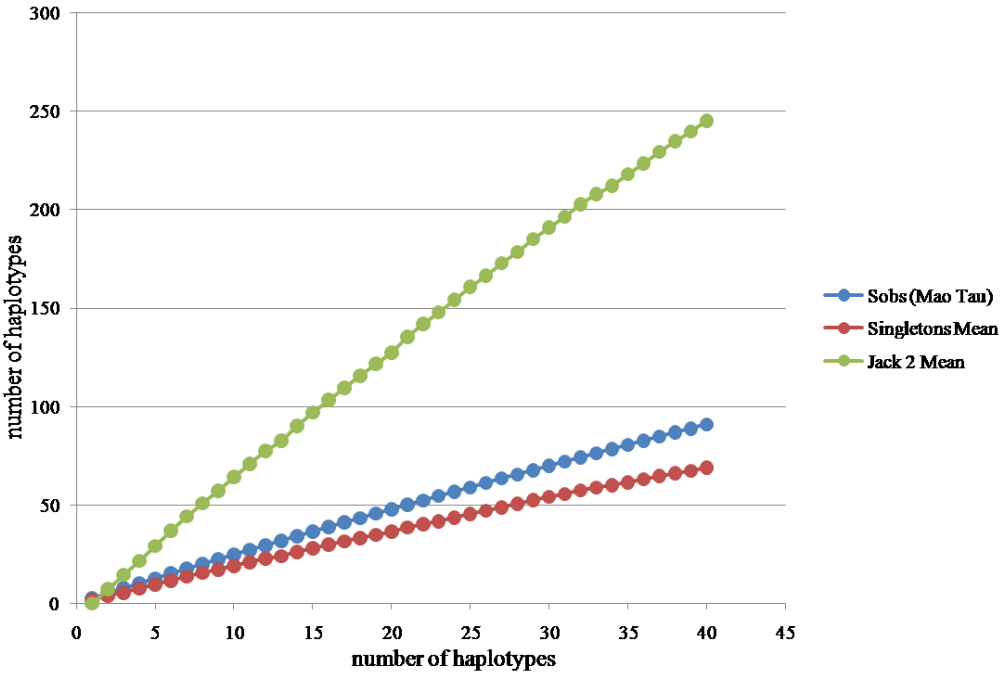

Figure 41: Sample based rarefaction analysis of haplotypes of the *COI* nucleotide of *Achipteria coleoptrata*. Jack 2 Mean, second-order Jackknife richness estimator, Singletons, numbers of haplotypes each present in only one sample, Sob (Mao Tau) is the observed haplotype richness.

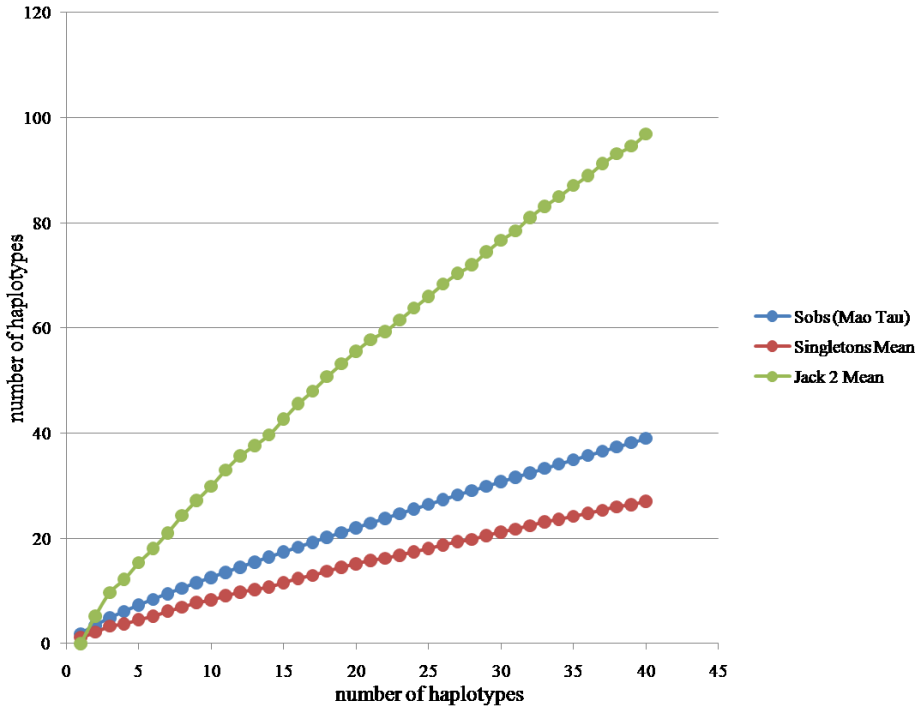

Figure 42: Sample based rarefaction analysis of haplotypes of the *COI* protein of *Achipteria coleoptrata*. Jack 2 Mean, second-order Jackknife richness estimator, Singletons, numbers of haplotypes each present in only one sample, Sob (Mao Tau) is the observed haplotype richness.

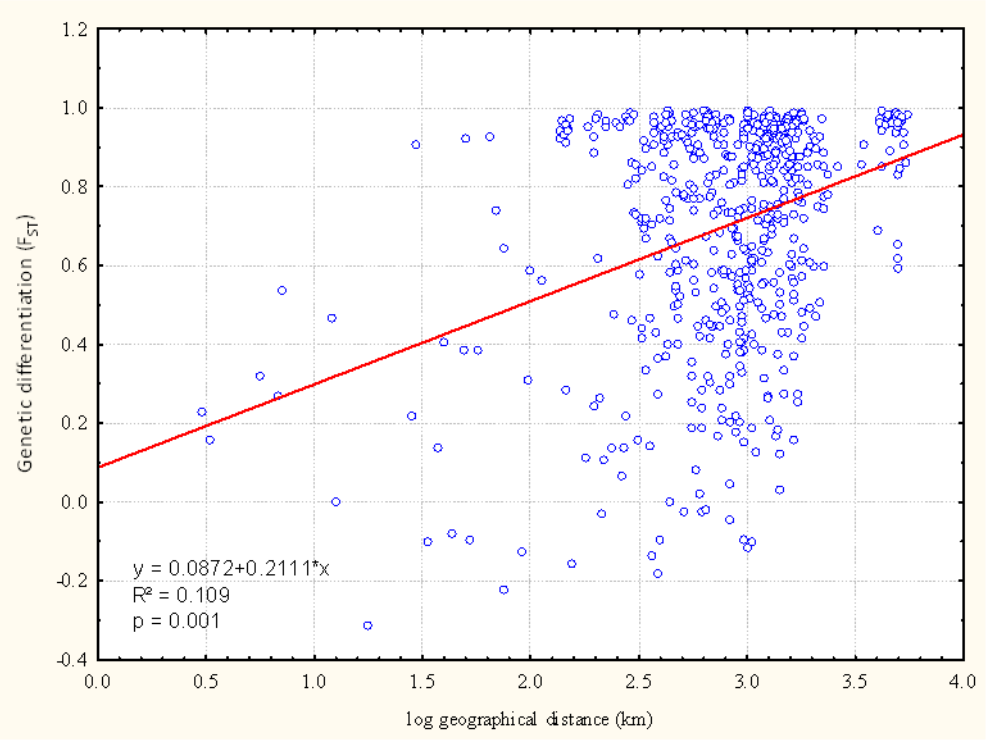

Figure 43: Linear regression of log10 geographical distances (kilometers) vs. genetic differentiations (F_{ST}) based on *COI* of *Achipteria coleoptrata*. Regression is significant using 1000 randomizations.

4.3.4 Steganacarus magnus

A total of 159 individuals (157 individuals of *S. magnus*, one individual of *R. duplicata* and one individual of *H. rufulus*) from 37 localities in 15 countries were sequenced. The sequences contained 27.5% A, 23.4% C, 17.1% G and 32% T. The 157 *COI* sequences comprised 96 haplotypes for the nucleotide (61.2%) (Fig. 44) with 314 variable sites (59.3%); 276 of these were parsimony informative sites (Table A37). The protein consisted of 59 haplotypes (37.6%) (Fig. 45). The haplotype diversity (H_d) was as high as in the *A. coleoptrata* with 0.98 in the nucleotide sequences.

Haplotype networks

The nucleotide haplotype network formed three main clades (black, blue dotted and red) with several subclades and isolated haplotypes. The main clades and subclades were separated each by large numbers of substitution steps (Fig. 44).

The black main clade formed eight subclades (subclade 1-8) with a total number of 87 individuals from 20 locations (D_1, D_2, D_3, D_5, D_8, D_9, DK_1, DK_3, F_2, F_3, GB_1, GB_2, I_1, I_2, NL_1, PL_2, RUM_1, RUM_2, RUM_5 and RUM_6) and comprised 49 haplotypes and two isolated single individual haplotypes from two locations (RUM_6 and S_2). Subclade 1 comprised three subclades 1a-c and one single individual haplotype from one German location (D_1.13). Subclade 1a comprised nine haplotypes from six locations [seven single individual haplotypes: two each from one Italian location (I_1.1, I_2.2), two from one French location (F_2.2, 2.4), three from two German locations (D_1.12, D_2.2, 2.8). Haplotypes of more than one individual: one of two individuals from on location in Germany (D_2.6-7), one of twelve individuals from six locations [two individuals from one French location (F_2.1, 2,3); four individuals from two Italian location (I_1.4, 1.9-10, I_2.4); six individuals from three German locations (D_1.1, D_2.1, 2.4-5, 2.9, D_9.1)]. Subclade 1b comprised six haplotypes from four locations [five single individual haplotypes: two each from one Italian location (I_1.2, I_2.1), three from two German locations (D_1.5, 1.14, D_9.2), one haplotype of six

individuals from two locations (D_1.2, I_1.3, 1.5-8)]. Subclade 1c comprised four haplotypes from six locations [three single individual haplotypes each from one location (D_1.7, DK_1.3, DK_3.3), one haplotype of 13 individuals from five locations {one from one Italian (I_2.3), two from one Danish (DK_3.1-2), ten from three German (D_1.3-4, 1.6, 1.8-11, D_2.3, D_9.3-4)}].
Subclade 2 comprised three single individual haplotypes from one German location (D_3.1-3). Subclade 3 comprised eight haplotypes from five locations [single individual haplotypes: one from one German (D_8.3), one from one Dutch (NL_1.2), one from one French (F_3.1), three from two British (GB_1.1-2, GB_2.1). Haplotypes of more than one individual: two of three individuals each of two locations (one of one German and one Dutch location (D_8.1-2, NL_1.3), one of one Scottish and one Dutch location (GB_2.2-3, NL_.11))]. Subclade 4 comprised three haplotypes from one French location [two single individual haplotypes (F_3.2, 3.4) and one haplotype of two individuals (F_3.3, 3.5)]. Subclade 5 comprised one haplotype of three individuals from one German location (D_5.1-3). Subclade 6 comprised six single individual haplotypes from two Romanian locations (RUM_5.1-4, RUM_6.1-2). Subclade 7 comprised two haplotypes from one Polish location [one single individual (PL_2.2), one haplotype of two (PL_2.1, 2.3)]. Subclade 8 comprised six haplotypes from two Romanian locations [single individual haplotypes: one from one Romanian (RUM_1.3), four from another Romanian (RUM_2.1-4); one haplotype of two from one Romanian (RUM_1.1-2)].
The blue dotted main clade formed three subclades (subclade 9-11) with a total number of 16 individuals from seven locations (CZ_1, D_4, D_6, DK_1, F_1, PL_1 and S_2) and comprised 13 haplotypes and one isolated haplotype of two individuals from one Norwegian location (N_1.1, 1.3). Subclade 9 comprised five single individual haplotypes from four locations [one Czech (CZ_1.4), one German (D_6.1), one Swedish (S_2.1), and two Polish (PL_1.1, 1.3)]. Subclade 10 comprised three haplotypes from two locations [two single individual each from one location (CZ_1.5, F_1.2), one of two individuals from one Czech location (CZ_1.1-2)]. Subclade 11 comprised five haplotypes from two locations [single individual haplotypes: two of one Danish location (DK_1.1-2), two of one German location (D_4.1, 4.5); one haplotype of three individuals from one German location (D_4.2, 4.6-7)].
The red main clade formed seven subclades (subclade 12-18) with a total number of 38 individuals from 13 locations (D_4, D_5, DK_2, F_1, F_4, FIN_1, PL_1, RUM_3, RUM_4, RUS_1, RUS_2, S_1 and S_2) and comprised 21 haplotypes, two isolated haplotypes of two individuals each from one location (A_1.1-2 and D_7.2, 7.4) and eight single individual haplotypes from seven locations (CZ_1.3, D_4.4, D_5.5, D_7.1, 7.3, F_1.3, N_1.2 and RUS_1.4).
Subclade 12 comprised two subclades 12a-b and one single individual from one Romanian location (RUM_3.3). Subclade 12a comprised three single individuals from one Romanian location (RUM_4.1-3). Subclade 12b comprised one haplotype of three individuals from one Romanian location (RUM_3.1-2, 3.4). Subclade 13 comprised three single individuals from one Russian location (RUS_1.1-3). Subclade 14 comprised one haplotype of four Russian individuals (RUS_2.1-4). Subclade 15 comprised one haplotype of four French individuals (F_1.1, 1.4-6). Subclade 16 comprised three individuals from two Scandinavian locations [two single individuals each from one location (FIN_1.3, S_1.1), one haplotype of two Finish individuals (FIN_1.1-2)]. Subclade 17 comprised four haplotypes from four locations [three single individuals from two locations (PL_1.4-5, S_2.3), one haplotype of eight individuals from two locations (D_4.3, 4.8-9, DK_2.1-5)]. Subclade 18 comprised four haplotypes from three locations [three single individuals from two locations (D_5.4, PL_1.2, 1.6), one haplotype of two from one French location (F_4.1-2)].

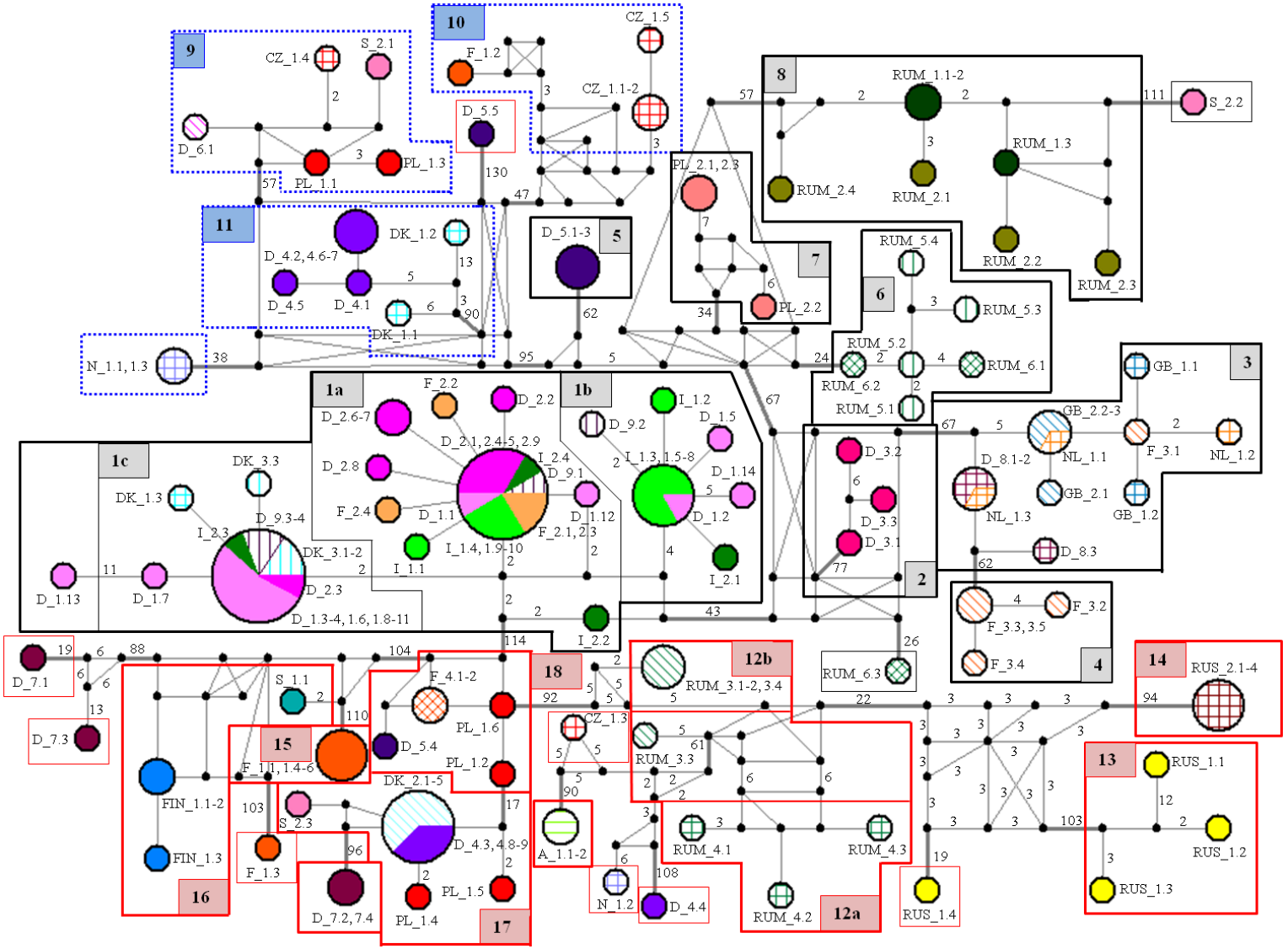

Figure 44: Median-joining haplotype network for the *COI* nucleotide of 96 haplotypes from *Steganacarus magnus*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one substitution step between haplotypes). Major subclades are marked by boxes.

The protein haplotype network consisted of 59 haplotypes and formed the three main clades (black, blue and red), which were separated by large numbers of amino acid changes (Fig. 45).
The black main clade comprised six subclades and two isolated individuals each from one location (RUM_6.3, S_2.2). Subclades 1a-c and the isolated individual (D_1.13) fused two one subclade of four haplotypes [three single individuals from two locations (D_1.13-14, I_1.3), one haplotype of 46 individuals from eight locations]. The three single individuals of subclade 2 fused to one haplotype. Subclades 3 and 4 fused and comprised eight haplotypes from five locations [single individuals: four each of one location (D_8.3, GB_1.1, GB_2.1, NL_1.2), two of one French location (F_3.2, 3.4); haplotypes with more than one individual: one of two French (F_3.3, 3.5), one of eight from five locations (D_8.1-2, F_3.1, GB_1.2, GB_2.2-3, NL_1.1, 1.3)]. Subclade 5 comprised one haplotype of three individuals from one German location (D_5.1-3). Subclades 6 and 7 fused and comprised four haplotypes from three locations [two single individuals each from one location (PL_2.2, RUM_5.1), one haplotype of two from one Polish location (PL_2.1, 2.3), one haplotype of five from two Romanian locations (RUM_5.2-4, RUM_6.1-2)]. Subclade 8 comprised two haplotypes from two Romanian locations [one single individual (RUM_2.4), one of six individuals from two Romanian locations (RUM_1.1-3, RUM_2.1-3)].
The blue clade formed three subclades. Subclade 9 and the isolated haplotype (N_1.1, 1.3) fused and comprised four haplotypes from five locations [two single individuals each from one location (D_6.1, PL_1.3), one haplotype of two individuals from one Norwegian location (N_1.1, 1.3), one haplotype of three individuals from three locations (CZ_1.4, PL_1.1, S_2.1)]. Subclade 10 comprised two haplotypes from two locations [one single individual (CZ_1.5), one haplotype of three individuals from two locations (CZ_1.1-2, F_1.2)]. Subclade 11 comprised five haplotypes from two locations [single individuals: two from one Danish (DK_1.1-2), two from one German (D_4.1, 4.5), one haplotype of three individuals from one German location (D_4.2, 4.6-7)].
The red clade formed six subclades, three isolated single individuals each from one location (D_4.4, D_5.5, F_1.3), one isolated haplotype of two individuals from one German location (D_7.2, 7.4) and two isolated individuals from one German location grouped together (D_7.1, 7.3). Subclade 12a, b, isolated individuals RUM_3.3, CZ_1.3, N_1.2, RUS_1.4 and the haplotype of the two Austrian individuals (A_1.1-2) fused and comprised six haplotypes [three single individuals each from one location (CZ_1.3, N_1.2, RUM_3.3), one haplotype of two individuals from one Austrian location (A_1.1-2), one of three individuals from one Romanian location (RUM_3.1-2, 3.4), one of four individuals from two locations (RUM_4.1-3, RUS_1.4)]. Subclades 13, 14, 15 and 16 had no changes to the nucleotide. Subclade 17 and 18 fused and comprised six haplotypes from six locations [single individuals: two from one Polish location (PL_1.4-5), one from one German (D_5.4), one from one Swedish (S_2.3); haplotype with more than one individual: one of four individuals from two locations (F_4.1-2, PL_1.2, 1.6), one of eight individuals from two locations (D_4.3, 4.8-9, DK_2.1-5)].
Haplotypes of individuals from the same location existed in different subclades like CZ_1, D_4, D_5, F_1, N_1, PL_1 and S_2 and among these subclades there were several high amino acid changes between individuals from the same location (CZ_1.1/CZ_1.4, CZ_1.4/CZ1.5, D_1.13/D_1.14, D_4.3/D_4.4, D_7.1/D_7.2, D7.2/D_7.3, F_1.1/F_1.3, F_3.1/F_3.2, RUM6.1/RUM6.3, RUS_1.1/RUS_1.4, RUS_1.2/RUS_1.4 and RUS_13/RUS_1.4).

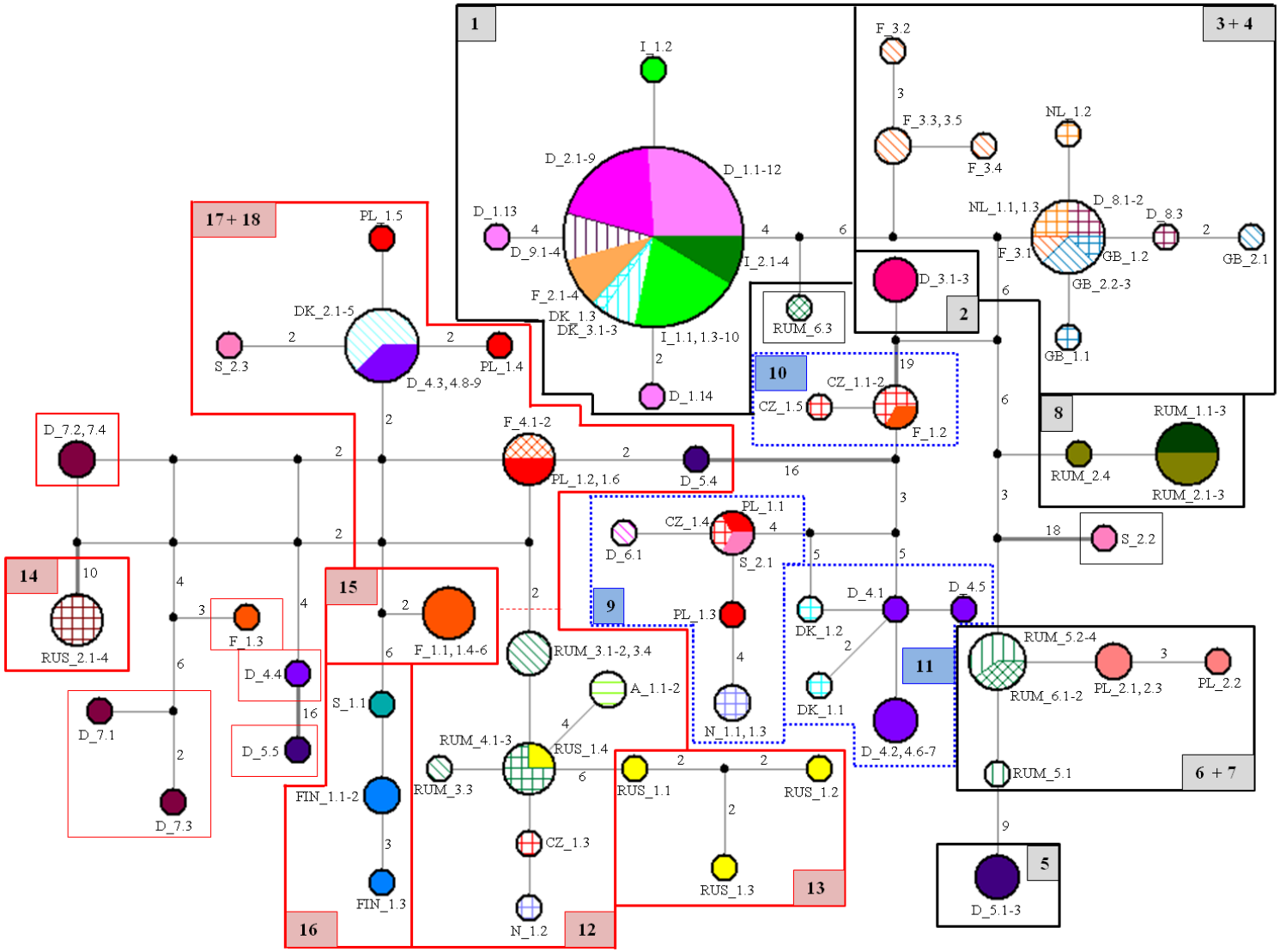

Figure 45: Median-joining haplotype network for the *COI* protein of 58 haplotypes from *Steganacarus magnus*. Each location has a specific colour marking. The size of the circles is proportional to the number of sequences per haplotype. Numbers on the lines represent the number of substitution steps separating the haplotypes (no number: only one amino acid change between haplotypes). Major subclades are marked by boxes.

Phylogenetic and population genetic analyses

All phylogenetic analyses (NJ without and with model of sequence evolution, ML, MrBayes and Beast) showed a maximum supported monophyletic group of *S. magnus* with three main clades (black, blue and red), 25 subclades and ten isolated individuals in the nucleotide (Fig. 46, A52-56). The three main subclades were supported by high bootstrap values and posterior probabilities. The black and the blue main clade were sister clades and supported by high bootstrap values and posterior probabilities. Only the arrangement of the 14 subclades was variable in the analyses. The different subclades and the individuals which built these clades are explained in Table 28.

In the phylogenetic trees of the protein the arrangement of the three main clades changed (Fig. 47, A57-59). The blue and the red clade were sister clades and supported by high bootstrap values and posterior probabilities. The number of subclades and isolated individuals shrunk from 25 to 17 subclades and from ten to five isolated individuals, respectively. Two subclades 3 and 4, 6 and 7, 9 and N_1.1, 1.3, 17 and 18 in the nucleotide grouped to one subclade in the protein, respectively. Subclade 12, A_1.1-2 and the two isolated individuals CZ_1.3 and N_1.2 grouped in one subclade.

The minimum and maximum mean average pairwise differences for the nucleotide sequences between populations were 0.3% (D_2/F_2) and 31.3% (RUM_5/RUS_1). Excluding Russia the maximum mean average pairwise differences was 29.9% (A_1/D_3). Within populations the minimum and maximum mean average pairwise differences were 0% (A_1, DK_2, F_4 andRUS_2) and 25.8% (S_2) (Table 29). For the protein the minimum and maximum mean average pairwise differences between populations were 0% (A_1/RUM_4, D_2/D_9/DK_3/F_2/I_1/I_2/RUM_1/RUM_2) and 4.2% (F_3/RUS_1); excluding Russia it was 4% (A_1/D_3, D_3/DK_2, D_3/F_1, D_3/F_4, D_3/RUM_3 and D_3/RUM_4). The minimum and maximum mean average pairwise differences within population were 0% (A_1, D_2, D_3, D_9, DK_2, DK_3, F_2, F_4, FIN_1, GB_1, I_1, I_2, RUM_1, RUM_2 and RUS_2) and 2% for the protein (D_5) (Table A38).

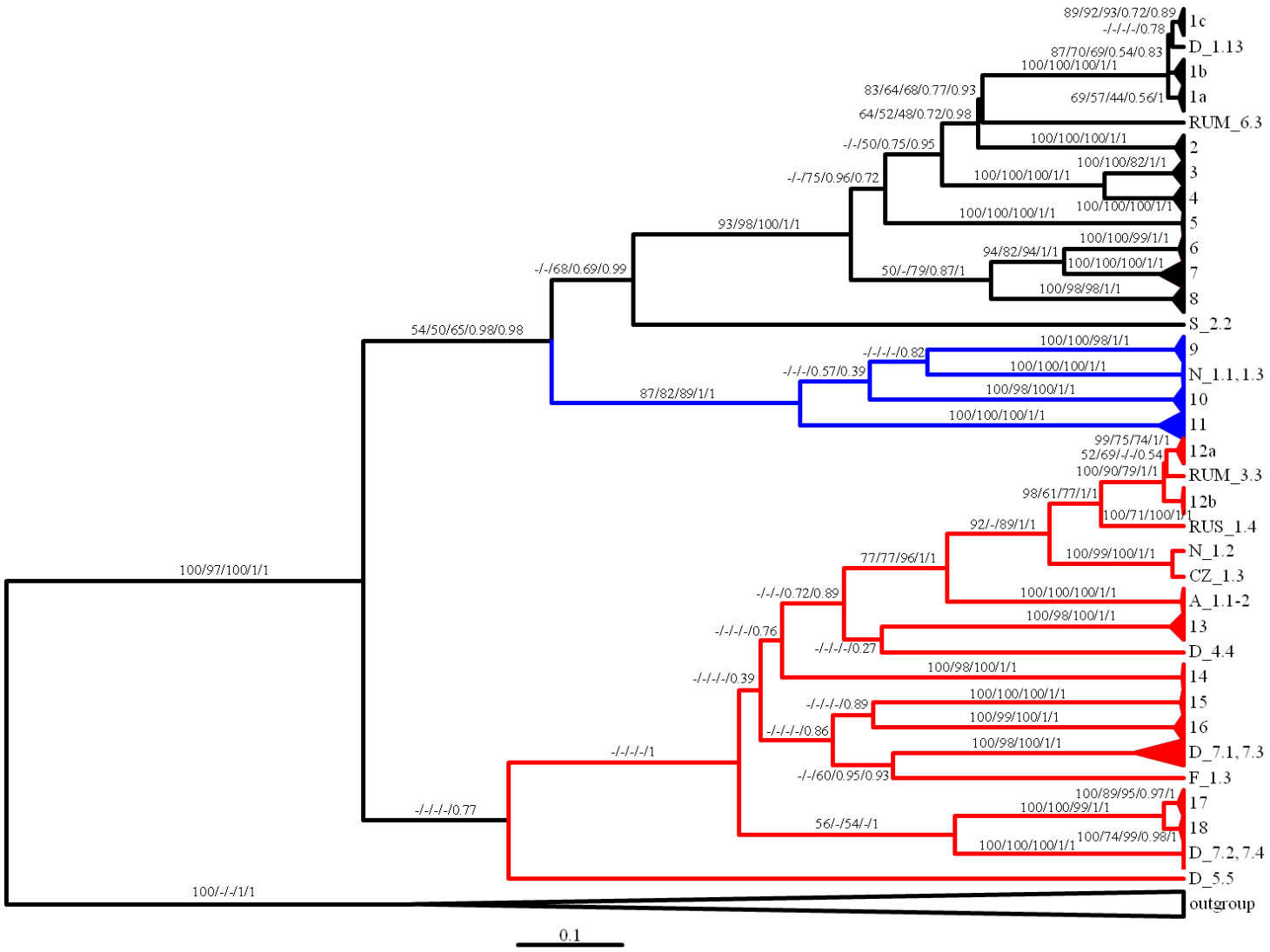

Figure 46: Bayesian phylogeny approach after $10x10^6$ generations from the 157 *COI* nucleotide sequences of *Steganacarus magnus*. Outgroups are *Hypochthonius rufulus* and *Rhysotritia duplicata*. Numbers on the branches are bootstrap values from NJ without and with model of sequence evolution (GTR+I+G) and ML analysis and posterior probabilities from MrBayes and Beast. Coloured branches are the three main clusters (black, blue and red). Tip numbers are the different subclades and explained in Table 28.

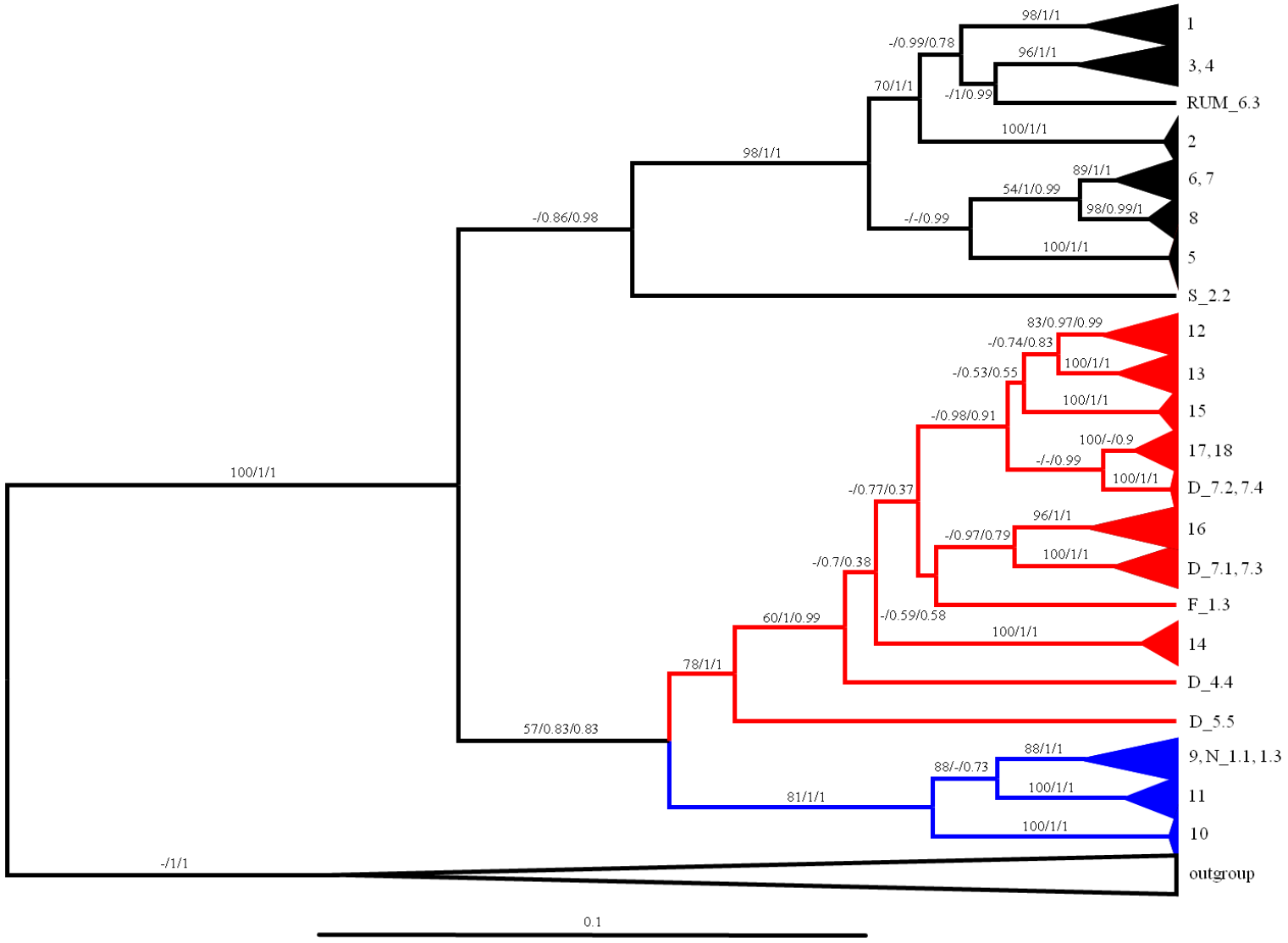

Figure 47: Bayesian phylogeny approach after $10x10^6$ generations from the 157 COI protein sequences of *Steganacarus magnus*. Outgroups are *Hypochthonius rufulus* and *Rhysotritia duplicata*. Numbers on the branches are bootstrap values from NJ analysis and posterior probabilities from MrBayes and Beast. Outgroups are HD_D_1 and RD_D_1.1. Branch colour (black, blue and red) show the different main clades. Tip numbers are the different subclades and explained in Table 28.

Table 28: Subclades of Bayesian phylogenetic trees of *Steganacarus magnus* based on *COI* nucleotide sequences (#ind=number or individuals, pp=posterior probabilities, sampling sites, individuals and ind. pop.=quantity of individuals from the population).

Clade I

Subclade	# ind	pp	sampling sites		individuals	ind. pop.
1	**49**	**1**	**8**			
1a	**20**	**1**	KW	D_1	1, 12	2/14
			Goettingen	D_2	1-2, 4-9	8/9
			Bonn	D_9	1	1/4
			Loire	F_2	1-4	4/4
			Grosseto	I_1	1, 4, 9-10	4/10
			Parma	I_2	4	1/4
1b	**12**	**0.83**	KW	D_1	2, 5, 14	3/14
			Bonn	D_9	2	1/4
			Grosseto	I_1	2-3, 5-8	6/10
			Parma	I_2	1-2	2/4
1c	**16**	**0.89**	KW	D_1	3-4 6-11	8/14
			Goettingen	D_2	3	1/9
			Bonn	D_9	3-4	2/4
			Parma	I_2	3	1/4
			Copenhagen	DK_1	1	3/3
			Arhus	DK_3	1-3	3/3
isol. ind.	**1**	**0.78**	KW	D_1	13	1/14
isol. ind.	**1**		Sinaia Lake	RUM_6	3	1/3
2	**3**	**1**	Constance	D_3	1-3	3/3
3	**12**	**1**	Cuxhaven	D_8	1-3	3/3
			St. Isidore	F_3	1	1/5
			Ascot	GB_1	1-2	2/2
			Braemar	GB_2	1-3	3/3
			Wageningen	NL_1	1-3	3/3
4	**4**	**1**	St. Isidore	F_3	2-5	3/5
5	**3**	**1**	Moerfelden	D_5	1-3	3/5
6	**6**	**1**	Busteni	RUM_5	1-4	4/4
			Sinaia	RUM_6	1-2	2/3
7	**3**	**1**	Warsaw	PL_2	1-3	3/3
8	**7**	**1**	Sibiu_1	RUM_1	1-3	3/3
			Sibiu_2	RUM_2	1-4	4/4
isol. ind.	**1**		Stroemstad	S_2	2	1/3

Table 28 continue:

Clade II

Subclade	# ind	pp	sampling sites		individuals	ind. pop.
9	**5**	**1**	Decin	CZ_1	4	1/5
			Black Forest	D_6	1	1/1
			Krakow	PL_1	1, 3	2/6
			Stroemstad	S_2	1	1/3
isol. ind.	**2**		Narvik	N_1	1, 3	2/3
10	**4**	**1**	Decin	CZ_1	1-2, 5	3/5
			Mont Blanc	F_1	2	1/6
11	**7**	**1**	Meckl. Seenpl.	D_4	1-2, 5-7	5/9
			Copenhagen	DK_1	1-2	2/3

Clade III

Subclade	# ind	pp	sampling sites		individuals	ind. pop.
12	**7**	**1**	**2**			
12a	**3**	**1**	Cluj Napoca	RUM_4	1-3	3/3
isol. ind.	**1**		Bagau	RUM_3	3	1/4
12b	**3**	**1**	Bagau	RUM_3	1-2, 4	3/4
isol. ind.	**1**		Altai Mountains	RUS_1	4	1/4
isol. ind.	**1**		Narvik	N_1	2	1/3
isol. ind.	**1**		Decin	CZ_1	3	1/5
isol. ind.	**2**		Villach	A_1	1-2	2/2
13	**3**	**1**	Altai Mountains	RUS_1	1-3	3/4
isol. ind.	**1**		Meckl. Seenpl.	D_4	4	1/9
14	**4**	**1**	Novosibirsk	RUS_2	1-4	4/4
15	**4**	**1**	Mont Blanc	F_1	1, 4-6	4/6
16	**3**	**0.96**	Lahti	FIN_1	1-3	3/3
isol. ind.	**1**	**1**	Umea	S_1	1	1/1
isol. ind.	**1**		Uelzen	D_7	1	1/4
isol. ind.	**1**		Uelzen	D_7	3	1/4
isol. ind.	**1**		Mont Blanc	F_1	3	1/6
17	**11**	**1**	Meckl. Seenpl.	D_4	3, 8-9	3/9
			Hjørring	DK_2	1-5	5/5
			Krakow	PL_1	4-5	2/6
			Stroemstad	S_2	3	1/3
18	**5**	**1**	Moerfelden	D_5	4	1/5
			Haute Loire	F_4	1-2	2/2
			Krakow	PL_1	2, 6	2/6
isol. ind.	**2**	**1**	Uelzen	D_7	2, 4	2/4
isol. ind.	**1**		Moerfelden	D_5	5	1/5

Table 29: Mean percentage pairwise differences of uncorrected p-distances of the nucleotide of *Steganacarus magnus* from 35 locations. The diagonal is the within population differences and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Bold letters in pink is the maximum divergences, if Russia is excluded. Locations with less than two individuals are excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	**A 1**	**0**																																		
2	**CZ 1**	25.2	**16.1**																																	
3	**D 1**	28.1	23.8	**1.2**																																
4	**D 2**	27.9	23.8	1.1	**0.3**																															
5	**D 3**	**29.9**	25.1	16.9	16.4	**1**																														
6	**D 4**	26	22.9	24.6	24.5	24.5	**16.3**																													
7	**D 5**	27.4	24.8	21.4	21.4	21.3	23.4	**18.4**																												
8	**D 7**	23.4	26.3	26.9	26.6	26	24.5	25.6	**16**																											
9	**D 8**	28.2	26.4	20.4	19.9	21.3	26.8	22.6	26.9	**0.3**																										
10	**D 9**	28.1	23.7	1.1	0.9	16.6	24.5	21.3	26.8	20.2	**1.2**																									
11	**DK 1**	28.9	22.3	16.8	16.8	22.2	16.8	23.1	26.9	24.1	16.8	**17.9**																								
12	**DK 2**	21.9	24	23.2	23.1	23	16	20.3	20.7	27.9	23	23.5	**0**																							
13	**DK 3**	27.8	23.7	0.8	0.8	16.9	24.4	21.3	26.9	20.2	0.7	16.5	23	**0.1**																						
14	**F 1**	25	24.5	26.3	26.2	26.1	25.9	26.8	23.1	28.9	26.1	28	22.4	26.3	**15.4**																					
15	**F 2**	27.9	23.8	1.2	**0.3**	16.6	24.5	21.5	26.7	20	0.9	16.8	23.2	0.9	26.3	**0.2**																				
16	**F 3**	29.4	26	20.7	20.2	21.1	27.2	23.7	28.5	10	20.6	24.5	27.9	20.6	29.4	20.3	**5.4**																			
17	**F 4**	21.3	23.7	22.7	22.6	22.8	17.2	20	20.9	27.6	22.6	23.2	3.8	22.5	22.1	22.7	27.8	**0**																		
18	**FIN 1**	23.5	27.2	25.4	25.2	25.4	26	25.9	21.5	27.8	25.3	28.3	20.9	25.3	22.6	25.2	28.3	20.7	**0.4**																	
19	**GB 1**	28.5	27	21.5	21.1	22	27.8	23.4	27.5	1.6	21.3	25.2	28.4	21.3	29.2	21.1	10.3	28.1	27.7	**0.4**																
20	**GB 2**	28.4	27	21.6	21.1	22	27.6	23.3	27.4	1.6	21.3	25.1	28.4	21.4	29.1	21.1	10.3	28.1	27.6	0.7	**0.6**															
21	**I 1**	28.4	23.8	1.3	1.1	16.5	24.5	21.3	26.7	20.5	1.1	16.9	23.1	1.3	26.1	1	20.8	22.8	25.2	21.6	21.7	**0.9**														
22	**I 2**	28.1	23.8	1.2	1	16.6	24.5	21.3	26.8	20.3	1.1	16.8	23.1	1	26.2	1	20.7	22.6	25.3	21.5	21.5	1.1	**1.4**													
23	**N 1**	25.8	19.3	25.9	25.9	26.2	24	25.6	26.8	26.8	25.9	23.5	25.9	25.8	26.9	25.9	26.9	24.9	27.5	27.4	27.3	25.9	25.8	**18**												
24	**NL 1**	28.3	26.7	21.2	20.8	21.7	27.3	23	27.2	1.1	21	24.8	28.3	21	28.9	20.8	10.2	27.9	27.6	0.8	0.9	21.3	21.2	27.1	**1.1**											
25	**PL 1**	23.7	21.7	23.8	23.6	23.4	18.8	21.7	22.9	27.4	23.6	22.9	9.5	23.6	24.1	23.7	26.9	9.8	23.3	27.9	27.9	23.7	23.6	23.9	27.8	**1.9**										
26	**PL 2**	29.8	23.8	16.8	16.5	19.1	24.8	21.7	24.7	20.7	16.6	21.9	24.4	16.6	26.8	16.5	16.5	24.4	26.4	21	20.8	16.7	16.5	25.3	20.8	24.2	**1.9**									
27	**RUM 1**	28.3	24.4	17.7	17.8	18.9	24.6	21.5	26.3	18.9	17.6	22	24.2	17.7	27.4	17.8	19.5	24.9	25.6	19.5	19.3	17.4	17.3	26.8	19.2	24	15.7	**0.4**								
28	**RUM 2**	28.3	24.5	17.7	17.8	19	24.7	21.6	26.5	19.1	17.6	22.2	24.2	17.8	27.4	17.8	19.7	24.9	25.8	19.7	19.6	17.5	17.3	27	19.4	24.1	15.8	0.8	**1.3**							
29	**RUM 3**	19	22.4	26.8	26.9	25.8	23.6	25.7	21.8	27.1	26.8	26.8	19.6	26.8	22.5	27	27.8	19.2	22.8	27.5	27.4	26.6	26.7	23.6	27.2	21.4	27.3	28	28	**1.7**						
30	**RUM 4**	19.1	22.2	27.3	27.3	25.7	23.3	26.2	22.2	27.6	27.2	26.5	20	27.2	23.1	27.3	27.7	19.8	22.5	28	27.9	27.1	27.2	23.3	27.7	21.7	27.8	27.8	27.9	2.9	**0.9**					
31	**RUM 5**	29	24.4	18	18.1	19.6	25.5	21.3	26.5	22	17.9	23.1	25.4	17.8	27	18.1	21.2	25	25.6	22.4	22.2	18.2	18	25.5	22	25.2	12.7	16	16.1	29.7	29.5	**0.7**				
32	**RUM 6**	29.2	24	16.7	16.8	18.5	25.2	21.2	26.7	20.3	16.5	22.4	24.9	16.5	27.5	16.8	20.3	24.6	26.1	20.8	20.7	16.7	16.6	25.4	20.4	24.7	14.5	16.2	16.4	28.8	28.7	6.2	**11.7**			
33	**RUS 1**	21.5	26.6	28.8	28.4	27.5	26.5	28.1	23.7	30.2	28.6	29.1	23.4	28.6	24.2	28.3	30.9	22.3	24.3	30.8	30.8	28.6	28.6	27.3	30.5	24.9	30.3	30.2	30.4	19.6	20	**31.3**	30.7	**12.5**		
34	**RUS 2**	24.5	28.6	30	29.6	29.3	26.6	28.4	23.2	29.3	29.7	29	25.4	30	23.7	29.6	31	25.1	26.4	30.1	30	29.6	29.7	28.8	29.8	26.6	29.4	28.1	28.3	23.1	22.6	30.1	30.3	23.5	**0**	
35	**S 2**	26.6	22.3	24.9	24.9	23.5	22.5	23.8	25.3	27	24.8	24.3	17.7	24.9	25.7	24.9	26.4	19.1	25.9	27.8	27.7	24.8	24.8	24.7	27.5	17.9	24.6	22.7	22.9	24.5	24.4	24.7	24.3	27.6	27.2	**25.8**

The results of the AMOVA showed that the nucleotide variation among samples within countries (60.8%) and the variation within samples (27.7%) were significant and high. In contrast, variation among countries was not significant (Table 30). The neutrality test (Tajima's D) was only significant for one population in the nucleotide (F_3: Tajima's D=-1.26296, p-value=0.0033) and one population in the protein (D_1: Tajima's D=-1.67053, p-value=0.0289) and Fu's F_s was neither significant for the nucleotide nor for the protein (Table A39 and A40).

Table 30: AMOVA table on variations among countries and within populations in nucleotide (black) and protein (red) sequences of *COI* of *Steganacarus magnus*. Each population was considered as separate groups. Populations with less than two individuals were excluded from the analysis. Significance level is p<0.05 (d.f.: degree of freedom).

Source of variance	d.f.	sum of squares	variance components	percent of total variation	fixation indices
among countries	13 / 13	2986.96 / 65	6.7 Va* / -0.03 Va	11.49 / -1.6	FCT: 0.12 / -0.02
among populations	21 / 21	3719.61 / 120.15	35.44 Vb* / 1.16 Vb*	60.79 / 72.92	FSC: 0.69* / 0.72*
within population	120 / 120	1939.64 / 54.71	16.16 Vc* / 0.46 Vc*	27.72 / 28.68	FST: 0.72* / 0.71*
total	154 / 154	8793.72 / 239.86	58.49 / 1.59		

The McDonald-Kreitman test showed that the differences between 64 of 595 populations (10.8%) were significant, between another 64 of 595 populations (10.8%) the differences were highly significant and between 41 of 595 populations (6.9%) they were very highly significant (Table 31, for more detail Table A41). All computed neutrality indices were 0 (Table A42).

None of the calculated rarefaction curves of the nucleotide and the protein reached saturation (Fig. 48 and 49). The Jackknife rarefaction curves ended at 256 haplotypes for the nucleotide and 149 haplotypes for the protein. The unique rarefaction curves ended at 73 haplotypes for the nucleotide and 38 haplotypes for the protein. The observed rarefaction curves (Sobs Mao Tau) ended at 95 haplotypes for the nucleotide and 59 haplotypes for the protein.

The results of the Mantel test indicate no evidence for isolation by distance in *S. magnus* when geographical distances were not log10 transformed [R^2=0.0215, p=0.084 (Fig. A60), and for log10 transformed data indicating isolation by distance R^2=0.0434, p=0.003 (Fig. A61) using 1000 randomizations]. Excluding the first three isolated data points from the scatterplot the regression changed little R^2=0.0272, p=0.000 (Fig. 50). However, this explained only 4% of the variation in the data.

The gmyc model had a significantly better fit in the single and the multiple analyses than the null model (Table 18). The model identified for the single analysis 21 and for the multiple analysis 26 distinct *COI* clusters. The clusters of the single analysis were mostly identical with the phylogenetic main clusters (Fig. A62). The clades 1a-c, 12a-b and the isolated individual RUM_3.3 formed single distinct clusters. Clusters 17 and 18 were grouped to one distinct cluster and the two isolated individuals CZ_1.3 and N_1.2 were one distinct cluster. In the multiple analysis of the gmyc model separated seven clades from the phylogenetic tree (in clade 1a the individual I_2.1 was excluded, the isolated individual D_1.13 was included in the clade 1c, individual PL_2.2 and F_1.2 were excluded from their clades, the isolated individual RUM_3.3 was included in clade 12b and the clades 3 and 6 persisted of two distinct clusters, respectively) (Fig. A63).

Table 31: Results of the McDonald-Kreitman test. The differences between 64 populations were significant (*0.01<P<0.05), between other 64 populations high significant (**0.001<P<0.01) and between 41 populations highly significant (***P<0.001).

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	A 1																																		
2	CZ 1	*																																	
3	D 1	-	*																																
4	D 2	-	*	-																															
5	D 3	-	*	*	-																														
6	D 4	-	-	**	***	**																													
7	D 5	*	-	-	-	-	-																												
8	D 7	-	-	**	***	***	-	-																											
9	D 8	-	**	**	-	-	**	-	***																										
10	D 9	-	*	-	-	-	***	-	***	-																									
11	DK 1	-	-	-	-	**	-	-	-	**	-																								
12	DK 2	-	*	-	-	-	-	-	-	-	-	-																							
13	DK 3	-	*	-	-	-	***	-	**	-	-	-	-																						
14	F 1	-	-	*	**	***	-	-	-	**	**	-	*	**																					
15	F 2	-	*	-	-	-	***	-	***	-	-	-	-	-	**																				
16	F 3	**	**	-	-	-	***	-	***	-	-	*	**	-	**	-																			
17	F 4	-	*	-	-	-	-	-	-	-	-	-	-	-	*	-	**																		
18	FIN 1	**	-	-	-	-	-	*	-	*	-	-	**	**	-	-	-	**																	
19	GB 1	-	**	**	-	-	**	-	***	-	-	**	-	-	**	-	-	-	*																
20	GB 2	*	*	***	-	-	**	-	**	*	-	***	*	***	**	**	-	*	**	-															
21	I 1	-	*	-	-	-	**	-	**	-	-	-	-	-	**	-	-	-	-	-	**														
22	I 2	-	*	-	-	-	***	-	***	-	-	-	-	-	**	-	-	-	-	-	-	-													
23	N 1	**	-	-	-	-	-	*	-	*	-	*	**	-	-	-	**	**	*	-	-	-	-												
24	NL 1	-	**	**	-	-	**	-	***	-	-	**	-	-	**	-	-	-	-	-	-	-	-	*											
25	PL 1	*	-	-	-	*	-	-	*	-	-	-	-	-	-	-	*	-	*	-	-	-	-	**	-										
26	PL 2	-	**	-	-	-	**	-	***	-	-	-	-	-	**	-	-	-	-	-	-	-	-	-	-	-									
27	RUM 1	-	*	*	-	-	-	*	**	-	-	*	-	-	**	-	-	-	-	-	*	-	-	-	-	-	-								
28	RUM 2	-	*	*	-	-	-	-	**	-	-	*	-	-	**	-	-	-	-	-	-	-	-	-	-	-	-	-							
29	RUM 3	-	-	-	-	*	-	-	-	-	*	-	-	-	-	-	***	-	-	-	-	-	-	***	-	-	-	-	-						
30	RUM 4	-	*	-	*	**	-	*	-	-	*	-	-	-	*	-	***	-	-	-	-	-	-	**	-	-	-	-	-	-					
31	RUM 5	-	*	-	-	-	*	-	**	-	-	-	-	-	**	-	-	-	-	-	**	-	-	-	-	-	*	-	-	-	-				
32	RUM 6	-	**	-	-	-	**	-	***	-	-	-	-	-	**	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
33	RUS 1	-	-	*	***	***	-	-	-	***	***	-	-	***	*	***	***	-	-	***	***	***	***	*	***	-	***	***	***	*	-	***	***			
34	RUS 2	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	*	-	**	-	*	-	-	-	-	*	-	-	-	-	-	-	-	-	
35	S 2	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-

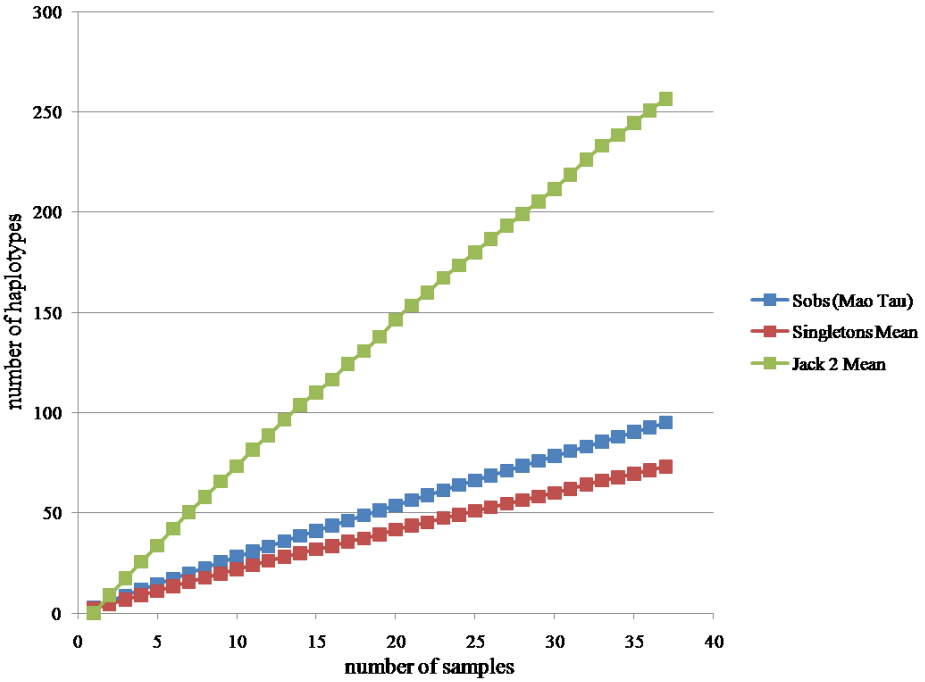

Figure 48: Sample based rarefaction analysis of haplotypes of the *COI* nucleotide of *Steganacarus magnus*. Jack 2 Mean, second-order Jackknife richness estimator, Singletons, numbers of haplotypes each present in only one sample, Sob (Mao Tau) is the observed haplotype richness.

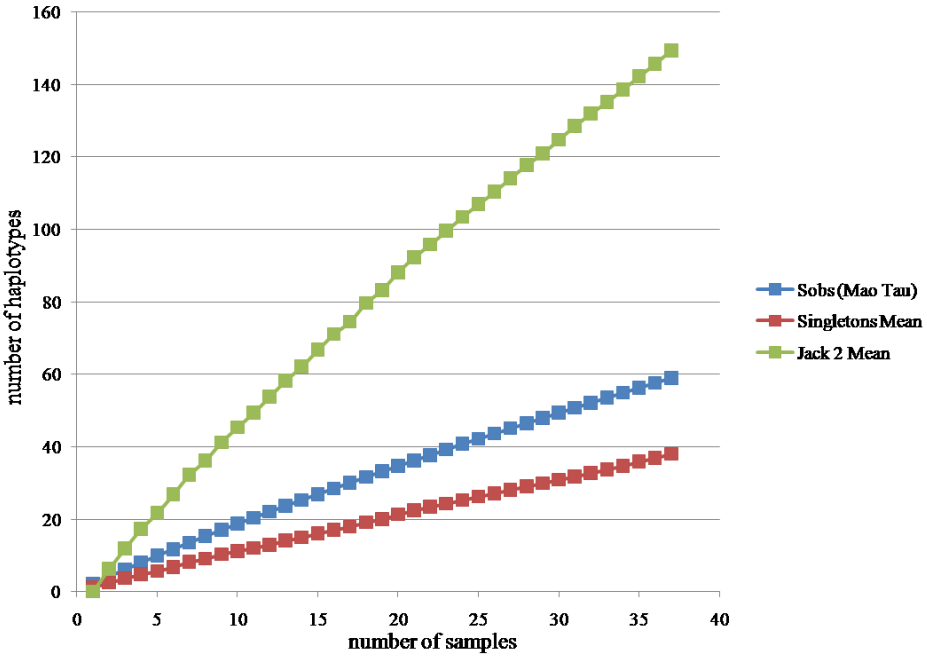

Figure 49: Sample based rarefaction analysis of haplotypes of the *COI* protein of *Steganacarus magnus*. Jack 2 Mean, second-order Jackknife richness estimator, Singletons, numbers of haplotypes each present in only one sample, Sob (Mao Tau) is the observed haplotype richness.

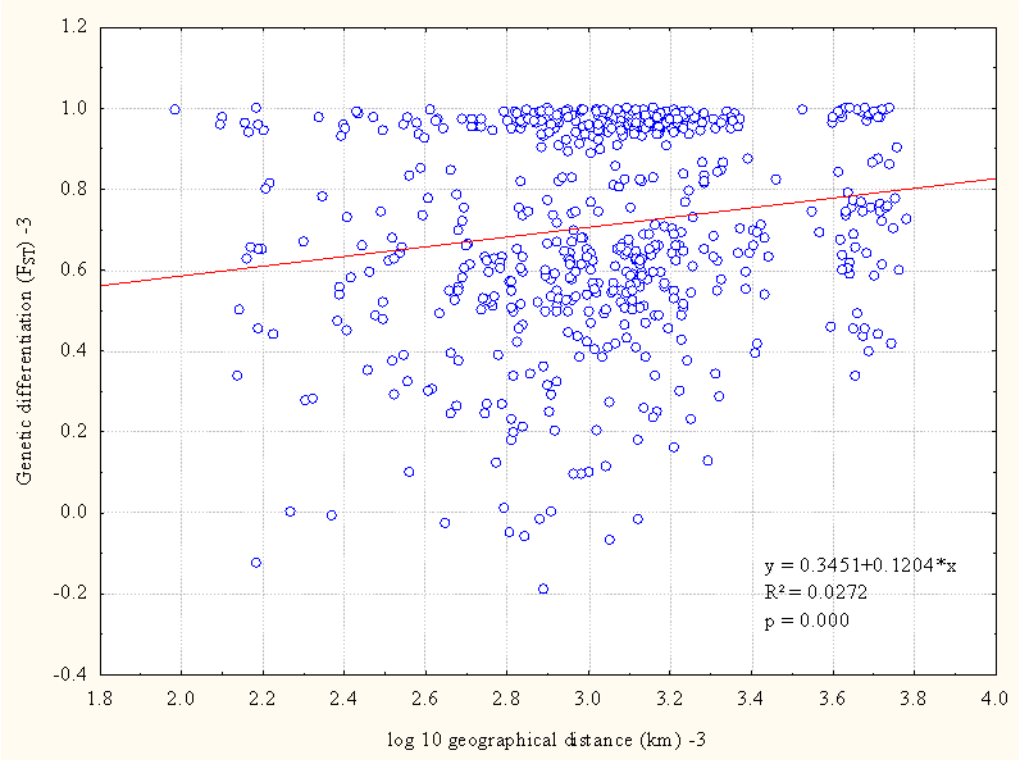

Figure 50: Linear regression of log10 geographical distances (kilometers) vs. genetic differentiations (F_{ST}) based on *COI* nucleotide sequences of *Steganacarus magnus*. The regression is significant using 1000 randomizations. Three isolated data points with no information were excluded. Linear regression of all data points is shown in Fig. A61.

4.4 Discussion

In contrast to the common aboveground patterns (Hewitt 1999, 2004, Hewitt and Ibrahim 2001, Habel *et al.*2009), the genetic structure of three of the four investigated oribatid mites species (*A. coleoptrata*, *S. magnus* and *P. peltifer*) is dominated by deep splits, indicating radiation of the European populations in the Miocene or earlier. Rarefaction plots suggest that the sampling of the four studied oribatid mite species were incomplete and much more haplotypes are to be expected and the genetic complexity is much higher than showed. The results suggest that the paradigm of Southern richness and Northern purity (Hewitt 1999) does not apply to at least three of the four oribatid mite species. Remarkably, each of the four oribatid mite species showed different patterns of colonization of Europe.

4.4.1 Nothrus silvestris

The low genetic variance in nucleotide and protein sequences indicates that there is only one clonal lineage of *COI* in *N. silvestris* which colonized Central and Northern Europe after the last ice age. The origin of this lineage, however, remains unclear; sequences from the studied countries are almost identical. One possible origin is Romania with colonization of Central and Northern Europe from East to West. Another possible origin is the Southwest Atlantic coast of France below the permafrost border and colonization from West to East. Rarity of *N. silvestris* in Southern Europe suggests that *N. silvestris* is a cold adapted species able to synthesize ethylene glycol (F. Horak, pers. comm.). Today, *N. silvestris* predominantly occurs in Central Europe (Subias 2004, 2009, Weigmann 2006), its lack in Scandinavia north of Lahti (Finland) may be due to dispersal limitation. Parthenogenetic reproduction has the advantage of faster colonization (Williams 1975; Bell 1982; Scheu and Schulz 1996; Lindberg and Bengtsson 2005, Schön 2007), one individual could establish a new population and that can

explain the existence of one clonal lineage of *N. silvestris* in Central Europe. In contrast, parthenogenetic oribatid mite species re-colonize litter and soil habitats not faster than sexual oribatid mite species (Domes et al. 2007c) and do not confirm with the theory.

4.4.2 Platynothrus peltifer

P. peltifer showed a very high genetic variance in the nucleotide but a low variance in the protein. Only synonymous substitutions existed in the parthenogenetic oribatid mite species *P. peltifer*. The existence of several separated Scandinavian clades and the Alpine clade of *COI* lineages in the nucleotide and the less abundant occurrence in the south suggests that *P. peltifer* survived the last glaciation in cryptic refugia in Central and presumably also Northern Europe. The common pattern of Northern purity and Southern richness (Hewitt and Ibrahim 2001) does not apply to *P. peltifer*; the genetic diversity in Northern locations even exceeded that in Southern locations. Cryptic refugia for *P. peltifer* likely existed in the Alps, Central and Northern Germany, Poland and Scandinavia.

4.4.3 Achipteria coleoptrata

A. coleoptrata showed a very high genetic variance in the nucleotide and in the protein. There were many non-synonymous substitutions which contrasts the only synonymous substitutions in the two parthenogenetic oribatid mites studied. Several geographical groups clustered together based on nucleotide variance. Clusters from the typical Southern refugia (Italy, Balkan and Romania) were not linked to Northern and Central clusters. This and the existence of several separated Central European clusters and one Alpine cluster indicates that similar to *P. peltifer A. coleoptrata* also survived the last glaciations in Northern refugia. Presumably, the Alps, Central and Northern Germany, Poland and Scandinavia likely functioned as cryptic refugia.

4.4.4 Steganacarus magnus

S. magnus showed the highest variance in the nucleotide and the protein ever found in one species (Edmands 2001, Boyer *et al*. 2007, Torricelli *et al.* 2010; see Chapter III). Non-synonymous substitutions existed in *S. magnus* like in the other sexual oribatid mite *A. coleoptrata*. Presumably, for *S. magnus* mountains functioned as geographical barrier as indicated by its absence in the Alps. For populations of *S. magnus* of the Canary Islands the substitution rate of *COI* has been estimated to be 2.15% per million years (Salomone *et al.* 2002). Assuming that this substitution rate also applies to the *S. magnus* populations investigated in this study, several preglacial splits exist in the European populations of this species. Unexpectedly, *S. magnus* presumably was little affected by the last ice ages; rather, main radiations of the European lineages of this species took place in the Miocene or the Oligocene. Cold resistance (Webb and Block 1993, Krantz and Walter 2009) and the high genetic divergences suggest that similar to the parthenogenetic *P. peltifer* and the other sexual oribatid mite species studied, *A. coleoptrata*, *S. magnus* survived the last glaciation in cryptic refugia. As indicated by nucleotide and protein variation Southern, Central and Northern Germany, Poland, Czech Republic, Great Britain and Scandinavia functioned as long-term refugia for *S. magnus*. In cryptic species complexes, as in the skipper butterflies *Astraptes fulgerator* (Hebert *et al.* 2004b) and of the genus *Perichares* (Burns and Janzen 2008), the Crustacean *Chydorus sphaericus* (Belyaeva and Taylor 2009), *COI* nucleotide variation may exceed 3% (Hebert *et al.* 2003a-b) but this is unlikely because the nuclear and the mitochondrial phylogenetic tree are not identically and a mixture of the different *COI* lineages existed in the nuclear lineages (Chapter III).

4.4.5 General explanation of high genetic variance

Generally, the results of our study suggest that using mitochondrial nucleotides to delineate species as commonly done (Pons *et al.* 2006, Fontaneto *et al.* 2007, Papadopoulou *et al.* 2009) is misleading in oribatid mites. In *A. coleoptrata*, *N. silvestris* and *S. magnus* several identical sequences were grouped in multiple distinct mitochondrial clusters. Only in *P. peltifer* the cluster delimitation based on *COI* nucleotide sequences may be used for identifying species. However, the results for *P. peltifer* showed six distinct mtDNA clusters which may be taken as evidence for the existence of six different species in *P. peltifer*. In each of these species, however, protein sequences of *COI* were identical questioning whether *P. peltifer* should be split into several species.
Mitochondria are inherited maternally (Wolstenholme 1992, Boore 1999). Therefore, nucleotide and protein divergence in mitochondria of sexual and parthenogenetic lineages are likely to be the same. In contrast to this assumption, only synonymous substitutions existed in the parthenogenetic species *P. peltifer* whereas in both sexual species studied also non-synonymous substitution existed. This might be due to the fact that in parthenogenetic species mitochondrial genes are fully linked with nuclear genes with non-synonymous substitutions breaking this linkage. This breaking of linked genes may be particularly fatal in cytochrome oxidase genes as the cytochrome oxidase complex is formed by subunits encoded by both mitochondrial and nucleotide genes. Variations in one of these subunits may result in a non-functional protein complex. In sexual species mitochondria and nuclear genomes are not fully linked, due to recombination non-synonymous substitutions presumably occur frequently since ten subunits of the thirteen cytochrome c oxidase subunits are located in the nucleus. Nuclear genes are mixed in every new generation and new cytochrome oxidase combinations are likely to be present. To prove this hypothesis the nuclear subunits of the cytochrome c oxidase and the other two mitochondrial subunits needs closer investigation.

Isolation by distance may be one reason for the high genetic divergences in *P. peltifer*, *A. coleoptrata* and *S. magnus*. However, the high variance in the protein in the two sexual oribatid mite species studied and the mixture of different *COI* lineages in the nuclear phylogenetic tree are unlikely caused by isolation by distance. Further, isolation by distance also cannot explain the high genetic divergence in individuals from the same locality and the fact that specimens of close geographical regions are strongly separated genetically and genetically identical to specimens of very distant geographical regions. The results of the isolation by distance analysis suggested that there was no significant relationship between geographic and genetic distance in each of the four oribatid mite species indicating that geographical distance did not affect genetic diversity. In three of the four oribatid mite species (*A. coleoptrata*, *P. peltifer* and *S. magnus*) studied genetic diversity on small geographical scales even exceeded than at large geographical scales.

The rarefaction analyses showed that sampling of haplotypes was very incomplete in each of the four oribatid mite species studied; the rarefaction curves of nucleotide and protein sequences increased in each of the four species.

The high genetic variance in the two sexual oribatid mites *A. coleoptrata* and *S. magnus* and in the parthenogenetic mite *P. peltifer* is unlikely to result from gene duplication, heteroplasmy, endosymbionts (Hurst and Jiggins 2005) or *COI* pseudogenes (Bensasson et al. 2001, Williams and Knowlton 2001, Song *et al.* 2008, Buhay 2009). The complete mitochondrial genomes of *S. magnus* and *P. peltifer* have been sequenced (Domes *et al.* 2008, Domes-Wehner 2009) and no gene duplication or heteroplasmy in *COI* was found in this or previous studies on oribatid mites. All *COI* nucleotide sequences of the four oribatid mite species can be translated into functional protein sequences without stop codons. Endosymbionts which indicate parthenogenesis such as *Wolbachia* (Plantard *et al.* 1998) and *Cardinium* (van Wilgenburg *et al.* 2006) could contaminate DNA and PCR

samples. *Wolbachia* was detected in an unidentified oribatid mite species (Cordaux et al. 2001) and in the parthenogenetic oribatid mite species *Oppiella nova* (Weeks *et al.* 2003). Further, *Cardinium* was detected in *Oppiella nova* (Weeks *et al.* 2003). In the four oribatid mite species studied no endosymbionts such as *Wolbachia* and *Cardinium* have ever been found. *Wolbachia* and *Cardinium* endosymbionts are known to induce parthenogenesis (Plantard *et al.* 1998, van Wilgenburg *et al.*2006, Bordenstein and Werren 2007). However, *A. coleoptrata* and *S. magnus* reproduce sexually whereas *P. peltifer* is assumed to form an old asexual lineage (Heethoff *et al.* 2007, Domes *et al.* 2008, Domes-Wehner 2009).

At the beginning of the Miocene (~23 mya) the vegetation and climate changed. The evergreen forests of the Oligocene shrunk and coniferous trees and deciduous trees took over (Mai 1989, 1991, 1996, Kvaček 2000). Potentially, the expansion of these forests was associated by the radiation of oribatid mite species which, according to our data, radiated in Europe during the Miocene or earlier. The expansion of forests of conifers and deciduous trees in Europe may have allowed co-occurring species to colonize new habitats and this typically is associated with radiation events (Hewitt 1999, Castagnoli *et al.* 2010). Evidence for the radiation of oribatid mite species in the Miocene or earlier are alpine clades of *A. coleoptrata* and *P. peltifer*. The rise of the Alps began in the Cretaceous (~100 Mya) and the last tectonic events occurred in the Oligocene and the Miocene (20 Mya and 6 Mya) (Cederbom *et al.* 2004).

The high variance in the *COI* gene provides evidence for high age, but it also prevents detailed determination of the age of the oribatid mite species studied. Variations in neutral mutations in mitochondrial DNA saturate after about 10-20 million years (Avise 2004). Compared to the nuclear genome mitochondrial genes evolve 10 times faster in *Drosophila* (Haag-Liautard *et al.* 2008) and 5-50 times faster in vertebrates (Lynch 2007) and have a simpler genetic code suggesting that the code is fourfold degenerated and every third position is synonymous (Baker 2000).

The high genetic variance in the protein of the two sexual species *A. coleoptrata* and *S. magnus* and the low variance in parthenogenetic species *P. peltifer* were unexpected but may actually be related to the mode of reproduction. Linkage between mitochondrial and nuclear genes in parthenogenetic species is complete. In proteins encoded in part by mitochondrial and nuclear genes, such as the cytochrome oxidase protein complex, variations in protein subunits encoded by mitochondrial genes may be fatal as it may reduce or eliminate the functioning of the protein complex. In sexual species such variations may be less fatal as genes are not closely linked due to outcrossing and recombination. Every generation nuclear genes are mixed, linked genes are broken up and new combinations are assembled. Variations in protein subunits encoded by mitochondrial genes may therefore be buffered by matching variations in the subunits encoded by nuclear genes keeping the protein complex functional.

The investigated fragment of the *COI* is widely used as barcoding gene (Hebert *et al.* 2003, 2004a-b, Ball *et al.* 2005, Hajibabaei *et al.* 2007) and a divergence of >3% is assumed to indicate separate species. The results of our study show that high genetic divergence is inappropriate for separating species in oribatid mites; the gene therefore is not useful for barcoding oribatid mite species. High genetic difference in *COI* nucleotide sequences were also found in other lineages of *S. magnus* (up to 28.6%; Salomone *et al.* 2002) and other arthropods, such as the springtail *Friesea grisea* (17.7%; Torricelli *et al.* 2010), the harvestman *Aoraki denticulata* (up to 19.2%; Boyer *et al.* 2007) and the intertidal copepod *Tigriopus californicus* (up to 23%; Edmands 2001). Potentially, soil and marine arthropods are able to accumulate high rates of mutations without splitting of species which is not the case in aboveground terrestrial arthropods (Edmands 2001, Boyer *et al.* 2007, Schaefer 2009, Torricelli *et al.* 2010). This may be due to the more pronounced changes in the aboveground habitat

due to the radiation of higher plants and associated coevolving arthropods in the Meso- and Cenozoic (Ehrlich and Raven 1964, Farrell and Mitter 1994). Soil and marine habitats presumably remained more constant allowing the accumulation of high rates of mutations without splitting of species. At least for oribatid mites this scenario is reasonable as the group is ancient (Shear *et al.*1984, Norton *et al.* 1988a, Heethoff *et al.* 2007, Schaefer *et al.* 2010) and in particular species which are abundant in boreal and temperate forest ecosystems, such as Desmonomata and Mixonomata, changed little for much of the Cenozoic (Palmer and Norton 1992). Further studies including other soil living invertebrates such as Collembola, nematodes and earthworms are needed to prove this assumption.

4.5 Conclusions

In contrast to aboveground animals, many oribatid mite species appear to have survived the glaciation of Europe in local refuges. However, colonization of Europe by oribatid mites was not uniform as indicated by the four species studied; rarefaction plots of the four species suggest that sampling of haplotypes was very incomplete. *N. silvestris* was the only species with a similar colonization pattern as documented for aboveground animals and plants; Central and Northern populations were characterized by low genetic variance in nucleotide and protein sequences and re-colonization of Central and Northern Europe likely occurred from Southern refugia but the location of these refugia remained unclear, potential regions include Romania (the Carpathians) and the Southwest Atlantic coast of France. The other three species studied (*A. coleoptrata*, *S. magnus* and *P. peltifer*) presumably were little affected by Pleistocene glaciations as indicated by high genetic divergences in the nucleotide and, in the two sexual species *A. coleoptrata* and *S. magnus*, also in the protein. Presumably, these species survived Pleistocene glaciations in cryptic refugia in Central and Northern Europe with the major lineages splitting earlier in the Miocene. The high genetic divergence in these species did not result from gene duplications, heteroplasmy, nuclear pseudogenes, endosymbionts or isolation by distance. Notably, the parthenogenetic species *P. peltifer* differed from the two sexual species in that only synonymous substitutions exist in this species whereas in the sexual species also non-synonymous substitutions occur. The high genetic divergence in the COI protein in sexual species may be linked to the sexual mode of reproduction. Recombination and associated variation in the subunits of the cytochrome oxidase encoded in the nucleus may buffer variations in the subunits of the gene encoded by mitochondrial genes and keep the cytochrome oxidase complex functional. The mitochondrial DNA evolves 10-15 times faster than the nuclear genome. Recombination of the nuclear genome may allow keeping up with these high mutation rates thereby rescuing the function of the protein. To prove this hypothesis variations in the mitochondrial and nuclear subunits of the cytochrome c oxidase need closer investigation. To estimate the age of the oribatid mite species studies other mitochondrial genes such as 16S, the other subunits of the cytochrome oxidase and ribosomal genes such as 18S need closer investigation.

Chapter Five

General Discussion

Belowground systems are characterized by high abundance and diversity of small animal species of very different phylogenetic affiliation including protozoa, lophotrochozoa (e.g. Annelida and Rotifera) and ecdysozoa (e.g. Crustacea, Insecta, Chelicerata and Nematoda). Therefore, soils harbor a variety of phylogenetically old taxa (Colemann *et al.* 2004, Schaefer *et al.* 2010). Soil organisms regulate major processes in ecosystems, such as the nutrient cycling (Scheu 2003), and they interact with the aboveground system (Bardgett 2005, Eisenhauer *et al.* 2007). Belowground organisms also provide a workscope to investigate evolutionary questions such as the existence and maintenance of sex and colonization of land (Schaefer *et al.* 2010) and new habitats after the ice ages.
The present study aimed at investigating biogeographical and evolutionary forces in the belowground system, using oribatid mite species as model organisms and molecular markers to detect post-glacial colonization events, cryptic refugia and cryptic species in belowground organisms. Using the intraspecific genetic variation of the mitochondrial cytochrome c oxidase I (*COI*) of the sexual oribatid mite *Steganacarus magnus* I investigated cryptic refugia and a possible cryptic species complex (Chapter II). The cryptic species complex in *S. magnus* was investigated and rejected using nuclear (*ef 1α*) and mitochondrial (*COI*) markers (Chapter III). To detect general post-glacial colonization patterns in oribatid mites, *COI* of two sexual *(Achipteria coleoptrata* and *S. magnus*) and two parthenogenetic (*Nothrus silvestris* and *Platynothrus peltifer*) oribatid mite species were investigated (Chapter IV). Oribatid mites are ideal model organisms to detect cryptic refugia since they are small, cold tolerant and have low dispersal abilities.

5.1 Barcoding

DNA barcoding is a well established tool for the discovery of new species and revolutionized taxonomy and species delineation of undescribed species and of species which are difficult to determine morphologically (Burns *et al.* 2007). However, DNA barcoding is discussed controversially on theoretical (Hickerson *et al*. 2006), methodical (Will and Rubinoff 2004, Cameron *et al*. 2006, Song *et al*. 2008) and empirical grounds (Hurst and Jiggins 2005). DNA barcoding is helpful for species taxonomy in groups with few or equivocal morphological characters (Ben-David *et al.* 2007). Importantly, it allows the determination of juvenile stages which often are hard to identify using morphological characters (Huang *et al.* 2007). Traditionally, a fragment of the mitochondrial *COI* gene (position 1-650 bp of the 5´-end of the Folmer fragment; Folmer 1994, Hebert *et al*. 2003a-b, 2004 a-b, Ball *et al*. 2005, Hajibabaei *et al*. 2007) is used for species delineation but in the last years the combined use of mitochondrial and nuclear genes has been advocated (Monaghan *et al.* 2005, Elias *et al.* 2007, Sevilla *et al*. 2007, Dasmahaptra *et al.* 2010).
Oribatid mites are species rich soil-living arthropods and represent an ecological important group of mainly decomposers but also with predatory, herbi- and lichenivorous species (Muraoka and Ishibashi 1976, Starý and Block 1998, Maraun and Scheu 2000, Schneider *et al.* 2004, K. Heidemann unpublished data). Species determination can be challenging, since morphological variation in body-size and color occurs in several species and the species determination of juveniles often is impossible.
Due to the high density and diversity of soil invertebrates (up to 400,000 individuals per square meter in temperate forest soils; Maraun and Scheu 2000) and the high numbers of undescribed species, especially in tropical regions, DNA barcoding is likely to be a valuable tool for assessing the number

of species present and for identifying new species. However, working with molecular methods can be demanding in oribatid mites. Species are small (0.2-1 mm body-size) and the amount of genomic DNA that can be extracted from a single individual is very limited. Additionally, oribatid mites are a phylogenetically old group (Norton *et al*. 1988a, Schaefer *et al.* 2010) and mutations in established primer binding regions in different species are frequent (I. Schaefer pers. comm.).

A number of barcoding genes have been tested in oribatid mites. The ribosomal internal transcribed spacer region 1 (*ITS1*, part of the *5.8*/*18*/*28S* complex) has been investigated in three species, *Heminothrus thori*, *N. silvestris* and *P. peltifer* and interspecific variation was sufficient to distinguish the three species (Heethoff *et al.* 2000). However, intraspecific and intraindividual variation of the *ITS1* region was high, in *P. peltifer* it ranged between 4.3% and 4.1% (Heethoff *et al.* 2000, Domes 2002), rendering this gene unsuitable for distinguishing species; the threshold proposed for species delineation is <2.7% (Hebert *et al.* 2003a, 2004a). Also, the *D3* domain of the ribosomal *28S* region has been tested as species marker (Maraun *et al*. 2003, 2004 Laumann *et al.* 2007) and no intraindividual or intraspecific variation was found but two closely related species of two different genera (*Eupelops hirtus* and *E. torulosus*, *Nanhermannia coronata* and *N. nana*; Maraun *et al.* 2003) had identical sequences; also rendering the *D3* domain unsuitable as a barcoding marker (Laumann *et al.* 2007). The conservative nuclear genes *18S*, *ef 1α*, heat shock protein (*hsp82*) and polymerase II (*pol II*) were applied successfully for phylogenies of oribatid mites (Schaefer *et al*. 2006, Domes *et al.* 2007a, 2007b, Laumann *et al.* 2007, Pachl 2010, Schaefer *et al*. 2010); closely related species had different DNA sequences, indicating that these genes might be good species markers and promising genes for barcoding. The mitochondrial gene *COI* was used successfully in a world wide phylogenetic study of the parthenogenetic oribatid mite species *P. peltifer* (Heethoff *et al.* 2007).

In the present study the genes *COI* and *ef 1α* of four oribatid mite species (*A. coleoptrata*, *N. silvestris* *P. peltifer* and *S. magnus*) were sequenced from 588 individuals sampled all over Europe. Based on this extensive dataset consisting of 581 individuals, *ef 1α*, *COI* and *18S* proofed to be unsuitable as DNA barcoding markers due to high intraspecific variance in the species *S. magnus* (31.8% in *COI*, Chapter II, 19.6% in *ef 1α*, Chapter III, and 0.7% in *18S*, M. Rosenberger, unpublished), *P. peltifer* (20.4% in *COI*, Chapter IV) and *A. coleoptrata* (19.4% in *COI*, Chapter IV) as well as relatively low interspecific variance between the two closely related taxa *S. magnus* and *Atropacarus* spec. of 0.5% in the *18S* gene (M. Rosenberger, unpublished). Intraspecific variance in these genes remain undetected as most phylogenetic studies amplify one sequence from only one individual per species (Dabert *et al.* 2010, Mutanen *et al.* 2010).

The conservative gene *pol II* is a good species marker for oribatid mites and its low mutation rate allows to infer deep phylogenetic splits between species (Pachl 2010), but more analyses with *pol II* and with polymerase III (*pol III*), another conservative gene, are needed to assess their suitability as barcoding genes in oribatid mites.

The mitochondrial *COI* gene is the most commonly used marker for barcoding (Hebert *et al.* 2003a-b, 2004a-b, Ball *et al.* 2005, Hajibabaei *et al.* 2007). However, the use of *COI* as barcoding marker for oribatid mites is problematic due to its high intraspecific variance in the two sexual (Chapter II, III and IV) and in the parthenogenetic oribatid mite species *P. peltifer* (Heethoff *et al.* 2007, Chapter IV). Similar to oribatid mites high intraspecific genetic divergence was also found in other soil-living arthropods including the springtails *Folsomia* spec. (13%; Hogg and Hebert 2004), *F. quadriculata* (18.3%; Schaefer 2009), *Ceratophysella denticulata* (21.5%; Schaefer 2009) and *Friesea grisea* (17.7%; Torricelli *et al.* 2010), the harvestman *Aoraki denticulata* (up to 19.2%; Boyer *et al.* 2007) and the intertidal copepod *Tigriopus californicus* (up to 23%; Edmands 2001). These and the present study question the use of *COI* as barcoding gene in arthropods and indicate that genetic variation in mitochondrial genes is maintained to a higher extend in soil-living and marine arthropods than in vertebrates and above-ground arthropods.

5.2 Colonization of Northern and Central Europe

Studies on aboveground animals and plants confirmed earlier suggestions that Central and Northern Europe were re-colonized via three routes after the last ice-ages (Hewitt 1999, 2004, Hewitt and Ibrahim 2001). The general pattern that few lineages started from countries south of the Alps and re-colonized empty habitats in Central and Northern Europe is reflected in the genetic structure of present day populations.

The present study is the first investigating these genetic patterns in soil animals allowing for the first time to compare patterns of above- and belowground invertebrates.

In this study, two sexual (*S. magnus* and *A. coleoptrata*) and two parthenogenetic taxa (*P. peltifer* and *N. silvestris*) were sampled from soil and litter throughout Europe and from three locations in east Russia and China. The genetic patterns of the mitochondrial *COI* gene, the standard gene for phylogeography, were very complex and indicate the colonization routes of Central and Northern Europe differ from the established routes known from aboveground organisms. Remarkably, the colonization routes of the four investigated oribatid mite species differed. The results suggest that three of the four species studied survived the Pleistocene glaciation of Europe in small refugia in Central and Northern Europe.

In contrast to plant and animal species above the ground, distinct mitochondrial lineages of oribatid mites from Central Europe co-occur in the same habitat or in habitats which are geographically close together. Further, closely related lineages are present at very different geographic locations. In addition, the results proved that the number of haplotypes (haplotype diversity index = 0.67 in *N. silvestris* and 0.98 in *A. coleoptrata*, *P. peltifer* and *S. magnus*) and the genetic distances between mitochondrial lineages (19-32%) are large, indicating that lineages of the same species that separated millions of years ago co-exist in one habitat. Presumably, these lineages survived in small populations in cryptic refugia in Central and Northern Europe and successfully spread into other habitats after glaciation.

I hypothesized that parthenogenetic species more quickly colonized Central and Northern Europe after the last ice age. In contrast to this assumption, the genetic pattern of the investigated sexual and parthenogenetic oribatid mite species suggests that the pace of colonization did not correlate with the mode of reproduction and that parthenogenetic species were not more vigorous in colonizing de-glaciated regions in Northern Europe after the last ice age. The low genetic variance in the parthenogenetic species as compared to the sexual species indicates that only one or two lineages re-recently colonized Central and Northern Europe. However, the high local genetic variance and genetic distances in the sexual species suggest that several lineages established populations in the past tens of millions of years and likely survived in small Central refugia from where re-colonization started when living conditions improved in the surrounding area.

The parthenogenetic oribatid mite species *P. peltifer* also survived the Pleistocene glaciation of Europe in cryptic refugia as indicated by its high genetic variance (divergence up to 20% in the nucleotide, see Chapter IV), but the two sexual oribatid mite species studied (*A. coleoptrata* and *S. magnus*) presumably survived as long as or even longer as *P. peltifer* in refugia and colonized Central and Northern Europe from there.

The investigated oribatid mite species have long generation times of at least one year from egg to adult (Palmer and Norton 1990) and a relatively low reproductive output with only one to two eggs per year (Domes *et al.* 2007c, Domes-Wehner 2009). The long generation time, the high abundance in forests, the cold hardiness of *S. magnus*, the dependency on dead lignified plant tissues (larval stages of *S. magnus* are endophagous) and the low dispersal capabilities render oribatid mites (especially *S. magnus*) ideal model organisms to detect cryptic refugia. Generally, cryptic refugia are difficult to detect as they may comprise small isolated islands. The dependence on plant material for development and cold hardiness provide *S. magnus* as model-organism ideally suited to reconstruct the

phylogeographic history of Central Europe. The results suggest that populations of *S. magnus* endured the ice-ages in isolated areas in Central Europe. Presumably, the species only relies on the presence of organic layers. Constant presence of plants may not be necessary as juvenile stages of *S. magnus* do not differentiate between living or dead lignified tissues for development.

5.3 Sex versus Parthenogenesis

The widespread distribution of sexual reproduction in the animal kingdom despite its high costs (production of two sexes and the dilution of the genome through meiosis) has puzzled evolutionary biologist for decades and is known as "the queen of problems in evolutionary biology" (Bell 1982). Parthenogenetic species are thought to go extinct since they cannot overcome the accumulation of negative substitutions (Maynard Smith1978, Bell 1982, Kondrashov 1993, Butlin 2002, Schön *et al.* 2008). The existence of so called 'ancient asexual scandals' namely darwinulid ostracods (Martens *et al.* 2003), bdelloid rotifers (Welch and Meselson 2000) and some taxa of oribatid mites (Maraun *et al.* 2003, Heethoff *et al.* 2007, 2009) is still debated. Theoretically, only sexual species radiate and form clusters of species that rarely contain single terminal parthenogenetic offshoots (Barraclough *et al*. 2003, Birky *et al.* 2005). Contrasting this pattern, there are two large parthenogenetic clusters in oribatid mites (Desmonomata and Enarthronota) which each likely evolved by radiation of a single parthenogenetic lineage (Maraun *et al.* 2004). Remarkably, in Desmonomata sex presumably re-evolved in Crotoniidae (Domes *et al.* 2007b). The origin of Desmonomata is dated back to 330 my (Schaefer 2009, Schaefer *et al.* 2010) suggesting that genes involved in mating and spermatogenesis have been functional or regained functionality after millions of years and generations.

Molecular approaches can be used for testing the long-term absence of sex. The long-term absence of recombination should affect the nuclear genome by accumulation of deleterious mutations (Muller's ratchet; Muller 1964, Mutational Load Theory; Kondrashov 1988). For proving absence of recombination and therefore anciency of parthenogenetic lineages the 'Meselson effect' has been proposed (Welch and Meselson 2000). However, in each of the above mentioned three 'ancient asexual scandals' no Meselson effect has been detected (Butlin 2002, Schaefer *et al.* 2006, Schön *et al.* 2008).

The high genetic variances in the nucleotide in three of the four investigated oribatid mite species, *A. coleoptrata*, *P. peltifer* and *S. magnus*, and in the COI protein of the sexual species and the low variance in parthenogenetic species were unexpected but may actually help to explain the maintenance of sexual reproduction. The linkage between mitochondrial and nuclear genes in parthenogenetic species is complete. In proteins encoded in part by mitochondrial and nuclear genes, such as the cytochrome oxidase protein complex, variations in the protein subunits encoded by mitochondrial genes may be fatal as it may reduce or eliminate the functioning of the protein complex. In sexual species such variations may be less fatal as genes are not closely linked due to outcrossing and recombination. Every generation nuclear genes are mixed, linked genes are broken up and new combinations are assembled. Variations in protein subunits encoded by mitochondrial genes may therefore be buffered by matching variations in the subunits encoded by nuclear genes keeping the protein complex functional. This interrelationship may actually be responsible for the existence of sex. In this scenario mixing of nuclear genes by sexual reproduction balances the high non-synonymous mitochondrial mutations. Further studies have to prove this hypothesis by analyzing mitochondrial and nuclear markers in parthenogenetic and sexual species.

5.3 Synopsis and Conclusion

The aim of this study was to infer phylogeographic patterns in soil-living arthropods on the basis the abundant and diverse group of oribatid mites. I expected that the extreme climatic conditions of the last glacial maximum (22-18 kya) had a similar impact on oribatid mites as on the already investigated above-ground taxa. The significance of the reproductive mode for re-colonization of empty habitats

was taken into account as two sexual (*A. coleoptrata* and *S. magnus*) and two parthenogenetic taxa (*N. silvestris* and *P. peltifer*), co-occurring in the same habitats, were sampled from forest litter throughout Europe. Molecular analyses concentrated on the standard gene for phylogenetic analyses and DNA barcoding, the mitochondrial *COI* gene (Folmer fragment, position 1-660). The nuclear encoded single copy gene elongation factor 1 α was also sequenced from a subset of the same individuals. In the course of this study it turned out that the well established above-ground re-colonization patterns are only to a limited extent transferable to soil-living oribatid mites.

In general, the genetic variance in oribatid mites throughout Europe is very high and phylogeographic patterns are only at the protein-level clearly comprehensible. On basis of nucleotide sequences, the genetic distance among individuals is unusually high (19-32%), except for *Nothrus silvestris*. In the three remaining species the *COI* gene appears to be saturated in European populations, indicating that lineages have existed for at least 20-50 million years.

The most complex genetic pattern was found in *S. magnus* (Chapter II), the species with the highest local genetic diversity and distances among individuals. With phylogenetic analyses, three distinct mitochondrial lineages were identified that even co-existed in the same habitat and probably separated at least 20 mya. This finding suggests a cryptic species complex in *S. magnus,* e.g. lineages differentiated recently and became reproductively isolated in the same habitat but remained morphologically indistinct. However, it is more likely that the LGM had only a limited effect on *S. magnus* but small populations rather survived in cryptic refugia in Central Europe and that three mitochondrial lineages re-colonized east-central, south-central and south-east of Europe.

A cryptic species complex, however, can be excluded (Chapter III). Phylogenetic analyses based on the mitochondrial *COI* gene and nuclear elongation factor 1α sequenced from the same individuals showed that sexual reproduction among different mitochondrial lineages occurred. One individual carried the nuclear haplotype of *ef 1α* from a different mitochondrial lineage, a combination that can only be explained by sexual reproduction between individuals of different mitochondrial lineages – both lineages were sampled in this population.

The pattern of high local and global genetic variance and high genetic distance found in *S. magnus* (Chapter II and III) indicative for pre-glacial diversification and survival in cryptic refugia was confirmed by the other sexual species (*A. coleoptrata*, Chapter IV).

The populations of the parthenogenetic species however are dominated by only one protein haplotype in *N. silvestris* and *P. peltifer*. This demonstrates that the LGM had a stronger impact on the parthenogenetic species than on the sexual species.

Oribatid mites are perfect model organisms for evolutionary and phylogeographic questions investigated in this study. Results presented in Chapter II showed that the oribatid mite *S. magnus* survived the last ice ages in cryptic refugia and the high genetic divergence in *COI* is not a result of a cryptic species complex (Chapter III). This is supported by the results presented in Chapter IV demonstrating that the last ice age did not shape the biodiversity of belowground organisms and oribatid mites radiated in Miocene or earlier (~20 mya). Sexual and parthenogenetic oribatid mites survived in Northern and Central Europe and colonized the habitat from there. The high genetic divergences in the protein of sexual species could explain the existence of sex since only in sexual species recombination is present. However, to support these hypotheses several studies with other belowground organisms such as Collembola, earthworms and nematodes must be carried out.

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Appendix

Table A1: Standard diversity measures of *COI* nucleotide sequences of *Steganacarus magnus*. Populations with less than two individuals were excluded (Chapter II).

population	locality	sample size n	invarible sites N_{is}	variable sites N_{vs}	parsimony inform. sites N_{pars}	number of singeltons N_s	number of haplotype haplotypes N_h	diversity H_d	variance	nucleotide diversity Π_n
A_1	Villach	2	513	0	0	0	1	0	0	0
CHINA_1	Nanjing	2	513	0	0	0	1	0	0	0
CZ_1	Decin	5	346	167	35	132	4	0.9	0.026	0.161
D_1	Kranichstein	14	486	27	12	15	8	0.769	0.014	0.013
D_2	Goettingen	9	508	5	0	5	4	0.417	0.036	0.002
D_3	Lake Const.	3	506	7	0	7	3	1	0.074	0.009
D_4	Meck. Seenpl.	9	326	187	124	63	4	0.75	0.013	0.162
D_5	Moerfelden	5	325	188	48	140	3	0.7	0.048	0.183
D_7	Uelzen	4	382	131	95	36	3	0.833	0.049	0.162
D_8	Cuxhaven	3	511	2	0	2	2	0.67	0.099	0.003
D_9	Bonn	4	502	11	2	9	3	0.833	0.049	0.011
DK_1	Copenhagen	3	377	136	0	136	3	1	0.074	0.181
DK_2	Hjørring	5	513	0	0	0	1	0	0	0
DK_3	Arhus 1	3	507	6	0	6	2	0.67	0.099	0.008
DK_4	Arhus 2	3	512	1	0	1	2	0.67	0.099	0.001
E_1	Teno	2	507	6	0	6	2	1	0.25	0.012
E_3	Anaga	4	496	17	5	12	4	1	0.031	0.019
F_1	Mont Blanc	6	327	186	48	138	3	0.6	0.046	0.155
F_2	Loire	4	511	2	0	2	3	0.833	0.049	0.002
F_3	Saint Isidore	5	445	68	0	68	4	0.9	0.026	0.053
F_4	Haute Loire	2	513	0	0	0	1	0	0	0
FIN_1	Lahti	3	510	3	0	3	2	0.67	0.099	0.004
GB_1	Bedford	5	446	67	5	62	4	0.9	0.026	0.054
GB_2	Ascot	2	511	2	0	2	2	1	0.25	0.004
GB_3	Braemar	3	508	5	0	5	2	0.67	0.099	0.007
I_1	Grosseto	10	503	10	8	2	4	0.711	0.014	0.009
I_2	Parma	4	500	13	2	11	4	1	0.031	0.013
IRL_1	Swords	2	511	2	0	2	2	1	0.25	0.004
N_1	Narvik	3	374	139	0	139	2	0.67	0.099	0.181
NL_1	Wageningen	3	505	8	0	8	3	1	0.074	0.01
PL_1	Krakow	6	375	138	131	7	6	1	0.009	0.143
PL_2	Warsaw	3	498	15	0	15	2	0.67	0.099	0.019
RUM_1	Sibiu 1	3	511	2	0	2	2	0.67	0.099	0.003
RUM_2	Sibiu 2	4	502	11	2	9	4	1	0.031	0.011
RUM_3	Bagau	4	495	18	0	18	2	0.5	0.070	0.018
RUM_4	Cluj	3	506	7	0	7	3	1	0.074	0.009
RUM_5	Busteni	4	507	6	1	5	4	1	0.031	0.006
RUM_6	Sinaia	3	425	88	0	88	3	1	0.074	0.115
RUS_1	Altai	4	387	126	4	122	4	1	0.031	0.127
RUS_2	Novosibirsk	5	477	36	0	36	2	0.4	0.056	0.028
S_2	Stroemstad	3	326	187	0	187	3	1	0.074	0.257
	all	174	203	310	275	35	103	0.98	0	0.212

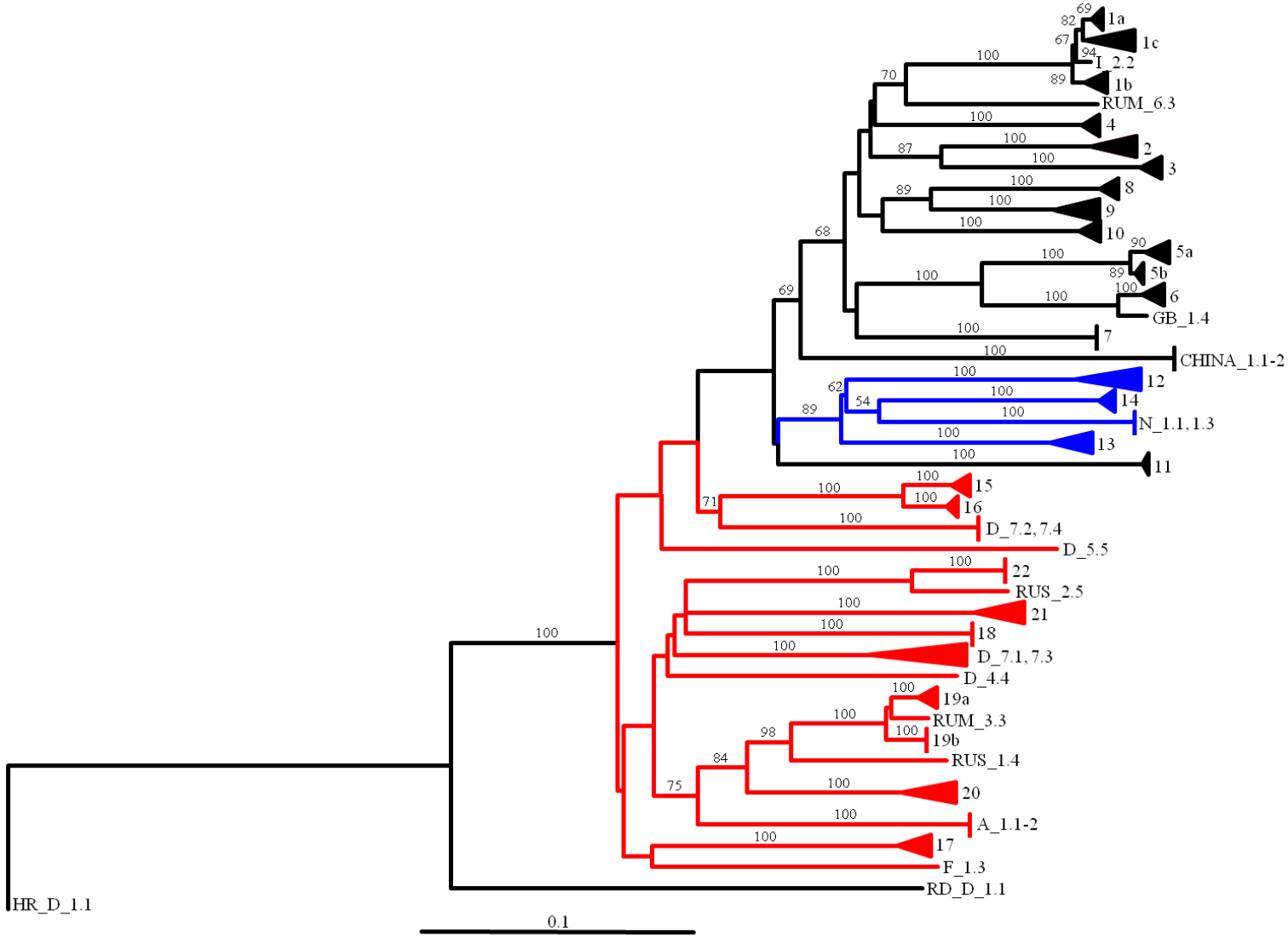

Figure A1: Neighbor-Joining tree of 180 *COI* nucleotide sequences of *Steganacarus magnus*. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Branch colours show the different clades (black, blue, red). Tip numbers are the different subclades and explain in Table 2.

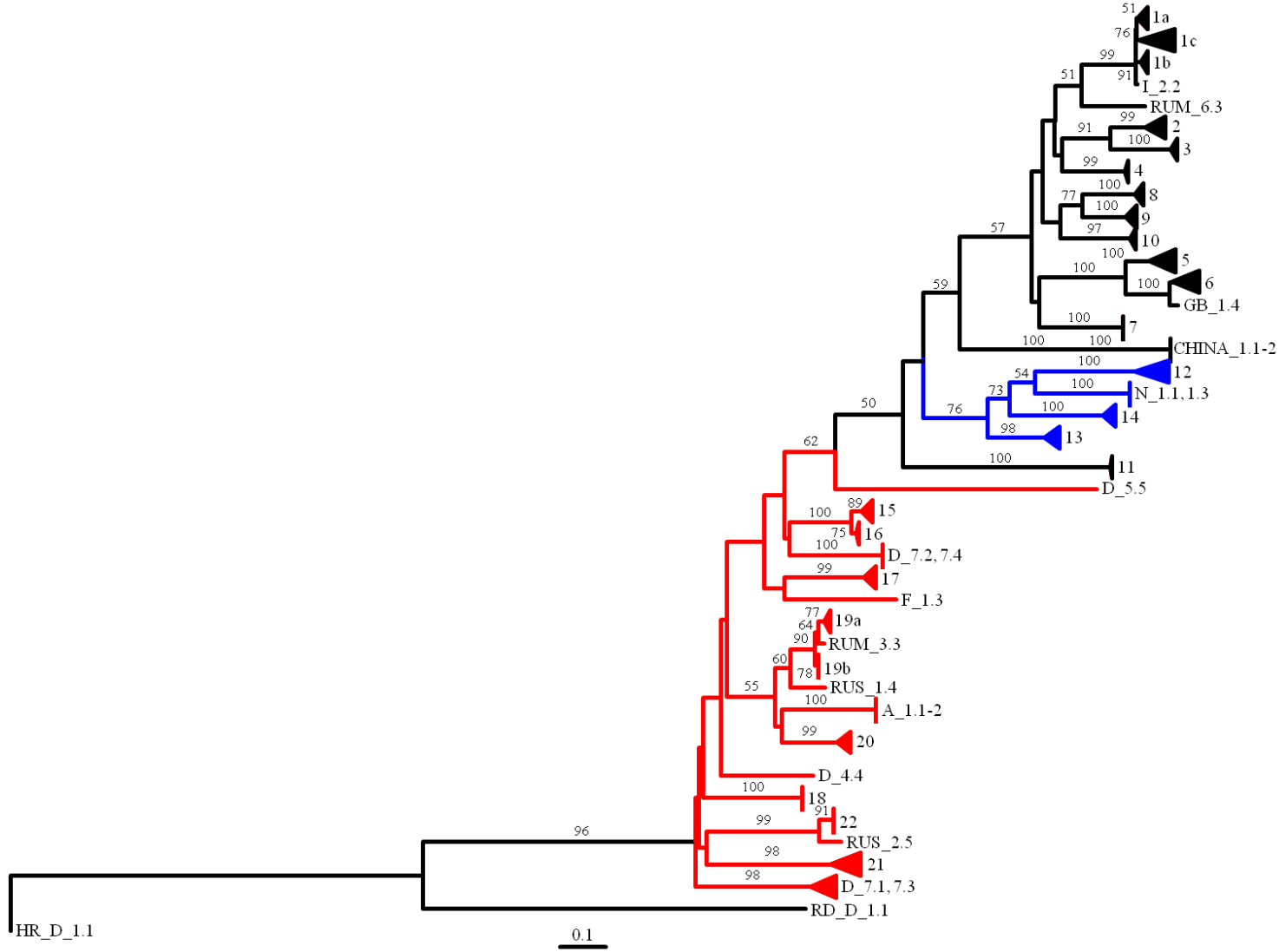

Figure A2: Neighbor-Joining tree of 180 *COI* nucleotide sequences of *Steganacarus magnus* with model of sequence evolution GTR+I+G. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Branch colours show the different clades (black, blue, red). Tip numbers are the different subclades and explain in Table 2.

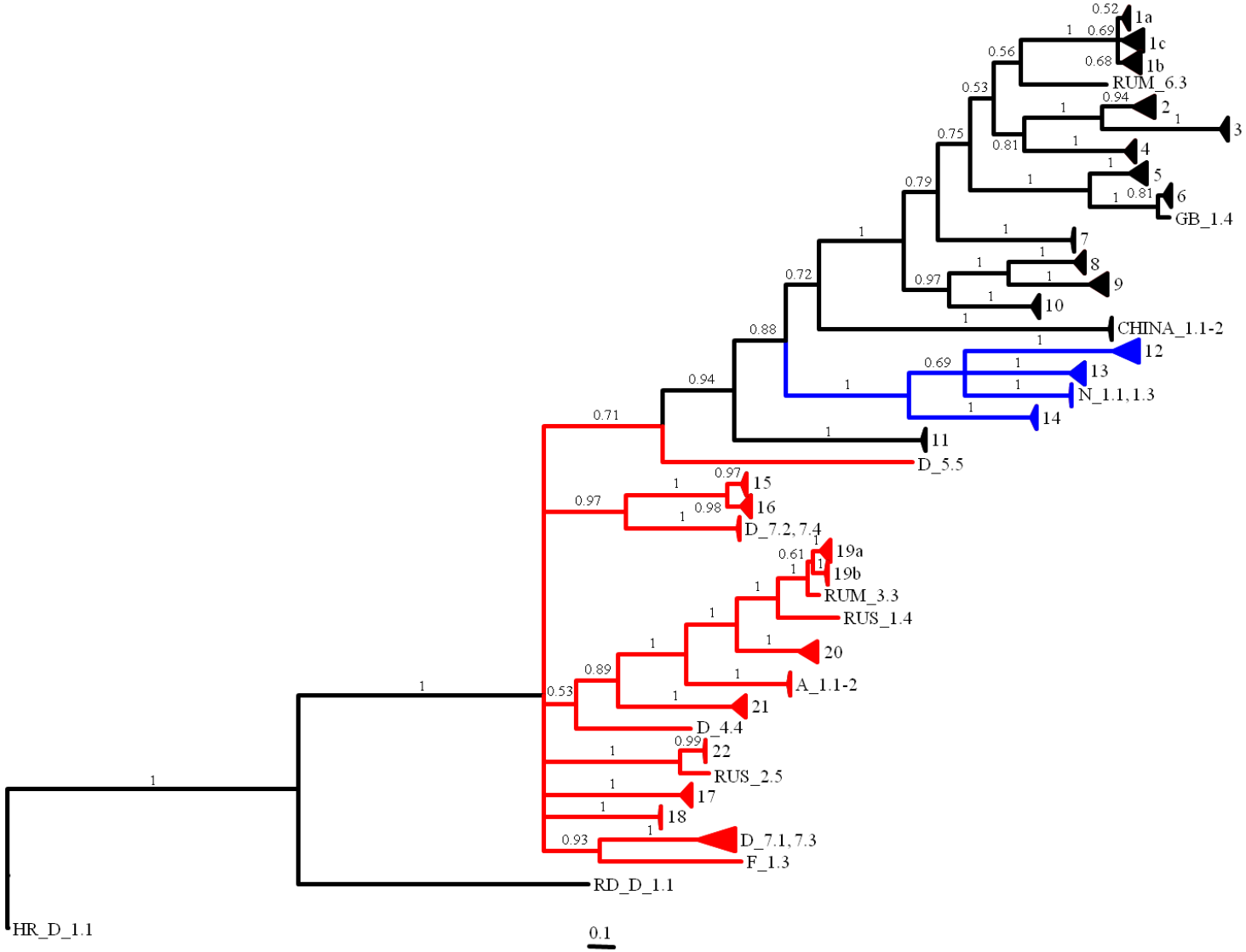

Figure A3: Bayesian tree after $10x10^6$ generations from the 180 *COI* nucleotide sequences of *Steganacarus magnus*. Split frequencies of 0.013955 and burnin of 25%. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are posterior probabilities. Branch colours show the different clades (black, blue, red). Tip numbers are the different subclades and explain in Table 2.

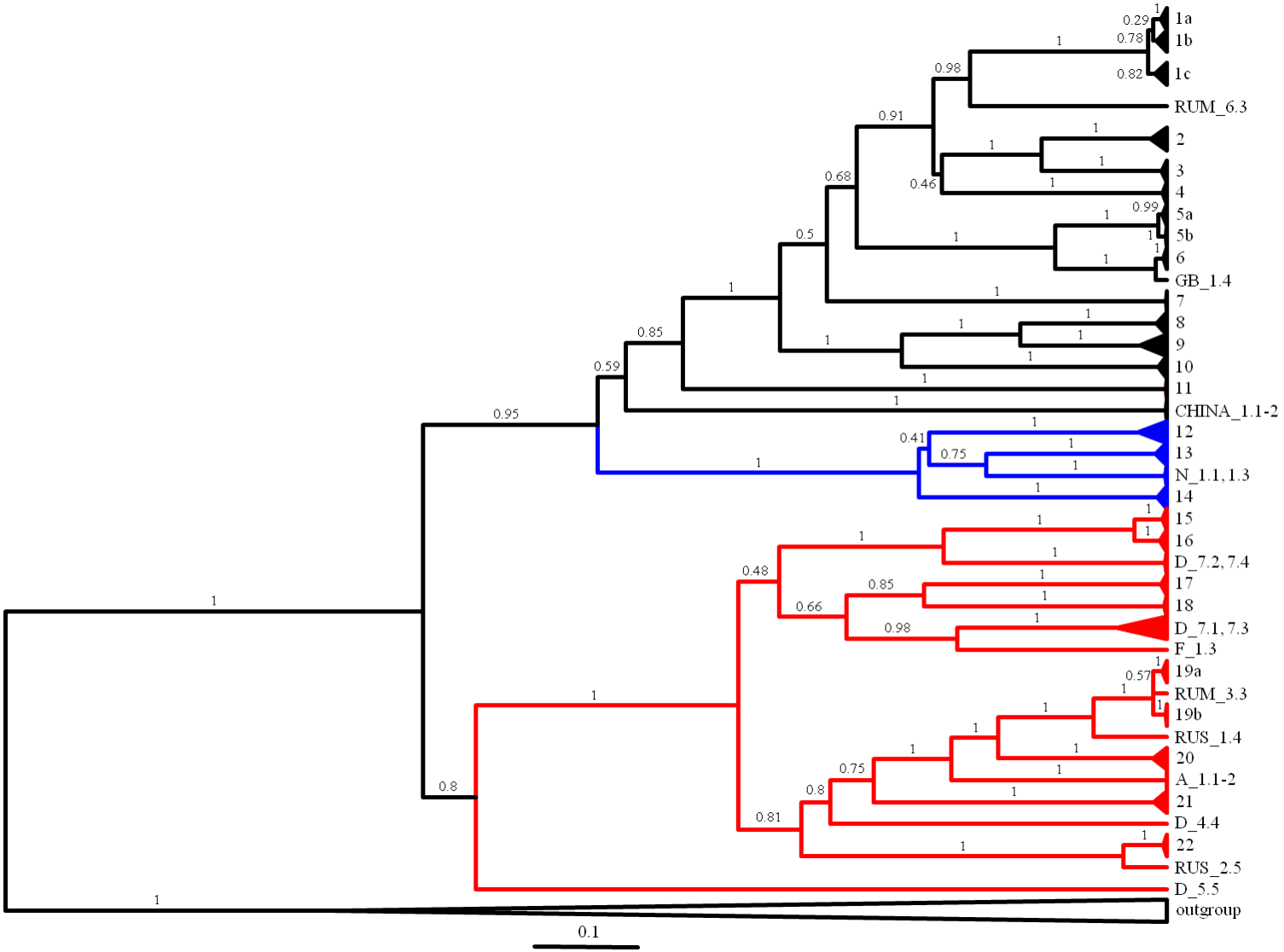

Figure A4: Bayesian phylogeny after $10x10^6$ generation with Beast v1.5.4 from the 180 *COI* nucleotide sequences of *Steganacarus magnus*. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are posterior probabilities. Branch colours show the different clades (black, blue, red). Tip numbers are the different subclades and explain in Table 2.

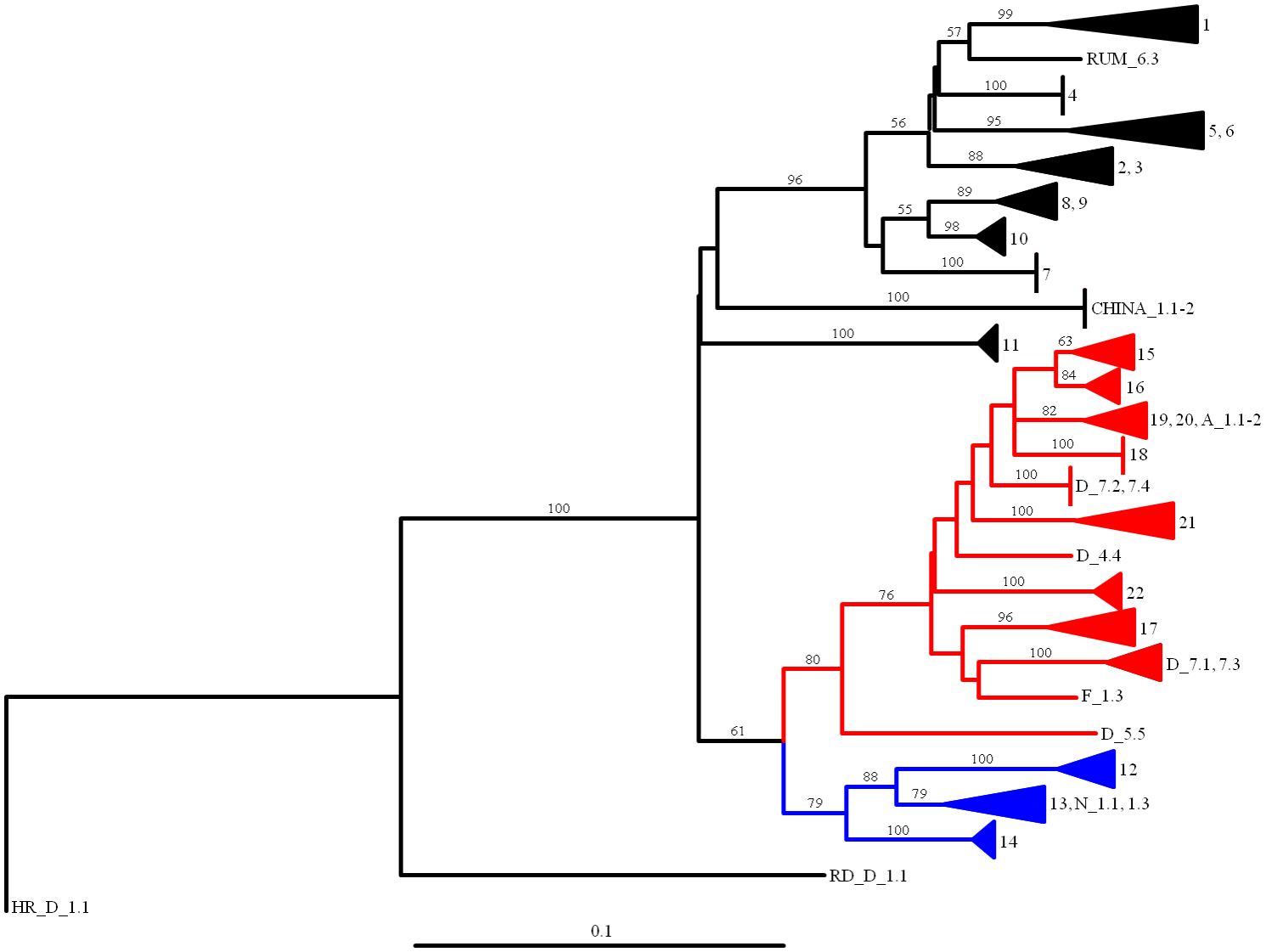

Figure A5: Neighbor-Joining tree of 180 *COI* protein sequences of *Steganacarus magnus*. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Branch colours show the different clades (black, blue, red). Tip numbers are the different subclades and explain in Table 2.

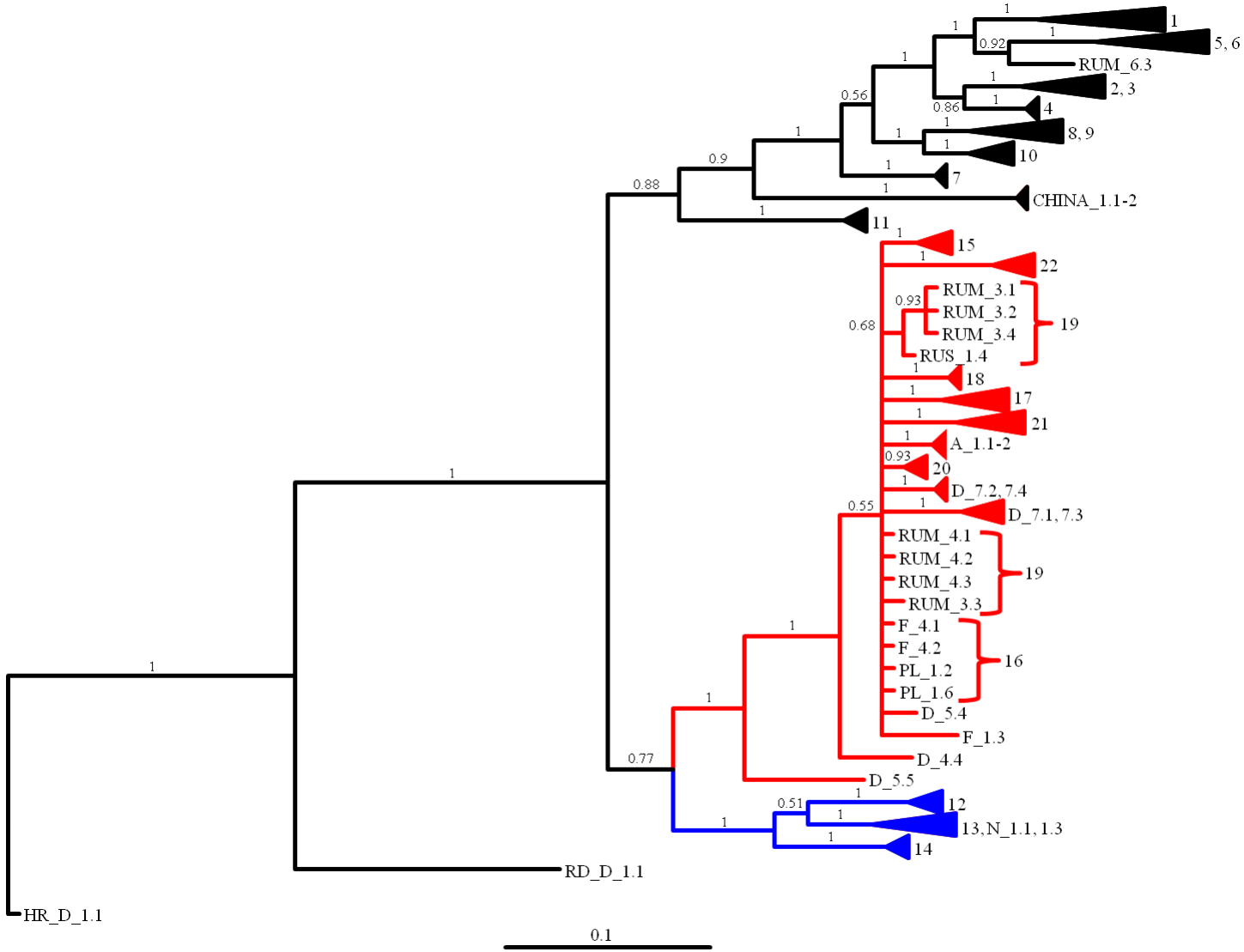

Figure A6: Bayesian tree after $10x10^6$ generations from the 180 *COI* protein sequences of *Steganacarus magnus*. Split frequencies of 0.008138 and burnin of 25%. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are posterior probabilities. Branch colours show the different clades (black, blue, red). Tip numbers are the different subclades and explain in Table 2.

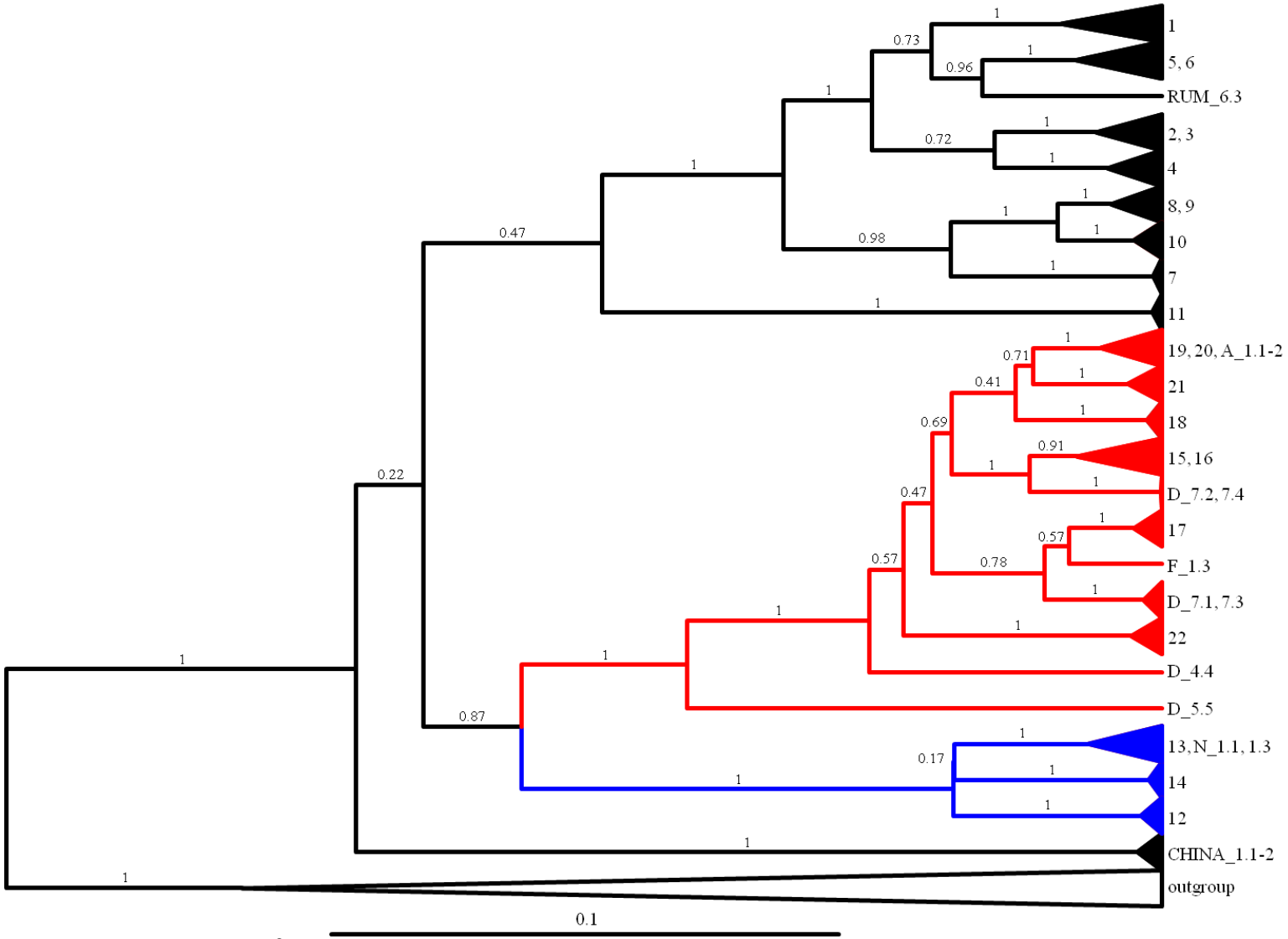

Figure A7: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 from the 180 *COI* protein sequences of *Steganacarus magnus*. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are posterior probabilities. Branch colours show the different clades (black, blue, red). Tip numbers are the different subclades and explain in Table 2.

Appendix

Table A2: Mean percentage pairwise differences of uncorrected p-distances of the protein of *Steganacarus magnus* from 41 locations in percent. The diagonal is the within population differences (bold) and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among populations. Bold letters in pink is the maximum divergences, if China and Russia were excluded. Locations with less than two individuals were excluded from the analysis.

	Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	A 1	0																										
2	CHINA 1	0.6	0																									
3	CZ 1	1.9	1.6	**1.1**																								
4	D 1	3	3.6	1.8	**0.4**																							
5	D 2	2.9	3.5	1.6	0.2	0																						
6	D 3	4.1	4.7	2.8	1.3	1.2	0																					
7	D 4	1.7	1.5	1.2	2.3	2.2	2.7	**1.2**																				
8	D 5	2.6	2.6	1.7	1.5	1.4	2.8	2.1	2.1																			
9	D 7	2.2	1.6	1.6	2.9	2.8	4	1.6	2.2	**1.1**																		
10	D 8	3.1	3.7	2.8	1.5	1.4	1.4	3.2	2.7	3.6	**0.4**																	
11	D 9	2.9	3.5	1.7	0.3	0.2	1.2	2.2	1.4	2.8	1.4	**0.3**																
12	DK 1	2.7	2.9	1.6	1.6	1.6	1.8	1.5	2	2.4	2.7	1.5	**1.6**															
13	DK 2	0.6	0	1.6	3	2.9	4.1	1.4	2.3	1.3	3.1	2.9	2.7	0														
14	DK 3	2.9	3.5	1.6	0.2	0	1.2	2.2	1.4	2.8	1.4	0.2	1.6	2.9	0													
15	DK 4	2.9	3.5	1.6	0.2	0	1.2	2.2	1.4	2.8	1.4	0.2	1.6	2.9	0	0												
16	E 1	2.9	4.1	2.1	0.7	0.6	1.8	2.5	2	2.8	2	0.6	2	2.9	0.6	0.6	0											
17	E 3	3.5	4.1	2.2	0.7	0.6	0.6	2.2	2.1	3.4	2	0.6	1.2	3.5	0.6	0.6	1.2	0										
18	F 1	1.6	1	1.9	2.6	2.5	4.1	1.8	2.4	2.2	3.9	2.6	2.6	1	2.5	2.7	3	3.5	**1.5**									
19	F 2	2.9	3.5	1.6	0.2	0	1.2	2.2	1.4	2.8	1.4	0.2	1.6	2.9	0	0	0.6	0.6	2.5	0								
20	F 3	3.2	3.7	2.8	1.5	1.4	1.4	3.2	2.6	3.6	0.4	1.4	2.8	3.2	1.4	1.4	2	2	3.9	1.4	**0.5**							
21	F 4	0.6	0	1.6	3	2.9	4.1	1.4	2.3	1.3	3.1	2.9	2.7	0	2.9	2.9	2.9	3.5	1	2.9	3.2	0						
22	FIN 1	1.2	1.2	1.3	2.4	2.3	3.5	1.3	2	1	2.5	2.3	2.2	0.6	2.3	2.3	2.3	2.9	1.6	2.3	2.6	0.6	0					
23	GB 1	2.9	3.5	2.6	1.3	1.2	1.2	3	2.5	3.4	0.2	1.2	2.5	2.9	1.2	1.2	1.8	1.8	3.7	1.2	0.2	2.9	2.3	0				
24	GB 2	2.9	3.5	2.6	1.3	1.2	1.2	3	2.5	3.4	0.2	1.2	2.5	2.9	1.2	1.2	1.8	1.8	3.7	1.2	0.2	2.9	2.3	0	0			
25	GB 3	3.1	3.7	2.8	1.5	1.4	1.4	3.2	2.7	3.6	0.3	1.4	2.7	3.1	1.4	1.4	2	2	3.9	1.4	0.4	3.1	2.5	0.2	0.2	**0.4**		
26	I 1	2.9	3.5	1.8	0.4	0.3	1.2	2.2	1.4	2.8	1.4	0.3	1.4	2.9	0.3	0.3	0.6	0.6	2.6	0.3	1.4	2.9	2.3	1.2	1.2	1.4	**0.3**	
27	I 2	2.9	3.5	1.7	0.3	0.2	1.2	2.2	1.4	2.7	1.4	0.2	1.5	2.9	0.2	0.2	0.6	0.6	2.6	0.2	1.4	2.9	2.3	1.2	1.2	1.4	0.2	**0.2**
28	IRL 1	2.9	3.5	2.6	1.3	1.2	1.2	3	2.5	3.4	0.2	1.2	2.5	2.9	1.2	1.2	1.8	1.8	3.7	1.2	0.2	2.9	2.3	0	0	0.2	1.2	1.2
29	N 1	1.8	1.6	1.3	2.3	2.2	3.3	1.6	2.2	1.8	3.1	2.2	2	1.8	2.2	2.2	2.5	2.7	2	2.2	3.2	1.8	1.6	2.9	2.9	3.1	2.2	2.2
30	NL 1	2.9	3.5	2.6	1.3	1.2	1.2	3	2.5	3.4	0.2	1.2	2.5	2.9	1.2	1.2	1.8	1.8	3.7	1.2	0.2	2.9	2.3	0	0	0.2	1.2	1.2
31	PL 1	1	0.6	1.3	2.4	2.3	3.5	1.3	2	1.4	2.9	2.3	2.2	0.6	2.3	2.3	2.5	2.9	1.2	2.3	3	0.6	0.6	2.7	2.7	2.9	2.3	2.3
32	PL 2	3.7	4.3	2.3	0.7	0.6	0.8	2.2	2.2	3.3	2.2	0.6	2.8	1.2	0.6	0.7	1.4	0.2	3.4	0.6	2.2	3.7	4.2	2	2	2.2	0.6	0.6
33	RUM 1	2.9	3.5	1.6	0.1	0	1.2	2.2	1.5	2.5	1.4	0	1.4	2.9	0	0	0.6	0.6	2.9	0	1.4	2.9	2.3	1.2	1.2	1.4	0	0
34	RUM 2	3.2	3.8	1.8	0.2	0.2	1.2	2.2	1.8	2.8	1.7	0.2	1.3	3.2	0.2	0.2	0.9	0.6	3	0.2	1.7	3.2	2.6	1.5	1.5	1.7	0.2	0.2
35	RUM 3	0	0.6	1.9	3	2.9	4.1	1.6	2.6	1.9	3.1	2.9	2.7	0.6	2.9	2.9	2.9	3.5	2	2.9	3.2	0.6	1.2	2.9	2.9	3.1	2.9	2.9
36	RUM 4	0	0.6	1.9	3	2.9	4.1	1.6	2.6	1.9	3.1	2.9	2.7	0.6	2.9	2.9	2.9	3.5	2	2.9	3.2	0.6	1.2	2.9	2.9	3.1	2.9	2.9
37	RUM 5	3.1	3.7	1.8	0.2	0.2	1.3	2.3	1.6	2.6	1.5	0.2	1.5	3.1	0.2	0.2	0.7	0.7	3	0.2	1.5	3.1	2.5	1.3	1.3	1.5	0.2	0.2
38	RUM 6	2.7	3.3	1.6	0.5	0.4	1.8	2.1	1.7	2.6	1.6	0.4	1.5	2.7	0.4	0.5	1.2	1.2	2.6	0.4	1.6	2.7	2.2	1.4	1.4	1.6	0.4	0.4
39	RUS 1	1.9	2.5	2.5	2.4	2.3	4	2.1	2.7	2.9	4.3	2.3	2.3	2.5	2.3	2.5	2.8	3.4	2.5	2.3	4.3	2.5	2.6	4.1	4.1	4.3	2.3	2.3
40	RUS 2	3.4	1.9	2.2	2.4	2.3	4.1	2.9	2.5	1.7	3.7	2.3	2.9	3	2.3	2.3	2.3	2.9	3	2.3	3.7	3	2.5	3.5	3.5	3.7	2.3	2.3
41	S 2	1.4	1.4	0.9	1.9	1.8	2.9	1.2	1.7	1.5	2.7	1.8	1.6	1.2	1.8	1.8	2.2	2.3	1.5	1.8	2.8	1.2	1	2.5	2.5	2.7	1.8	1.8

Table A2continue:

Population		28	29	30	31	32	33	34	35	36	37	38	39	40	41
28	**IRL 1**	**0**													
29	**N 1**	2.9	**1.6**												
30	**NL 1**	**0**	2.9	**0**											
31	**PL 1**	2.7	1.6	2.7	**0.9**										
32	**PL 2**	2	2.8	2.0	3.1	**0.4**									
33	**RUM 1**	1.2	2.2	1.2	2.3	0.8	**0**								
34	**RUM 2**	1.5	2.3	1.5	2.6	0.7	0.3	**0.6**							
35	**RUM 3**	2.9	1.6	2.9	1	3.7	2.9	3.2	**0**						
36	**RUM 4**	2.9	1.6	2.9	1	3.7	2.9	3.2	**0**	**0**					
37	**RUM 5**	1.3	2.3	1.3	2.5	0.9	0.2	0.4	3.1	3.1	**0.3**				
38	**RUM 6**	1.4	2.1	1.4	2.2	1.2	0.6	0.8	2.7	2.7	0.7	**1.2**			
39	**RUS 1**	4.1	2.2	4.1	2.3	3.3	2.8	2.9	2.3	2.3	2.9	2.6	**1.8**		
40	**RUS 2**	3.5	2.7	3.5	2.4	3.1	2.3	2.6	3.4	3.4	2.5	2.5	3.5	**0.2**	
41	**S 2**	2.5	1.2	2.5	1	2.5	1.8	2	1.4	1.4	1.9	1.8	2	2.4	**1.2**

Appendix

Table A3: Tajima's D and Fu's FS neutrality tests of *Steganacarus magnus COI* nucleotide sequences. Bold red letters show significance (P<0.05).

Neutrality tests	A 1	CHINA 1	CZ 1	D 1	D 2	D 3	D 4	D 5	D 7	D 8	D 9	DK 1	DK 2	DK 3	DK 4	E 1	E 3	F 1	F 2	F 3	F 4
Tajima's D test																					
Samplesize	2	2	5	14	9	3	9	5	4	3	4	3	5	3	3	2	4	6	4	5	2
S	0	0	168	27	6	7	188	188	131	2	11	137	0	6	1	6	18	187	2	68	0
Pi	0	0	82.9	6.44	1.5	4.67	83.53	94.1	83	1.33	5.83	93.67	0	4	0.67	6	10.17	79.93	1	27	0
Tajima's D	0	0	0.21	-1.03	-1.4	0	1.08	0.33	1.69	0	-0.28	6523804.68	0	0	0	0	0.36	-0.16	-0.71	-1.26	0
Tajima's D p-value	1	1	0.61	0.15	0.08	0.76	0.9	0.64	0.93	0.94	0.55	1	1	0.78	0.99	1	0.73	0.46	0.28	**0.003**	1
Fu's FS test																					
Real no. of alleles	1	1	4	8	4	3	4	3	3	2	3	3	1	2	2	2	4	3	3	4	1
Orig. no. of alleles	1	1	4	8	4	3	4	3	3	2	3	3	1	2	2	2	4	3	3	4	1
Theta_pi	0	0	82.9	6.44	1.5	4.67	83.53	94.1	83	1.33	5.83	93.67	0	4	0.67	6	10.17	79.93	1	27.2	0
Exp. no. of alleles	0	0	4.88	7.79	3.4	2.52	8.6	4.9	3.93	1.97	3.26	2.97	0	2.47	1.65	1.86	3.52	5.82	2.08	4.67	0
FS	0	0	5.38	0.27	-0.21	0.31	15.28	9.82	6.5	1.06	1.75	3.43	0	2.64	0.20	1.79	0.35	12.09	-0.89	3.31	0
FS p-value	N.A.	N.A.	0.96	0.55	0.4	0.38	1	1	0.98	0.58	0.75	0.58	N.A.	0.84	0.40	0.52	0.35	1.00	0.09	0.88	N.A.

Neutrality tests	GB 1	GB 2	GB 3	I 1	I 2	IRL 1	N 1	NL 1	PL 1	PL 2	RUM 1	RUM 2	RUM 3	RUM 4	RUM 5	RUM 6	RUS 1	RUS 2	S 2
Tajima's D test																			
Samplesize	5	2	3	10	4	2	3	3	6	3	3	4	4	3	4	3	4	5	3
S	67	2	5	10	13	2	139	8	139	15	2	11	18	7	6	89	127	36	188
Pi	27.8	2	3.33	4.67	6.83	2	92.67	5.33	73.8	10	1.33	5.83	9	4.67	3.17	59.67	65.5	14.4	132.67
Tajima's D	-1.03	0	0	1.42	-0.37	0	0	0	1.38	0	0	-0.28	-0.85	0	-0.31	1447938.65	-0.57	-1.25	14872900.98
Tajima's D p-value	0.13	1	0.8	0.94	0.52	1	0.82	0.74	0.96	0.7	0.93	0.55	0.07	0.76	0.55	1.00	0.39	**0.01**	1.00
Fu's FS test																			
Real no. of alleles	4	2	2	4	4	2	2	3	6	2	2	4	2	3	4	3	4	2	3
Orig. no. of alleles	4	2	2	4	4	2	2	3	6	2	2	4	2	3	4	3	4	2	3
Theta_pi	27.8	2	3.33	4.67	6.83	2	92.67	5.33	73.8	10	1.33	5.83	9	4.67	3.17	59.67	65.5	14.4	132.67
Exp. no. of alleles	4.67	1.67	2.39	5.7	3.34	1.67	2.97	2.57	5.81	2.74	1.97	3.26	3.47	2.52	2.89	2.95	3.91	4.42	2.98
FS	3.35	0.69	2.36	2.77	-0.12	0.69	8.4	0.46	1.52	4.17	1.06	-0.32	5.39	0.31	-1.16	2.98	2.36	8.12	3.78
FS p-value	0.88	0.37	0.81	0.91	0.27	0.37	0.99	0.39	0.49	0.93	0.59	0.22	0.98	0.38	0.09	0.57	0.55	1	0.6

Table A3 continue:

Neutrality tests	mean	s.d.
Tajima's D test		
Samplesize	4.24	2.43
S	49.76	66.94
Pi	27.14	37.55
Tajima's D	557186.37	2515606.36
Tajima's D p-value	0.70	0.32
Fu's FS test		
Real no. of alleles	2.95	1.36
Orig. no. of alleles	2.95	1.36
Theta_pi	27.14	37.55
Exp. no. of alleles	3.18	1.88
FS	2.72	3.63
FS p-value	N.A.	N.A.

Appendix

Table A4: Tajima's D and Fu's FS neutrality tests of *Steganacarus magnus COI* protein sequences. Bold red letters show significance (P<0.05).

Neutrality tests	A 1	CHINA 1	CZ 1	D 1	D 2	D 3	D 4	D 5	D 7	D 8	D 9	DK 1	DK 2	DK 3	DK 4	E 1	E 3	F 1	F 2	F 3	F 4	FIN 1
Tajima's D test																						
Samplesize	2	2	5	14	9	3	9	5	4	3	4	3	5	3	3	2	4	6	4	5	2	3
S	0	0	5	4	0	0	4	7	3	1	1	4	0	0	0	0	0	6	0	2	0	0
Pi	0	0	1.9	0.67	0	0	2.11	3.5	1.83	0.67	0.50	2.67	0	0	0	0	0	2.53	0	0.8	0	0
Tajima's D	0	0	-1.4	-1.54	0	0	1.77	0.29	1.09	0	-0.61	0	0	0	0	0	0	-0.21	0	-0.97	0	0
Tajima's D p-value	1	1	**0**	**0.04**	1	1	0.98	0.67	0.85	0.99	0.38	0.84	1	1	1	1	1	0.45	1	0.19	1	1
Fu's FS test																						
Real no. of alleles	1	1	4	4	1	1	4	3	3	2	2	3	1	1	2	1	3	3	1	4	1	2
Orig. no. of alleles	1	1	4	4	1	1	4	3	3	2	2	3	1	1	2	1	3	3	1	4	1	2
Theta_pi	0	0	1.9	0.67	0	0	2.11	3.5	1.83	0.67	0.5	2.67	0	0	0	0	0	2.53	0	0.8	0	0
Exp. no. of alleles	0	0	2.85	2.65	0	0	3.95	3.42	2.5	1.65	1.68	2.3	0	0	0	0	0	3.46	0	2.11	0	0
FS	0	0	0.56	-1.29	0	0	1.92	1.62	0.01	0.2	0.17	-0.34	0	0	0	0	0	1.47	0	1.04	0	0
FS p-value	N.A.	N.A.	0.54	0.06	N.A.	N.A.	0.86	0.77	0.29	0.39	0.34	0.19	N.A.	N.A.	N.A.	N.A.	N.A.	0.77	N.A.	0.62	N.A.	N.A.

Neutrality tests	GB 1	GB 2	GB 3	I 1	I 2	IRL 1	N 1	NL 1	PL 1	PL 2	RUM 1	RUM 2	RUM 3	RUM 4	RUM 5	RUM 6	RUS 1	RUS 2	S 2	mean	s.d.
Tajima's D test																					
Samplesize	5	2	3	10	4	2	3	3	6	3	3	4	4	3	4	3	4	5	3	4.24	2.43
S	0	0	1	1	1	0	4	0	3	1	0	2	0	0	1	3	6	1	3	1.56	2.01
Pi	0	0	0.67	0.44	0.33	0	2.67	0	1.6	0.67	0	1	0	0	0.5	2	3	0.4	2	0.79	1.03
Tajima's D	0	0	0	0.66	-2.86	0	0	0	1.12	0	0	-0.71	0	0	-0.61	0	-0.81	-0.82	0	-0.14	0.73
Tajima's D p-value	1	1	0.99	0.8	**0**	1	0.84	1	0.88	0.99	1	0.29	1	1	0.38	0.88	0.16	0.30	0.88	0.77	0.34
Fu's FS test																					
Real no. of alleles	3	2	2	4	3	1	2	2	5	2	1	3	2	1	2	2	4	2	3	2.29	1.12
Orig. no. of alleles	3	2	2	4	3	1	2	2	5	2	1	3	2	1	2	2	4	2	3	2.29	1.12
Theta_pi	0	0	0.67	0.44	0.33	0	2.67	0	1.6	0.67	0	1	0	0	0.5	2	3	0.4	2	0.79	1.03
Exp. no. of alleles	0	0	1.65	2.03	1.49	0	2.3	0	2.94	1.65	0	2.08	0	0	1.68	2.17	2.85	1.66	2.17	1.25	1.28
FS	0	0	0.2	0.74	-0.32	0	2.02	0	2.51	0.2	0	1.1	0	0	0.17	1.61	0.73	0.09	1.61	0.39	0.77
FS p-value	N.A.	N.A.	0.39	0.46	**0.04**	N.A.	0.76	N.A.	0.87	0.39	N.A.	0.62	N.A.	N.A.	0.34	0.7	0.58	0.29	0.7	N.A.	N.A.

Table A5: Results of the McDonald-Kreitman test for *Steganacarus magnus*. The differences between 90 populations are significant (*0.01<P<0.05), between other 80 populations high significant (**0.001<P<0.01) and between 63 populations extremely high significant (***P<0.001). Number of fixed and polymorph synonymous and non-synonymous mutations are shown. Locations with less than two individuals were excluded.

Population		A1			CHINA_1			CZ_1			D_1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
CHINA_1	fixed	107	46	- ns									
Nanjing	poly	0	0										
CZ_1	fixed	48	3	0.0443*	52	23	0.0286*						
Decin	poly	145	31		145	31							
D_1	fixed	96	44	0.265 ns	82	35	0.3596 ns	30	17	0.0353*			
Kranichstein	poly	15	12		16	11		156	41				
D_2	fixed	97	45	0.1867 ns	84	37	0.3204 ns	32	17	0.0163*	0	0	- ns
Goettingen	poly	5	0		5	0		148	31		17	11	
D_3	fixed	105	47	0.1055 ns	88	37	0.1122 ns	40	18	0.0383*	67	11	0.0375*
Ittendorf	poly	7	0		7	0		151	31		23	11	
D_4	fixed	40	5	0.6384 ns	45	22	0.0039**	6	0	0.5995 ns	27	17	0.0082**
Meckl. Seenpl.	poly	161	29		161	29		243	41		171	39	
D_5	fixed	53	6	0.0181*	46	16	0.8666 ns	9	1	0.6929 ns	20	5	0.8169 ns
Moerfelden	poly	148	48		148	48		229	57		160	57	
D_7	fixed	61	10	1 ns	62	37	0.0001***	24	2	0.3919 ns	52	29	0.0066**
Uelzen	poly	114	19		114	19		218	40		124	29	
D_8	fixed	95	47	1 ns	97	41	1 ns	41	42	0.0083**	81	13	0.0028**
Cuxhaven	poly	1	1		1	1		145	32		17	12	
D_9	fixed	97	45	0.1746 ns	82	36	0.1763 ns	31	17	0.0095**	0	0	- ns
Bonn	poly	10	1		10	1		152	32		17	11	
DK_1	fixed	69	24	0.3051 ns	56	23	0.7602 ns	9	2	1 ns	0	0	- ns
Copenhagen	poly	92	45		93	44		194	59		106	55	
DK_2	fixed	96	15	- ns	104	41	- ns	46	3	0.0458*	76	38	0.5044 ns
Hjorring	poly	0	0		0	0		145	31		16	11	
DK_3	fixed	97	45	0.1781 ns	84	37	0.1797 ns	32	17	0.0163*	0	0	- ns
Arhus Stadion	poly	6	0		6	0		148	31		18	11	
DK_4	fixed	98	45	0.3194 ns	86	36	0.3008 ns	33	17	0.0199*	0	0	- ns
Arhus Mo	poly	0	1		0	1		145	32		16	12	
E_1	fixed	100	44	0.1807 ns	100	38	0.195 ns	41	18	0.0399*	76	14	0.0424*
Teno Mountains	poly	6	0		6	0		149	31		22	11	
E_3	fixed	95	48	0.4282 ns	96	39	0.5968 ns	27	19	0.0028**	63	13	0.0419*
Anaga Mountains	poly	14	4		14	4		154	35		28	15	
F_1	fixed	49	4	0.0876 ns	49	21	0.038*	0	0	- ns	25	16	0.0263*
Mont Blanc	poly	162	34		162	34		228	38		170	45	
F_2	fixed	99	45	0.5706 ns	84	37	0.5759 ns	31	17	0.0175*	0	0	- ns
Loire	poly	2	0		2	0		147	31		18	11	
F_3	fixed	77	45	0.0028**	75	35	0.0225*	28	19	0.0017**	64	10	0.114 ns
Saint Isidore	poly	57	11		57	11		180	40		70	22	
F_4	fixed	96	12	- ns	102	44	- ns	44	2	0.021*	73	39	0.6561 ns
Haute Loire	poly	0	0		0	0		145	31		16	11	
FIN_1	fixed	102	17	0.0039**	98	43	0.0311*	60	3	0.1912 ns	83	37	0.1313 ns
Lahti	poly	0	3		0	3		145	34		16	14	
GB_1	fixed	78	45	0.0000***	73	36	0.0002***	27	19	0.0004***	62	10	0.5255 ns
Bedford	poly	61	6		61	6		186	35		74	17	
GB_2	fixed	95	48	1 ns	97	42	1 ns	44	23	0.0097**	85	14	0.003**
Ascot	poly	1	1		1	1		145	32		17	12	
GB_3	fixed	95	47	0.0493*	98	41	0.0332*	44	22	0.0265*	86	13	0.0003***
Braemar	poly	1	4		1	4		146	35		17	15	
I_1	fixed	98	45	1 ns	83	36	1 ns	31	17	0.0176*	0	0	- ns
Grosseto	poly	7	3		7	3		151	34		16	13	
I_2	fixed	97	44	0.3464 ns	82	36	0.3448 ns	31	16	0.0258*	0	0	- ns
Parma	poly	11	2		11	2		154	33		18	12	
IRL_1	fixed	94	47	0.5556 ns	98	41	1 ns	43	22	0.0086**	85	13	0.0058**
Swords	poly	2	0		2	0		146	31		18	11	
N_1	fixed	64	5	0.0099**	67	28	0.2239 ns	2	0	1 ns	53	23	0.6446 ns
Narvik	poly	109	30		108	31		181	36		118	44	

Table A5 continue:

Population		A1			CHINA_1			CZ_1			D_1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
NL_1	fixed	97	45	0.1867 ns	96	41	1 ns	41	22	0.0084*	82	13	0.0057**
Wageningen	poly	5	0		6	2		149	33		22	13	
PL_1	fixed	63	8	0.0289*	68	30	0.3813 ns	1	0	1 ns	39	21	0.3266 ns
Krakow	poly	107	35		106	36		193	39		118	46	
PL_2	fixed	102	43	0.5574 ns	79	39	0.3873 ns	32	19	0.0069**	56	18	0.2833 ns
Warsaw	poly	12	3		12	3		150	33		27	14	
RUM_1	fixed	100	3	0.5799 ns	89	40	0.5725 ns	40	17	0.0581 ns	68	14	0.0363*
Sibiu_1	poly	2	3		2	0		147	31		18	11	
RUM_2	fixed	97	42	0.5094 ns	87	39	0.505 ns	40	17	0.0601 ns	66	13	0.0557 ns
Sibiu_2	poly	9	2		9	2		149	32		25	13	
RUM_3	fixed	83	5	0.133 ns	96	45	0.2759 ns	23	1	0.1362 ns	83	42	0.8541 ns
Bagau	poly	15	3		15	3		152	32		31	14	
RUM_4	fixed	87	5	1 ns	96	46	0.1 ns	24	1	0.0903 ns	88	43	1 ns
Cluj	poly	7	0		7	0		148	31		23	11	
RUM_5	fixed	105	42	0.6769 ns	96	38	0.6775 ns	40	18	0.0409*	66	18	0.1055 ns
Busteni	poly	5	1		5	1		148	32		21	12	
RUM_6	fixed	83	36	0.1571 ns	72	29	0.3155 ns	17	13	0.004**	36	5	0.0827 ns
Sinaia	poly	70	19		70	19		191	44		84	29	
RUS_1	fixed	58	3	0.1278 ns	66	14	0.0000***	20	2	0.5457 ns	64	33	0.0065**
Altai Mountains	poly	114	16		114	16		213	42		124	28	
RUS_2	fixed	94	14	0.5628 ns	96	48	0.0019**	53	7	0.4178 ns	95	42	0.3076 ns
Novosibirsk	poly	33	3		33	3		160	32		47	14	
S_2	fixed	54	6	0.0398*	60	18	1 ns	0	0	- ns	28	14	0.251 ns
Stroemstad	poly	152	44		152	44		223	51		163	53	

Population		D_2			D_3			D_4			D_5		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D_3	fixed	68	12	0.2133 ns									
Ittendorf	poly	12	0										
D_4	fixed	28	17	0.0013**	29	14	0.0143*						
Meckl. Seenpl.	poly	163	23		165	29							
D_5	fixed	20	7	1 ns	27	7	0.8275 ns	0	0	- ns			
Moerfelden	poly	151	48		151	48		132	55				
D_7	fixed	52	29	0.0003***	47	33	0.0000***	12	1	0.6989 ns	17	1	0.1388 ns
Uelzen	poly	117	19		120	19		220	40		218	57	
D_8	fixed	82	15	1 ns	88	17	1 ns	40	22	0.0018**	39	9	0.4509 ns
Cuxhaven	poly	6	1		8	1		161	30		148	49	
D_9	fixed	0	0	- ns	67	11	0.4536 ns	27	17	0.0012**	20	6	1 ns
Bonn	poly	11	1		17	1		166	30		155	48	
DK_1	fixed	0	0	- ns	41	4	0.0018**	0	0	- ns	5	1	0.6891 ns
Copenhagen	poly	97	44		97	45		187	50		184	67	
DK_2	fixed	78	38	0.1794 ns	77	40	0.0952 ns	0	0	- ns	8	1	0.4627 ns
Hjorring	poly	5	0		9	0		161	29		148	48	
DK_3	fixed	0	0	- ns	68	12	0.2055 ns	27	17	0.0011**	20	7	0.8134 ns
Arhus Stadion	poly	6	0		13	0		164	29		152	48	
DK_4	fixed	0	0	- ns	70	12	1 ns	28	17	0.0017**	20	7	1 ns
Arhus Mo	poly	5	1		7	1		161	30		148	49	
E_1	fixed	38	15	0.2116 ns	89	13	0.3561 ns	47	14	0.0583 ns	34	7	0.4166 ns
Teno Mountains	poly	11	0		13	0		164	29		152	48	
E_3	fixed	66	14	1 ns	61	10	1 ns	35	17	0.0109*	30	8	0.6863 ns
Anaga Mountains	poly	19	4		21	4		170	33		158	52	
F_1	fixed	26	16	0.0129*	30	19	0.0016**	6	0	0.5978 ns	5	1	1 ns
Mont Blanc	poly	164	34		167	34		241	42		240	59	
F_2	fixed	0	0	- ns	68	12	0.353 ns	29	17	0.0016**	23	7	1 ns
Loire	poly	7	0		9	0		162	29		150	48	
F_3	fixed	64	12	1 ns	66	13	0.8271 ns	23	18	0.0002***	23	6	0.8196 ns
Saint Isidore	poly	61	11		63	11		200	39		185	58	

Table A5 continue:

Population		D_2			D_3			D_4			D_5		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
F_4	fixed	74	39	0.1692 ns	75	41	0.0941 ns	6	1	1 ns	0	1	0.2487 ns
Haute Loire	poly	5	0		7	0		161	29		148	48	
FIN_1	fixed	87	37	0.698 ns	87	39	1 ns	39	6	0.6596 ns	43	4	0.0108*
Lahti	poly	5	3		7	3		161	32		148	51	
GB_1	fixed	62	12	0.2091 ns	68	13	0.1515 ns	22	18	0.0000***	24	6	1 ns
Bedford	poly	65	6		67	6		201	34		188	53	
GB_2	fixed	87	16	1 ns	89	18	1 ns	43	23	0.0023**	42	9	0.3541 ns
Ascot	poly	5	1		8	1		161	30		148	49	
GB_3	fixed	87	15	0.0644 ns	90	17	0.2215 ns	43	22	0.0079**	42	8	0.194 ns
Braemar	poly	6	4		8	4		162	33		149	52	
I_1	fixed	0	0	- ns	67	11	0.711 ns	27	17	0.0017**	21	6	0.8192 ns
Grosseto	poly	10	3		14	3		165	32		153	50	
I_2	fixed	0	0	- ns	66	11	0.7317 ns	27	17	0.0012**	20	6	1 ns
Parma	poly	12	2		18	2		167	30		155	48	
IRL_1	fixed	86	15	0.5899 ns	89	17	0.352 ns	42	22	0.0018**	42	8	0.2584 ns
Swords	poly	7	0		9	0		163	29		150	48	
N_1	fixed	53	24	0.1424 ns	59	23	0.3375 ns	11	0	0.3702 ns	15	0	0.0467*
Narvik	poly	112	31		112	32		225	37		214	61	
NL_1	fixed	83	15	1 ns	86	17	1 ns	40	22	0.0019**	39	8	0.3392 ns
Wageningen	poly	11	2		13	2		165	31		153	49	
PL_1	fixed	39	21	0.1209 ns	40	23	0.0657 ns	0	0	- ns	0	0	- ns
Krakow	poly	112	35		112	35		205	40		195	61	
PL_2	fixed	54	20	0.3825 ns	72	17	0.7591 ns	31	15	0.0107*	29	6	0.5137 ns
Warsaw	poly	17	3		19	3		166	30		154	48	
RUM_1	fixed	71	16	0.351 ns	78	16	0.3507 ns	37	13	0.092 ns	37	4	0.0394*
Sibiu_1	poly	7	0		8	0		163	29		149	48	
RUM_2	fixed	69	15	0.7327 ns	76	15	0.7363 ns	37	13	0.0943 ns	36	4	0.0584 ns
Sibiu_2	poly	14	2		15	2		166	30		153	49	
RUM_3	fixed	88	42	0.0817 ns	78	45	0.0185*	29	3	0.5851 ns	39	5	0.0726 ns
Bagau	poly	20	3		22	3		168	29		157	49	
RUM_4	fixed	93	43	0.0189*	80	45	0.0049**	28	3	0.5846 ns	42	5	0.0486*
Cluj	poly	12	0		14	0		166	29		151	48	
RUM_5	fixed	67	20	0.454 ns	74	18	0.4546 ns	34	15	0.0227*	31	5	0.1984 ns
Busteni	poly	9	1		12	1		163	30		149	49	
RUM_6	fixed	38	6	0.3566 ns	44	7	0.3713 ns	18	12	0.0057**	11	2	0.534 ns
Sinaia	poly	73	19		74	19		197	40		185	60	
RUS_1	fixed	66	34	0.0001***	51	36	0.0000***	16	1	0.4824 ns	22	1	0.0572 ns
Altai Mountains	poly	117	16		120	16		228	39		222	60	
RUS_2	fixed	95	42	0.0019**	91	46	0.0003***	35	7	0.6351 ns	47	6	0.0864 ns
Novosibirsk	poly	38	3		40	3		184	30		170	49	
S_2	fixed	30	15	0.1236 ns	29	13	0.2309 ns	0	0	- ns	2	0	1 ns
Stroemstad	poly	156	44		157	44		238	49		219	64	

Population		D_7			D_8			D_9			DK_1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D_8	fixed	53	32	0.0002***									
Cuxhaven	poly	115	20										
D_9	fixed	52	29	0.0004***	82	14	1 ns						
Bonn	poly	119	20		11	2							
DK_1	fixed	31	12	0.7017 ns	50	9	0.0098**	0	0	- ns			
Copenhagen	poly	176	57		93	46		101	45				
DK_2	fixed	50	4	0.2284 ns	97	44	1 ns	76	38	0.1704 ns	44	17	0.5119 ns
Hjørring	poly	114	19		1	1		10	1		92	45	
DK_3	fixed	52	29	0.0003***	82	15	1 ns	0	0	- ns	0	0	- ns
Arhus Stadion	poly	118	19		7	1		12	1		97	44	
DK_4	fixed	54	29	0.0008***	85	15	0.07 ns	0	0	- ns	0	0	- ns
Arhus Mo	poly	114	20		1	2		10	2		93	45	

Table A5 continue:

Population		D_7			D_8			D_9			DK_1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
E_1	**fixed**	63	34	0.0002***	97	17	1 ns	76	14	0.4566 ns	53	5	0.0005***
Teno Mountains	**poly**	120	19		7	1		16	1		97	45	
E_3	**fixed**	52	34	0.0007***	82	14	0.3169 ns	64	13	1 ns	42	4	0.0011**
Anaga Mountains	**poly**	123	23		15	5		24	5		103	49	
F_1	**fixed**	15	0	0.1382 ns	44	24	0.004**	27	16	0.0066**	11	2	0.739 ns
Mont Blanc	**poly**	222	41		162	35		166	35		207	62	
F_2	**fixed**	53	29	0.0004***	83	15	1 ns	0	0	- ns	4	0	0.3108 ns
Loire	**poly**	116	19		3	1		12	1		94	44	
F_3	**fixed**	43	30	0.0001***	2	0	1 ns	65	11	1 ns	38	6	0.0371*
Saint Isidore	**poly**	154	30		57	12		64	12		134	55	
F_4	**fixed**	52	3	0.0903 ns	95	45	1 ns	73	39	0.1011 ns	42	17	0.6189 ns
Haute Loire	**poly**	114	19		1	1		10	1		92	45	
FIN_1	**fixed**	54	5	0.1808 ns	102	39	0.0265*	84	37	1 ns	61	20	0.1756 ns
Lahti	**poly**	114	22		1	4		10	4		93	47	
GB_1	**fixed**	43	30	0.0000***	0	0	- ns	63	11	0.3263 ns	36	6	0.1122 ns
Bedford	**poly**	160	25		61	7		68	7		137	50	
GB_2	**fixed**	55	33	0.0002***	5	1	0.5 ns	86	15	1 ns	55	10	0.0073**
Ascot	**poly**	115	20		2	2		11	2		92	46	
GB_3	**fixed**	55	32	0.0007***	5	0	0.0278*	87	14	0.1353 ns	55	9	0.0025**
Braemar	**poly**	115	22		2	5		11	5		93	49	
I_1	**fixed**	52	29	0.0009***	84	14	0.2092 ns	0	0	- ns	2	0	0.562 ns
Grosseto	**poly**	118	22		8	4		10	3		98	47	
I_2	**fixed**	52	29	0.0004***	81	14	0.6998 ns	0	0	- ns	0	0	- ns
Parma	**poly**	120	20		12	3		12	2		101	45	
IRL_1	**fixed**	55	32	0.0001***	5	0	0.4444 ns	86	14	0.6936 ns	54	9	0.0064**
Swords	**poly**	116	19		3	1		12	1		94	45	
N_1	**fixed**	32	5	0.6438 ns	56	30	0.0448*	53	23	0.1952 ns	25	3	0.0669 ns
Narvik	**poly**	198	43		109	31		116	33		172	67	
NL_1	**fixed**	53	32	0.0002***	0	0	- ns	83	14	1 ns	51	9	0.01*
Wageningen	**poly**	117	21		7	3		16	3		97	47	
PL_1	**fixed**	28	1	0.0239*	57	29	0.1759 ns	39	21	0.1242 ns	13	3	0.5674 ns
Krakow	**poly**	190	47		107	36		113	36		168	67	
PL_2	**fixed**	43	29	0.0001***	81	20	0.7475 ns	56	19	0.4191 ns	32	8	0.1733 ns
Warsaw	**poly**	121	21		13	4		21	4		101	47	
RUM_1	**fixed**	58	28	0.0014**	81	15	1 ns	68	15	0.4601 ns	43	6	0.0054**
Sibiu_1	**poly**	116	19		3	1		12	1		94	45	
RUM_2	**fixed**	56	28	0.0012**	79	15	0.6915 ns	66	14	0.7598 ns	42	6	0.0087**
Sibiu_2	**poly**	122	20		10	3		19	3		99	46	
RUM_3	**fixed**	50	8	1 ns	85	46	0.2119 ns	84	42	0.0662 ns	54	23	0.8797 ns
Bagau	**poly**	125	22		16	4		24	4		102	47	
RUM_4	**fixed**	53	10	0.829 ns	91	47	0.272 ns	89	43	0.0148*	56	23	0.7618 ns
Cluj	**poly**	119	19		8	1		17	1		97	45	
RUM_5	**fixed**	53	29	0.0007***	92	17	0.6145 ns	66	19	0.5131 ns	41	9	0.0689 ns
Busteni	**poly**	118	20		6	2		15	2		96	45	
RUM_6	**fixed**	36	25	0.0004***	57	7	0.0876 ns	37	5	0.2423 ns	17	3	0.2922 ns
Sinaia	**poly**	160	35		71	20		77	20		142	55	
RUS_1	**fixed**	31	4	0.6243 ns	61	42	0.0000***	64	34	0.0001***	43	14	1 ns
Altai Mountains	**poly**	192	34		115	17		118	17		179	59	
RUS_2	**fixed**	50	8	1 ns	95	43	0.0122*	94	42	0.0028**	61	22	0.7668 ns
Novosibirsk	**poly**	136	22		34	4		42	4		120	48	
S_2	**fixed**	20	1	0.141 ns	46	17	0.501 ns	29	14	0.1649 ns	9	2	0.7362 ns
Stroemstad	**poly**	227	52		152	45		159	44		200	68	

Table A5 continue:

Population		DK_2			DK_3			DK_4			E_1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
DK_3	fixed	78	38	0.1753 ns									
Arhus Stadion	poly	6	0										
DK_4	fixed	79	38	0.3305 ns	0	0	- ns						
Arhus Mo	poly	0	1		6	1							
E_1	fixed	92	43	0.1775 ns	76	15	0.2061 ns	78	15	1 ns			
Teno Mountains	poly	6	0		12	0		6	1				
E_3	fixed	81	45	0.3005 ns	66	14	1 ns	67	14	0.5155 ns	61	5	0.2191 ns
Anaga Mountains	poly	14	4		20	4		14	5		18	4	
F_1	fixed	33	1	0.0357*	29	16	0.0128*	30	16	0.0141*	41	22	0.0043**
Mont Blanc	poly	162	34		165	34		162	34		166	34	
F_2	fixed	80	38	0.5636 ns	0	0	- ns	4	0	0.4286 ns	78	15	0.3569 ns
Loire	poly	2	0		8	0		2	1		8	0	
F_3	fixed	80	41	0.0106*	64	12	1 ns	67	12	0.824 ns	72	15	0.8305 ns
Saint Isidore	poly	57	11		62	11		57	12		62	11	
F_4	fixed	17	3	- ns	74	39	0.1008 ns	75	39	0.3478 ns	95	42	0.1801 ns
Haute Loire	poly	0	0		6	0		0	1		6	0	
FIN_1	fixed	91	15	0.0039**	87	37	1 ns	89	37	0.0089**	108	42	0.7134 ns
Lahti	poly	0	3		6	3		0	4		6	3	
GB_1	fixed	80	41	0.0001***	62	12	0.2082 ns	65	12	0.4609 ns	70	15	0.105 ns
Bedford	poly	61	6		66	6		61	7		65	6	
GB_2	fixed	100	45	1 ns	87	16	1 ns	89	16	0.0714 ns	95	18	1 ns
Ascot	poly	1	1		6	1		1	2		7	1	
GB_3	fixed	101	44	0.0364*	87	15	0.0874 ns	90	15	0.0006***	93	18	0.2113 ns
Braemar	poly	1	4		7	4		1	5		7	4	
I_1	fixed	76	38	1 ns	0	0	- ns	2	0	0.5385 ns	76	14	0.7185 ns
Grosseto	poly	7	3		11	3		7	4		13	3	
I_2	fixed	76	37	0.2301 ns	0	0	- ns	0	0	- ns	76	14	0.7331 ns
Parma	poly	11	2		13	2		11	3		17	2	
IRL_1	fixed	101	44	0.5777 ns	86	15	0.3708 ns	89	15	0.3879 ns	95	17	0.3677 ns
Swords	poly	2	0		8	0		2	1		8	0	
N_1	fixed	73	5	0.0022**	52	24	0.14 ns	55	24	0.2594 ns	68	25	0.2748 ns
Narvik	poly	108	31		113	31		108	32		115	30	
NL_1	fixed	98	44	1 ns	83	15	1 ns	86	15	0.1634 ns	94	17	1 ns
Wageningen	poly	6	2		12	2		6	3		12	2	
PL_1	fixed	0	0	- ns	39	21	0.1201 ns	40	21	0.2326 ns	50	23	0.2553 ns
Krakow	poly	107	35		113	35		107	36		113	35	
PL_2	fixed	84	34	0.5576 ns	54	20	0.2663 ns	56	2	1 ns	80	17	1 ns
Warsaw	poly	12	3		18	3		12	4		18	3	
RUM_1	fixed	88	36	1 ns	71	16	0.344 ns	72	16	0.4664 ns	86	20	0.3469 ns
Sibiu_1	poly	2	0		8	0		2	1		8	0	
RUM_2	fixed	86	35	0.5163 ns	69	15	0.7301 ns	70	15	0.6908 ns	84	15	0.7339 ns
Sibiu_2	poly	15	3		15	2		9	3		15	2	
RUM_3	fixed	85	11	0.6951 ns	88	42	0.0539 ns	88	42	0.4291 ns	94	43	0.0844 ns
Bagau	poly	5	1		21	3		15	4		20	3	
RUM_4	fixed	86	13	0.5934 ns	93	43	0.0114*	93	43	0.4352 ns	99	43	0.0201*
Cluj	poly	7	0		13	0		7	1		13	0	
RUM_5	fixed	92	35	0.6816 ns	66	20	0.4471 ns	68	20	1 ns	89	14	1 ns
Busteni	poly	5	1		10	1		5	2		11	1	
RUM_6	fixed	63	29	0.1845 ns	38	6	0.3566 ns	38	6	0.258 ns	58	5	0.0425*
Sinaia	poly	69	20		73	19		70	20		75	19	
RUS_1	fixed	62	9	1 ns	66	34	0.0001***	67	34	0.0002***	76	36	0.0001***
Altai Mountains	poly	114	16		118	16		114	17		117	16	
RUS_2	fixed	96	21	0.1991 ns	95	42	0.0019**	97	43	0.013*	99	41	0.0021**
Novosibirsk	poly	33	3		39	3		33	4		39	3	
S_2	fixed	0	0	- ns	30	15	0.1226 ns	31	15	0.1853 ns	35	14	0.347 ns
Stroemstad	poly	152	44		157	44		152	45		157	44	

Table A5 continue:

Population		E_3			F_1			F_2			F_3		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
F_1	fixed	32	21	0.0017**									
Mont Blanc	poly	169	38										
F_2	fixed	68	14	0.7492 ns	31	16	0.0153*						
Loire	poly	16	4		163	34							
F_3	fixed	59	12	1 ns	30	19	0.0038**	65	12	1 ns			
Saint Isidore	poly	70	15		194	44		59	11				
F_4	fixed	81	44	0.3043 ns	33	1	0.0357*	76	39	0.5517 ns	77	43	0.0044**
Haute Loire	poly	14	4		162	34		2	0		57	11	
FIN_1	fixed	96	42	0.8028 ns	35	1	0.0136*	88	37	0.3228 ns	80	37	0.0911 ns
Lahti	poly	14	7		162	37		2	3		57	14	
GB_1	fixed	60	12	0.4927 ns	29	19	0.0006***	63	12	0.2149 ns	0	0	- ns
Bedford	poly	72	10		200	39		63	6		67	11	
GB_2	fixed	85	15	0.3236 ns	45	25	0.0042**	87	16	1 ns	0	0	- ns
Ascot	poly	15	5		163	35		3	1		58	12	
GB_3	fixed	84	15	0.0401*	45	24	0.0122*	88	15	0.0082**	0	0	- ns
Braemar	poly	15	8		162	38		2	4		58	15	
I_1	fixed	64	13	0.4023 ns	27	16	0.0129*	0	0	- ns	67	11	0.5171 ns
Grosseto	poly	21	7		166	37		9	3		62	14	
I_2	fixed	64	13	0.7836 ns	27	15	0.02*	0	0	- ns	65	11	0.8246 ns
Parma	poly	25	6		167	36		13	2		64	13	
IRL_1	fixed	85	14	0.5017 ns	44	24	0.0034**	87	15	1 ns	0	0	- ns
Swords	poly	16	4		164	34		4	0		59	11	
N_1	fixed	50	28	0.0417*	9	1	0.704 ns	55	24	0.1949 ns	41	27	0.0033**
Narvik	poly	120	34		227	46		110	31		151	39	
NL_1	fixed	83	14	0.3618 ns	43	24	0.0034**	84	15	0.6536 ns	0	0	- ns
Wageningen	poly	19	6		166	35		8	2		60	12	
PL_1	fixed	38	26	0.0337*	15	1	0.3239 ns	41	21	0.174 ns	39	24	0.0213*
Krakow	poly	117	39		212	45		109	35		153	45	
PL_2	fixed	71	16	0.7963 ns	30	18	0.0051**	55	20	0.5468 ns	59	17	0.5518 ns
Warsaw	poly	26	7		167	35		14	3		63	14	
RUM_1	fixed	71	17	1 ns	33	17	0.0174*	73	16	0.5991 ns	59	12	1 ns
Sibiu_1	poly	16	4		164	34		4	0		59	11	
RUM_2	fixed	69	17	1 ns	32	17	0.0102*	71	15	1 ns	57	12	1 ns
Sibiu_2	poly	23	6		168	35		11	2		71	14	
RUM_3	fixed	82	46	0.0711 ns	38	2	0.0558 ns	85	42	0.266 ns	66	44	0.0005***
Bagau	poly	29	7		170	34		22	6		62	11	
RUM_4	fixed	88	46	0.0993 ns	41	2	0.0354*	93	43	0.0581 ns	71	45	0.0006***
Cluj	poly	21	4		165	34		9	0		61	11	
RUM_5	fixed	70	15	0.7674 ns	27	18	0.0022**	69	20	0.6823 ns	66	14	0.8273 ns
Busteni	poly	19	5		165	35		7	1		62	11	
RUM_6	fixed	41	5	0.1145 ns	17	13	0.0044**	41	6	0.2551 ns	37	6	0.3878 ns
Sinaia	poly	79	23		200	47		71	19		115	29	
RUS_1	fixed	61	40	0.0000***	23	0	0.0317*	65	35	0.0000***	46	39	0.0000***
Altai Mountains	poly	122	20		224	44		116	16		159	27	
RUS_2	fixed	100	43	0.025*	38	7	1 ns	96	42	0.0034**	85	41	0.0078*
Novosibirsk	poly	44	7		179	35		35	3		67	15	
S_2	fixed	27	14	0.1651 ns	6	0	0.5957 ns	32	15	0.1849 ns	31	14	0.2532 ns
Stroemstad	poly	161	48		241	54		154	44		185	54	

Population		F_4			FIN_1			GB_1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
FIN_1	fixed	91	14	0.0033**						
Lahti	poly	0	3							
GB_1	fixed	77	43	0.0000***	83	37	0.0051**			
Bedford	poly	61	6		61	9				
GB_2	fixed	97	46	1 ns	101	40	0.0289*	0	0	- ns
Ascot	poly	1	1		1	4		62	7	

Table A5 continue:

Population		F_4			FIN_1			GB_1			GB_2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
GB_3	fixed	98	45	0.0414*	101	39	0.0012**	0	0	- ns	0	1	1 ns
Braemar	poly	1	4		1	7		62	10		2	5	
I_1	fixed	73	39	1 ns	85	37	1 ns	65	11	0.811 ns	88	15	0.2117 ns
Grosseto	poly	7	3		7	6		66	9		8	4	
I_2	fixed	72	38	0.2186 ns	83	37	1 ns	63	11	0.4694 ns	85	15	0.7025 ns
Parma	poly	11	2		11	5		68	8		12	3	
IRL_1	fixed	98	45	0.569 ns	102	39	0.1434 ns	0	0	- ns	0	1	0.4 ns
Swords	poly	2	0		2	3		63	9		3	1	
N_1	fixed	70	4	0.0016**	80	10	0.0163*	40	27	0.0004***	59	31	0.0475*
Narvik	poly	109	30		108	34		157	34		110	31	
NL_1	fixed	95	45	1 ns	99	39	0.3022 ns	0	0	- ns	0	0	- ns
Wageningen	poly	6	2		6	5		62	7		7	3	
PL_1	fixed	0	0	- ns	60	8	0.0198*	38	24	0.0064**	60	30	0.1813 ns
Krakow	poly	107	35		107	38		157	40		108	36	
PL_2	fixed	81	37	0.5516 ns	92	38	0.7848 ns	59	17	0.0887 ns	80	21	1 ns
Warsaw	poly	12	3		12	6		68	9		13	4	
RUM_1	fixed	89	39	0.5767 ns	93	37	0.1542 ns	60	12	0.2083 ns	82	16	1 ns
Sibiu_1	poly	2	0		2	3		63	6		3	1	
RUM_2	fixed	87	38	0.5072 ns	91	36	0.7574 ns	58	12	0.2388 ns	80	16	0.6957 ns
Sibiu_2	poly	9	2		9	5		70	8		10	3	
RUM_3	fixed	85	9	0.4054 ns	96	16	0.1969 ns	65	44	0.0000***	87	47	0.2125 ns
Bagau	poly	15	3		15	6		75	9		16	4	
RUM_4	fixed	88	10	0.6182 ns	95	17	0.3653 ns	71	45	0.0000***	93	48	0.2723 ns
Cluj	poly	7	0		7	3		65	6		8	1	
RUM_5	fixed	87	38	0.6687 ns	89	38	0.4574 ns	69	14	0.2425 ns	92	18	0.6015 ns
Busteni	poly	5	1		5	4		65	7		5	2	
RUM_6	fixed	58	32	0.0699 ns	69	31	0.4224 ns	40	5	0.4795 ns	57	9	0.2134 ns
Sinaia	poly	69	20		69	23		117	24		70	20	
RUS_1	fixed	57	6	0.6372 ns	67	10	0.8388 ns	46	39	0.0000***	62	43	0.0000***
Altai Mountains	poly	114	16		114	19		162	22		115	17	
RUS_2	fixed	97	18	0.4081 ns	101	18	1 ns	83	41	0.0002***	99	44	0.0126*
Novosibirsk	poly	33	3		33	6		82	10		34	4	
S_2	fixed	8	1	0.687 ns	49	6	0.0406*	31	14	0.1696 ns	49	18	0.5102 ns
Stroemstad	poly	152	44		152	47		190	49		152	45	

Population		GB_3			I_1			I_2			IRL_1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
I_1	fixed	89	14	0.0055**									
Grosseto	poly	8	7										
I_2	fixed	86	14	0.0801 ns	0	0	- ns						
Parma	poly	12	6		11	4							
IRL_1	fixed	0	0	- ns	88	14	0.3336 ns	85	14	1 ns			
Swords	poly	3	4		114	35		13	2				
N_1	fixed	59	30	0.0991 ns	53	23	0.1685 ns	53	22	0.3267 ns	58	30	0.0444*
Narvik	poly	110	34		13	5		116	34		110	30	
NL_1	fixed	0	0	- ns	85	14	0.1685 ns	82	14	0.7381 ns	0	0	- ns
Wageningen	poly	7	6		13	5		17	4		8	2	
PL_1	fixed	61	29	0.3764 ns	39	21	0.1776 ns	39	20	0.2265 ns	61	29	0.2279 ns
Krakow	poly	108	39		111	38		114	37		109	35	
PL_2	fixed	79	20	0.2394 ns	56	19	1 ns	55	18	0.6013 ns	80	20	1 ns
Warsaw	poly	13	7		18	6		22	5		14	3	
RUM_1	fixed	81	16	0.0244*	68	15	0.6929 ns	66	14	1 ns	83	15	1 ns
Sibiu_1	poly	3	4		9	3		13	2		4	0	
RUM_2	fixed	76	16	0.0847 ns	66	14	0.5364 ns	64	13	1 ns	81	15	1 ns
Sibiu_2	poly	10	6		16	5		20	4		11	2	
RUM_3	fixed	87	46	0.8138 ns	85	42	0.266 ns	83	41	0.0795 ns	87	46	0.1212 ns
Bagau	poly	16	7		22	6		26	2		17	3	

Table A5 continue:

Population		GB_3			I_1			I_2			IRL_1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM_4	fixed	93	47	1 ns	90	43	0.273 ns	88	42	0.062 ns	93	47	0.0576 ns
Cluj	poly	8	4		14	3		18	5		9	0	
RUM_5	fixed	91	17	0.0299*	68	19	1 ns	66	18	0.7571 ns	92	17	1 ns
Busteni	poly	6	5		12	4		16	3		7	1	
RUM_6	fixed	57	8	0.067 ns	38	5	0.166 ns	36	5	0.3358 ns	56	8	0.2021 ns
Sinaia	poly	70	23		76	22		79	20		72	19	
RUS_1	fixed	63	42	0.0000***	64	34	0.0002***	63	33	0.0002***	63	42	0.0000***
Altai Mountains	poly	115	20		117	19		119	18		116	16	
RUS_2	fixed	99	43	0.1131 ns	95	42	0.0002***	94	42	0.0041**	99	43	0.0035**
Novosibirsk	poly	34	7		39	6		43	5		35	3	
S_2	fixed	49	17	0.8688 ns	29	14	0.2402 ns	29	14	0.1643 ns	49	17	0.6134 ns
Stroemstad	poly	153	48		157	46		160	44		154	44	

Population		N_1			NL_1			PL_1			PL_2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
NL_1	fixed	56	30	0.0454*									
Wageningen	poly	113	32										
PL_1	fixed	26	0	0.0066**	57	29	0.1747 ns						
Krakow	poly	191	46		113	37							
PL_2	fixed	50	24	0.1031 ns	79	20	1 ns	42	18	0.4882 ns			
Warsaw	poly	118	33		17	4		118	38				
RUM_1	fixed	65	25	0.3468 ns	79	15	1 ns	47	20	0.4039 ns	66	9	0.6893 ns
Sibiu_1	poly	110	31		8	2		109	35		14	3	
RUM_2	fixed	63	25	0.2731 ns	77	15	0.7378 ns	46	20	0.4 ns	63	8	0.325 ns
Sibiu_2	poly	115	32		15	4		113	36		21	5	
RUM_3	fixed	44	1	0.0013**	83	46	0.1156 ns	47	8	0.1806 ns	87	42	0.138 ns
Bagau	poly	120	33		21	5		115	36		26	6	
RUM_4	fixed	46	1	0.0012**	89	47	0.1452 ns	50	9	0.1913 ns	90	43	0.0835 ns
Cluj	poly	114	30		13	2		110	35		19	3	
RUM_5	fixed	55	24	0.1935 ns	89	17	0.7019 ns	50	19	0.7378 ns	53	3	0.0833 ns
Busteni	poly	113	31		11	3		111	36		17	4	
RUM_6	fixed	32	18	0.0674 ns	55	7	0.1319 ns	24	14	0.1598 ns	35	3	0.1242 ns
Sinaia	poly	165	47		74	20		157	51		79	20	
RUS_1	fixed	30	1	0.0376*	60	42	0.0000***	33	4	0.2584 ns	72	34	0.0004***
Altai Mountains	poly	194	43		119	17		196	47		122	18	
RUS_2	fixed	66	10	0.2774 ns	95	43	0.0102*	62	11	0.2924 ns	91	43	0.0126*
Novosibirsk	poly	136	33		39	5		134	37		40	6	
S_2	fixed	15	0	0.051 ns	45	17	0.4952 ns	0	0	- ns	30	11	0.5422 ns
Stroemstad	poly	222	55		158	46		160	49		159	45	

Population		RUM_1			RUM_2			RUM_3			RUM_4		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM_2	fixed	0	0	- ns									
Sibiu_2	poly	9	2										
RUM_3	fixed	96	41	0.1938 ns	93	40	0.2516 ns						
Bagau	poly	17	3		22	5							
RUM_4	fixed	97	42	0.0609 ns	94	41	0.1012 ns	5	0	1 ns			
Cluj	poly	9	0		16	2		20	3				
RUM_5	fixed	70	8	1 ns	69	7	0.3828 ns	101	42	0.3187 ns	102	42	0.1156 ns
Busteni	poly	7	1		14	3		19	4		12	1	
RUM_6	fixed	71	16	0.351 ns	41	3	0.0319*	72	35	0.1226 ns	73	36	0.0828 ns
Sinaia	poly	7	0		77	21		79	23		74	20	
RUS_1	fixed	70	35	0.0001***	70	34	0.0004***	27	0	0.0457*	33	1	0.2001 ns
Altai Mountains	poly	116	16		120	18		123	19		118	16	
RUS_2	fixed	89	41	0.003**	88	41	0.0038**	84	15	0.4541 ns	85	15	0.2728 ns
Novosibirsk	poly	35	3		42	5		45	5		39	3	
S_2	fixed	27	10	0.5271 ns	26	10	0.5195 ns	38	7	0.4198 ns	39	8	0.5527 ns
Stroemstad	poly	154	44		158	45		160	45		155	44	

Table A5 continue:

Population		RUM_5			RUM_6			RUS_1			RUS_2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM_6	**fixed**	0	0	- ns									
Sinaia	**poly**	72	20										
RUS_1	**fixed**	77	35	0.0005***	50	30	0.0005***						
Altai Mountains	**poly**	119	17		165	34							
RUS_2	**fixed**	98	41	0.0127*	80	34	0.0929 ns	50	11	0.2818 ns			
Novosibirsk	**poly**	37	4		95	23		135	19				
S_2	**fixed**	35	9	0.8432 ns	13	7	0.2646 ns	26	2	0.1284 ns	43	7	0.4244 ns
Stroemstad	**poly**	154	45		194	54		228	56		175	44	

Appendix

Table A6: Neutrality indices of *Steganacarus magnus* computed in the McDonald-Kreitman test with DnaSP v5 of 41 locations. Locations with less than two individuals were excluded.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1 A 1																																								
2 CHINA 1	-																																							
3 CZ 1	3.4	0.5																																						
4 D 1	1.7	1.6	0.5																																					
5 D 2	0	0	0.4	-																																				
6 D 3	0	0	0.5	2.9	0																																			
7 D 4	1.4	0.4	-	0.4	0.3	0.4																																		
8 D 5	2.9	0.9	2.2	1.2	0.9	1.2	-																																	
9 D 7	1.0	0.3	2.2	0.4	0.3	0.2	2.2	4.4																																
10 D 8	2.0	2.4	0.4	4.4	1	0.6	0.3	1.4	0.3																															
11 D 9	0.2	0.2	0.4	-	-	0.4	0.3	1	0.3	1.1																														
12 DK 1	1.4	1.2	1.4	-	-	4.8	-	1.8	0.8	2.7	-																													
13 DK 2	-	-	3.3	1.4	0	0	-	2.6	2.1	2.2	0.2	1.3																												
14 DK 3	0	0	0.4	-	-	0	0.3	0.9	0.3	0.8	-	-	0																											
15 DK 4	-	-	0.4	-	-	0.8	0.3	0.9	0.3	11.3	-	-	-	-																										
16 E 1	0	0	0.5	2.7	0	0	0.5	1.5	0.3	0.8	0.3	4.9	0	0	0.9																									
17 E 3	0.6	0.7	0.3	2.6	1	1.2	0.4	1.2	0.3	2	1	5	0.5	0.9	1.7	2.7																								
18 F 1	2.6	0.5	-	0.4	0.4	0.3	-	1.2	-	0.4	0.4	1.6	6.9	0.4	0.4	0.4	0.3																							
19 F 2	0	0	0.4	-	-	0	0.3	1.1	0.3	1.8	-	-	0	-	-	0	1.2	0.4																						
20 F 3	0.3	0.4	0.3	2	1	0.9	0.2	1.2	0.3	-	1.1	2.6	0.4	0.9	1.2	0.9	1.1	0.4	1																				
21 F 4	-	-	4.7	1.3	0	0	1.1	0	2.9	2.1	0.2	1.2	-	0	-	0	0.5	6.9	0	0.3																			
22 FIN 1	-	-	1.8	2	1.4	1	1.3	3.7	2.1	10.5	0.9	1.5	-	1.2	-	1.3	1.1	8	3.6	0.5	-																	
23 GB 1	0.2	0.2	0.3	1.4	0.5	0.5	0.2	1.1	0.2	-	0.6	2.2	0.2	0.5	0.6	0.4	0.7	0.3	0.5	-	0.2	0.3																
24 GB 2	2.0	2.3	0.4	4.3	1.1	0.6	0.3	1.5	0.3	5	1	2.8	2.2	0.9	11.1	0.8	1.9	0.4	1.8	-	2.1	10.1	-															
25 GB 3	8.1	9.6	0.5	5.8	3.9	2.6	0.4	1.8	0.3	-	2.8	3.2	9.2	3.3	30	3	3	0.4	11.7	-	8.7	18.1	-	0														
26 I 1	0.9	1.0	0.4	-	-	1.3	0.3	1.1	0.3	3.0	-	-	0.9	-	-	1.3	1.6	0.4	-	1.4	0.8	2	0.8	2.9	5.6													
27 I 2	0.4	0.4	0.4	-	-	0.7	0.3	1	0.3	1.4	-	-	0.4	-	-	0.6	1.2	0.4	-	1.2	0.3	1	0.7	1.4	3.1	-												
28 IRL 1	0	0	0.4	4	0	0	0.3	1.7	0.3	-	0.5	2.9	0	0	3	0	1.5	0.4	0	-	0	3.9	-	0	-	2.1	0.9											
29 N 1	3.5	0.7	-	0.9	0.6	0.7	-	-	1.4	0.5	0.7	3.2	4.2	0.6	0.7	0.7	0.5	1.8	0.6	0.4	4.8	2.5	0.3	0.5	0.6	0.7	0.7	0.5										
30 NL 1	0	0.8	0.4	3.7	1	0.8	0.3	1.6	0.3	-	1.1	2.7	0.7	0.9	2.9	0.9	1.9	0.4	1.4	-	0.7	2.1	-	-	-	2.3	1.4	-	0.5									
31 PL 1	2.6	0.8	-	0.7	0.6	0.5	-	-	6.9	0.7	0.6	1.7	-	0.6	0.6	0.7	0.5	3.2	0.6	0.5	-	2.7	0.4	0.7	0.8	0.6	0.6	0.7	-	0.6								
32 PL 2	0.6	0.5	0.4	1.6	0.5	0.7	0.4	1.5	0.3	1.2	0.6	1.9	0.6	0.5	0.9	0.8	1.2	0.3	0.6	0.8	0.5	1.2	0.5	1.2	2.1	1	0.7	0.9	0.6	0.9	0.8							
33 RUM 1	0	0	0.5	3	0	0	0.5	3	0.3	1.8	0.4	3.4	0	0	2.3	0	1	0.4	0	0.9	0.0	3.8	0.5	1.7	6.8	1.5	0.7	0	0.7	1.3	0.8	1.6							
34 RUM 2	0.5	0.5	0.5	2.6	0.7	0.7	0.5	2.9	0.3	1.6	0.7	3.3	0.5	0.6	1.6	0.6	1.1	0.4	0.9	0.9	0.5	1.4	0.6	1.5	3	1.5	1	1	0.7	1.4	0.7	1.9	-						
35 RUM 3	3.3	0.4	4.8	0.9	0.3	0.2	1.7	2.4	1.1	0.5	0.3	1.1	1.5	0.3	0.6	0.3	0.4	3.8	0.6	0.3	1.9	2.4	0.2	0.5	0.8	0.6	0.4	0.3	12.1	0.4	1.8	0.5	0.4	0.5					
36 RUM 4	0	0	5	1	0	0	1.6	2.7	0.8	0.2	0.1	1.1	0.0	0	0.3	0	0.4	4.2	0	0.3	0	2.4	0.1	0.2	1	0.4	0.2	0	12	0.3	1.8	0.3	0.0	0.3	-				
37 RUM 5	1	1	0.5	2.1	0.4	0.3	0.4	2	0.3	1.8	0.5	2.1	0.5	0.3	1.4	0.6	1.2	0.3	0.5	0.8	0.5	1.9	0.5	2	4.5	1.2	0.7	0.8	0.6	1.4	0.9	4.2	1.3	2.1	0.5	0.2			
38 RUM 6	1	1	0.3	2.5	1.6	1.6	0.3	1.8	0.3	2.3	1.9	2.2	0.6	1.6	1.8	2.9	2.4	0.3	1.8	1.6	0.5	0.7	1.6	1.8	2.3	2.2	1.8	1.8	0.5	2.1	0.6	3.0	0.0	3.7	0.6	0.5	-		
39 RUS 1	3	0	2	0.4	0.3	0.2	2.7	5.9	1.4	0.2	0.3	1	1	0.3	0.3	0.3	0.3	-	0.3	0.2	1.3	1.1	0.2	0.2	0.3	0.3	0.3	0.2	6.6	0.2	2	0.3	0.3	0.3	-	4.5	0.3	0.3	
40 RUS 2	1	0	1.5	0.7	0.2	0.1	0.8	2.3	1	0.3	0.2	1.1	0.4	0.2	0.3	0.2	0.4	1.1	0.2	0.4	0.5	1	0.2	0.3	0.5	0.3	0.3	0.2	1.6	0.3	1.6	0.3	0.2	0.3	0.6	0.4	0.3	0.6	0.6	
41 S 2	3	1	-	0.7	0.6	0.6	-	-	4.6	0.8	0.6	1.5	-	0.6	0.6	0.7	0.6	-	0.6	0.6	2.3	2.5	0.6	0.8	0.9	0.6	0.6	0.8	-	0.8	-	0.8	0.8	0.7	1.5	1.4	1.1	0.5	3.2	1.5

Table A7: Standard diversity measures of the *COI* gene of *Steganacarus magnus*. Populations with less than two individuals were excluded (Chapter III).

population	sample size n	invarible sites N_{is}	variable sites N_{vs}	parsimony inform. sites N_{pars}	number of singeltons N_s	number of haplotypes N_h	haplotype diversity H_d	variance	nucleotide diversity Π_n
A_1	2	597	0	0	0	1	0	0	0
CZ_1	5	405	192	38	154	5	1	0.02	0.158
D_1	14	562	35	16	19	9	0.84	0.01	0.02
D_2	9	383	214	142	72	5	0.83	0.01	0.16
DK_1	3	438	159	0	159	3	1	0.07	0.181
F_1	4	595	2	0	2	3	0.83	0.05	0.002
GB_1	5	521	76	6	70	4	0.9	0.03	0.053
I_1	10	583	14	11	3	5	0.76	0.02	0.011
PL_1	6	438	159	152	7	6	1	0.01	0.142
RUM_1	4	577	20	0	20	3	0.83	0.05	0.017
RUM_2	3	587	10	0	10	3	1	0.07	0.011
all	67	288	309	277	32	47	0.98	0	0.191

Table A8: Standard diversity measures of the *ef 1α* gene of *Steganacarus magnus*. Populations with less than two individuals were excluded (Chapter III).

population	sample size n	invarible sites N_{is}	variable sites N_{vs}	parsimony inform. sites N_{pars}	number of singeltons N_s	number of haplotypes N_h	haplotype diversity H_d	variance	nucleotide diversity Π_n
CZ_1	2	458	3	0	3	2	1	0.25	0.007
D_1	6	423	38	19	19	6	1	0.01	0.037
D_2	5	400	61	36	25	4	0.9	0.03	0.071
F_1	2	396	65	0	65	2	1	0.25	0.14
GB_1	5	453	8	1	7	4	0.9	0.03	0.007
I_1	4	385	76	13	63	4	1	0.03	0.090
PL_1	4	400	61	0	61	4	1	0.03	0.066
RUM_1	2	426	35	0	35	2	1	0.25	0.076
RUM_2	2	422	29	0	29	2	1	0.25	0.085
all	37	243	218	127	91	35	0.997	0	0.113

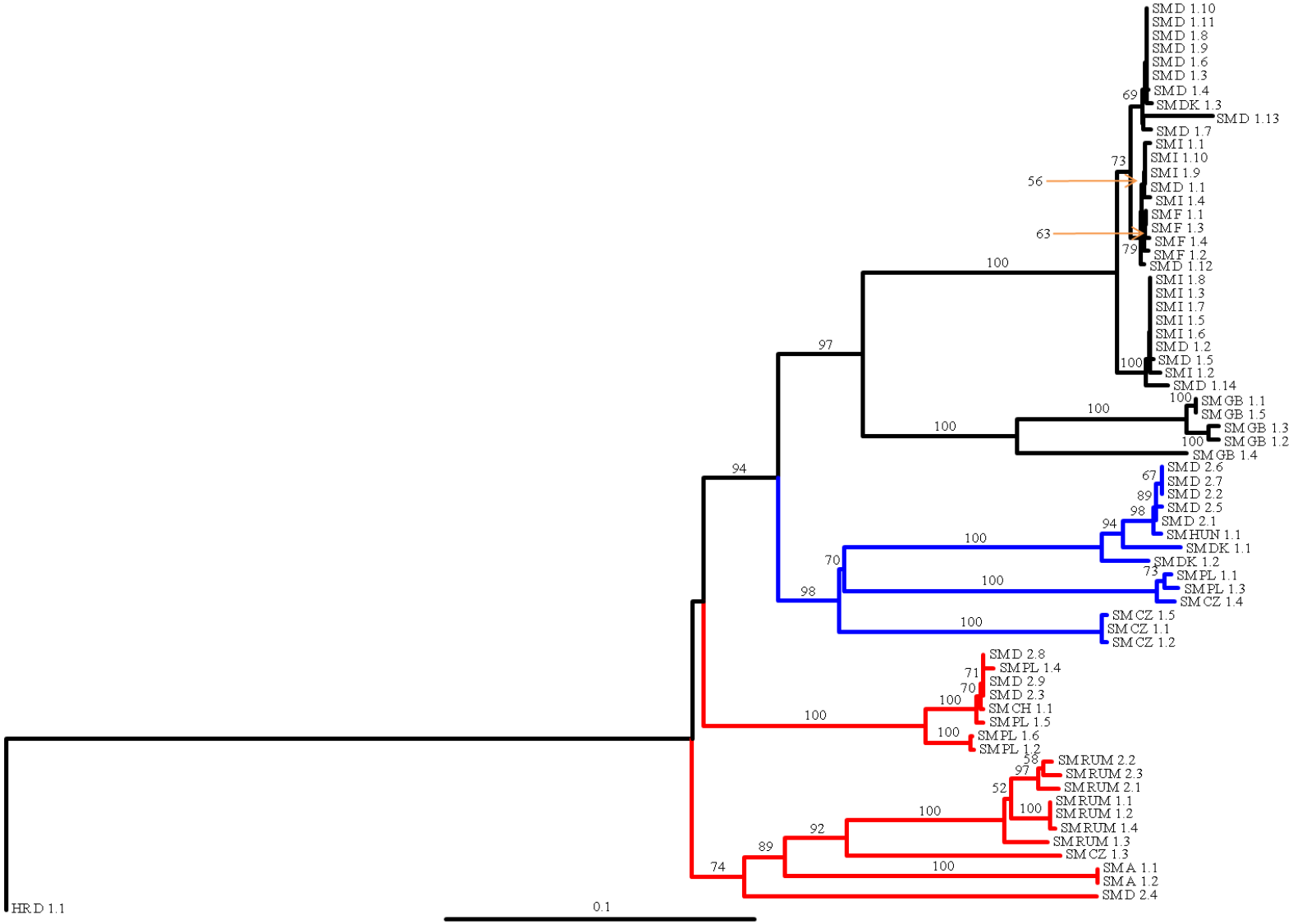

Figure A8: Neighbor-Joining tree of the 67 *COI* nucleotide sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are bootstrap values after 100,000 pseudo-replicates computed in PAUP*. Branch colours show the different clades (black, blue, red).

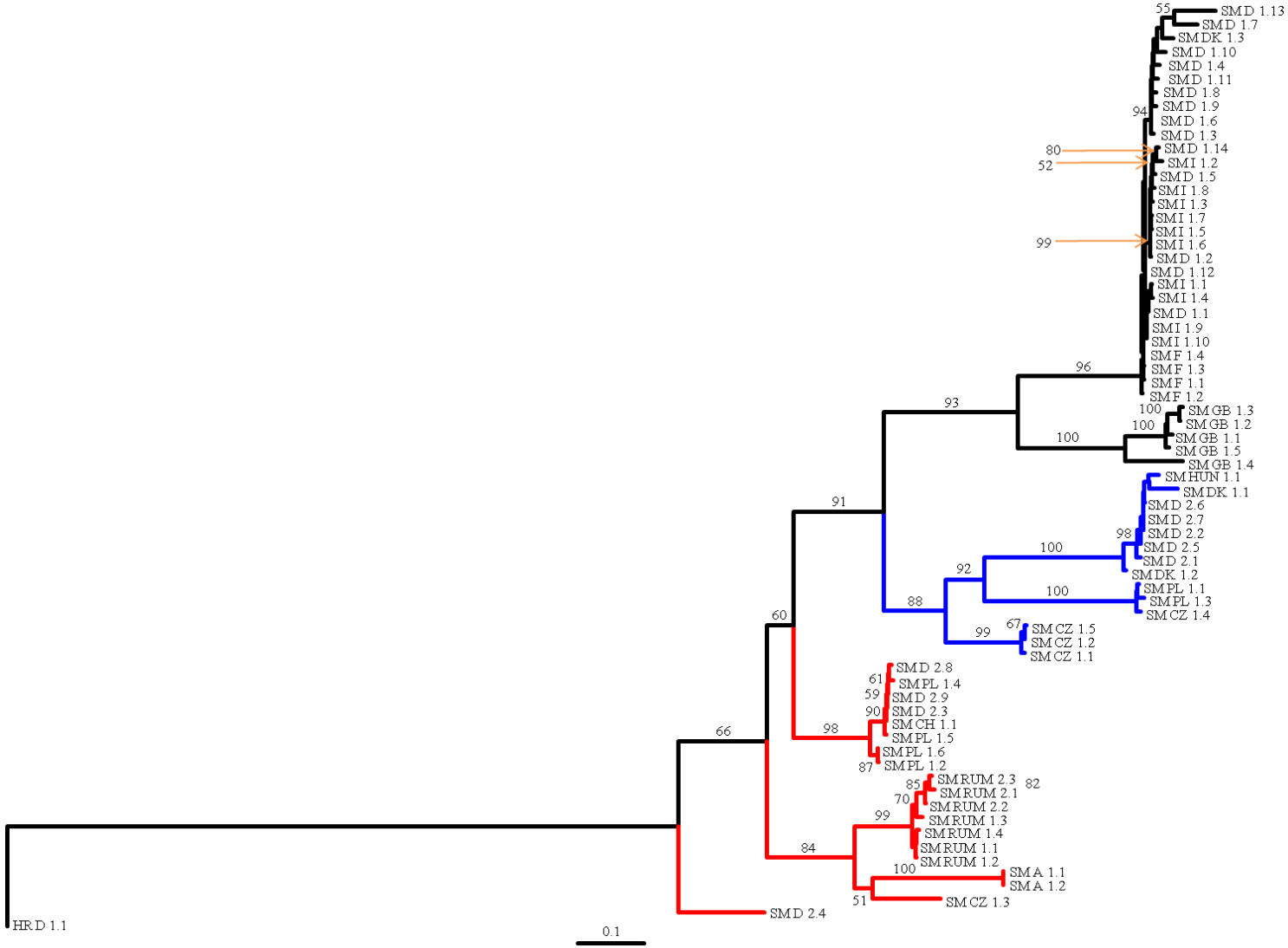

Figure A9: Neighbor-Joining tree with model of sequence evolution (TVM+I+G) of the 67 *COI* nucleotide sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are bootstrap values after 100,000 pseudo-replicates computed in PAUP*. Branch colours show the different clades (black, blue, red).

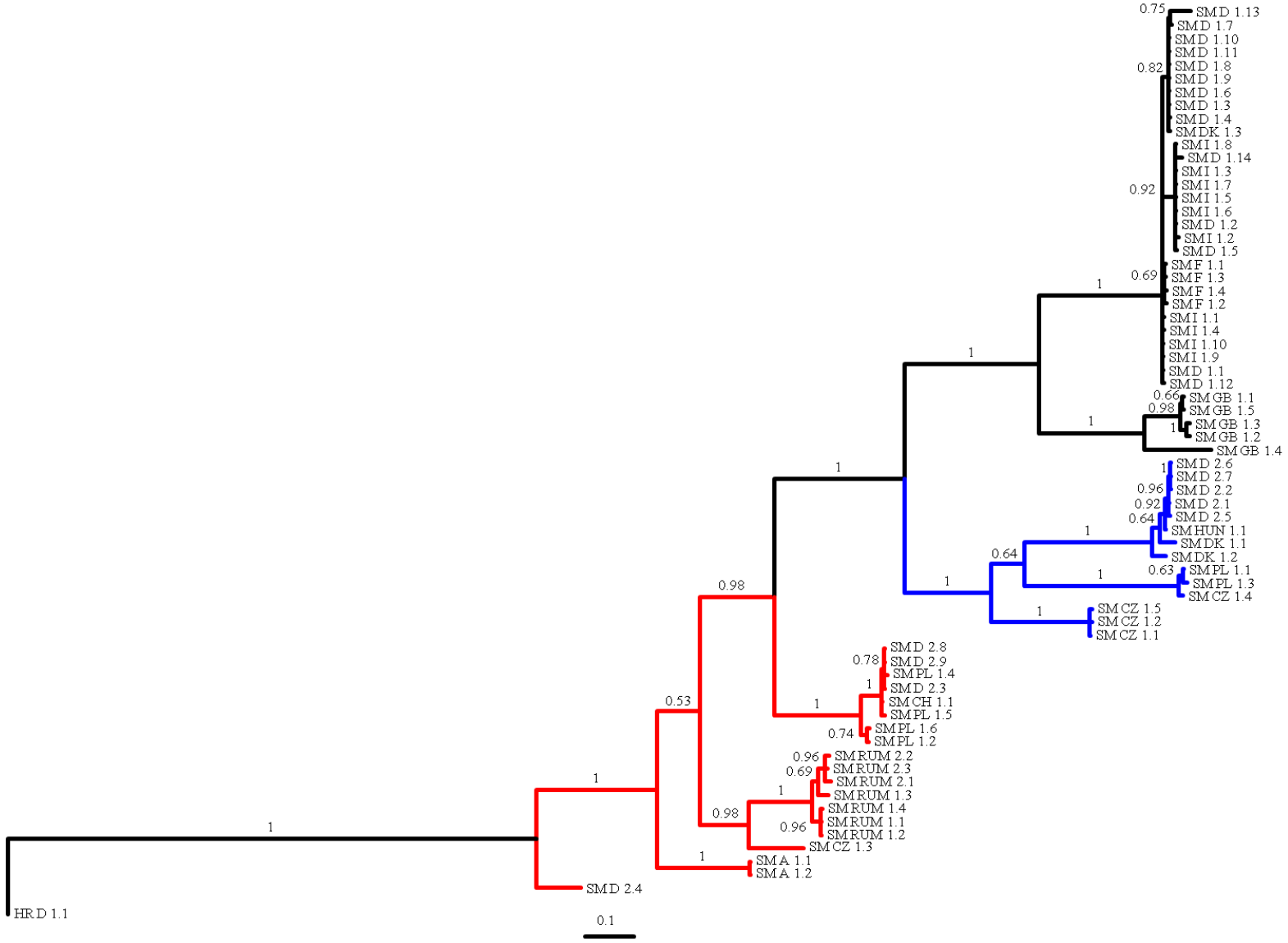

Figure A10: Bayesian phylogeny after $10x10^6$ generations from the 67 *COI* nucleotide sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are posterior probabilities from MrBayes. Branch colours show the different clades (black, blue, red).

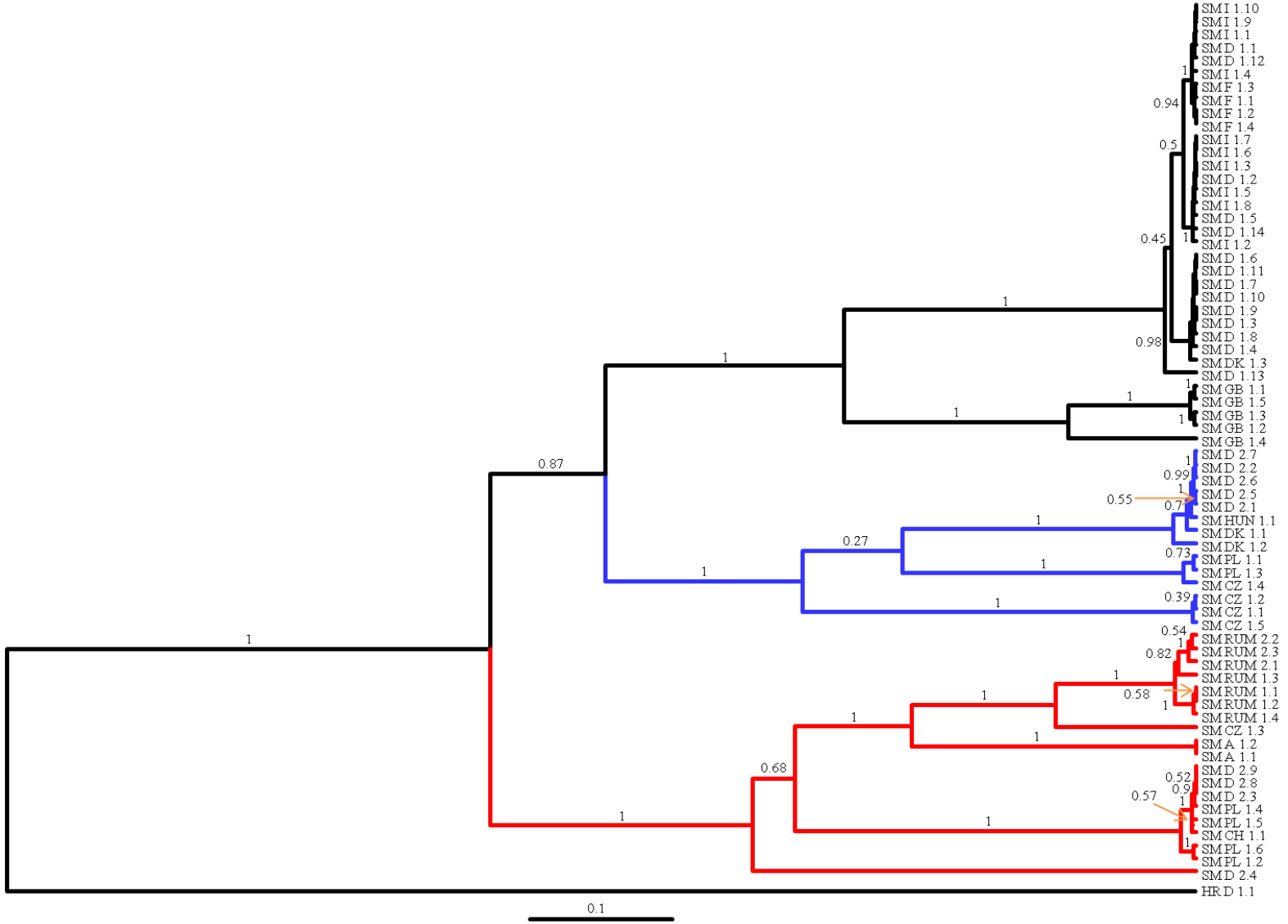

Figure A11: Bayesian phylogeny after $10x10^6$ generations from the 67 *COI* nucleotide sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are posterior probabilities from BEAST. Branch colours show the different clades (black, blue, red).

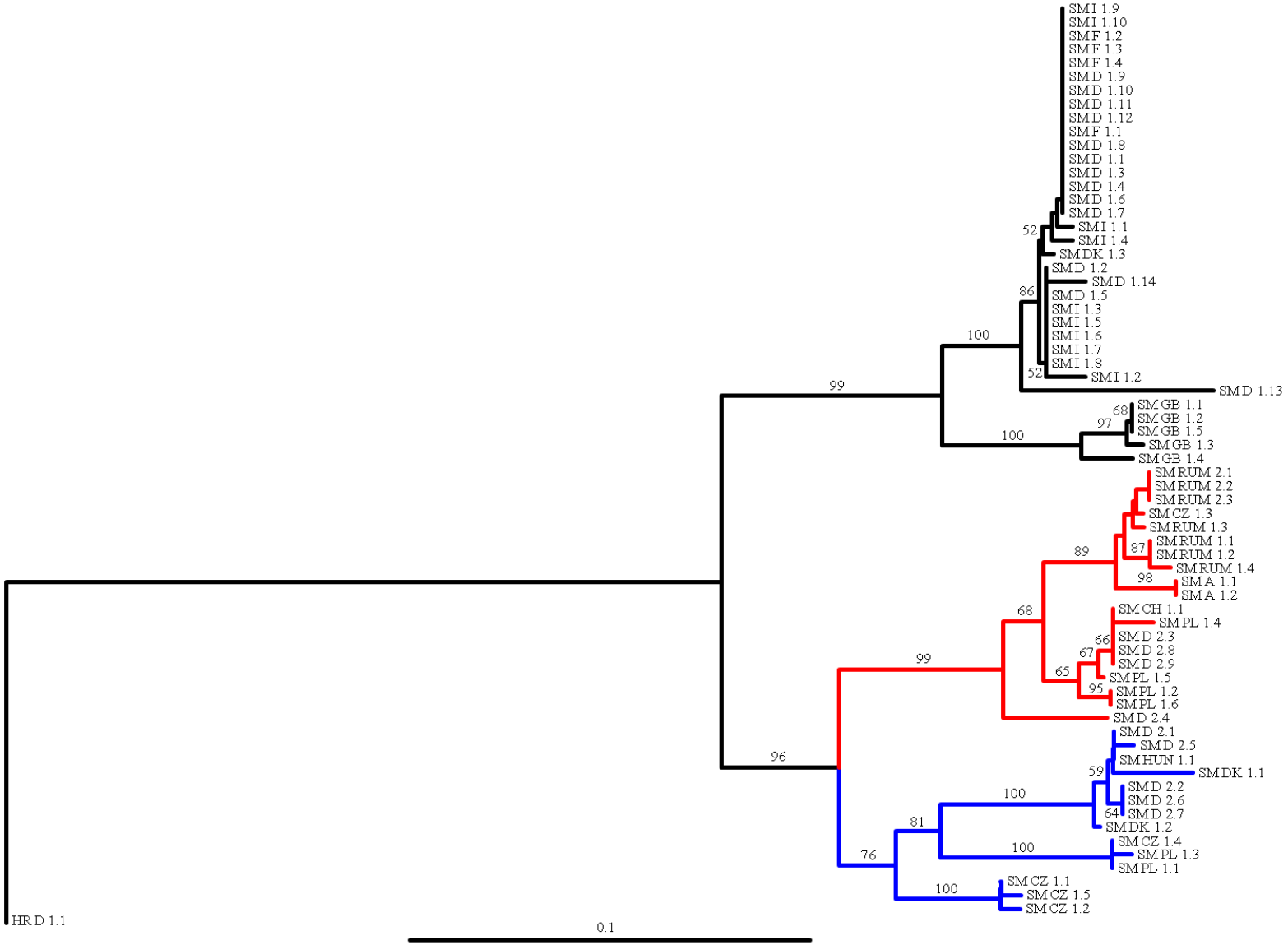

Figure A12: Neighbor-Joining tree from the 67 *COI* protein sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are bootstrap values after 100,000 pseudo-replicates computed in PAUP*. Branch colours show the different clades (black, blue, red).

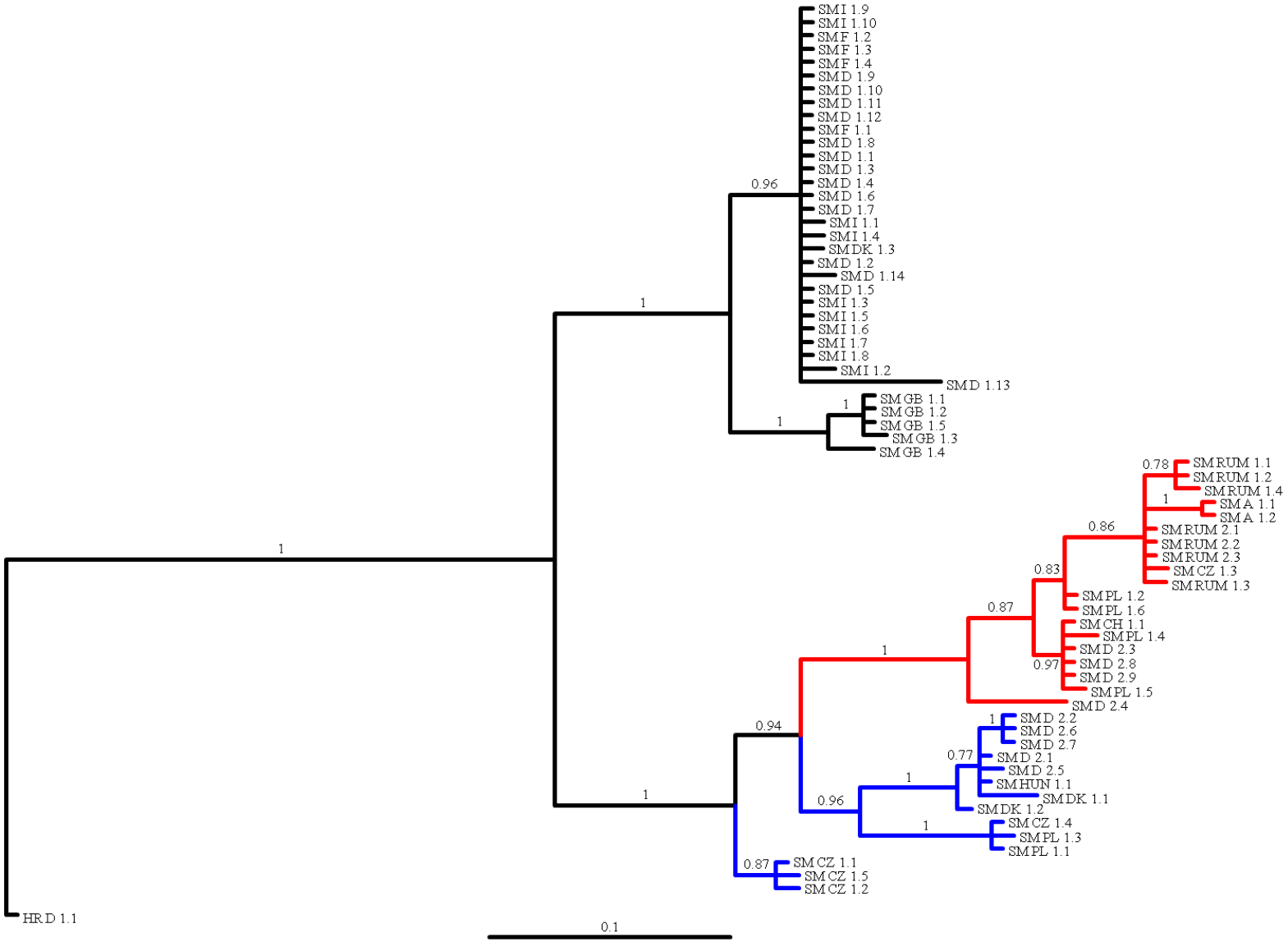

Figure A13: Bayesian phylogeny after $10x10^6$ generations from the 67 *COI* protein sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are posterior probabilities from MrBayes. Branch colours show the different clades (black, blue, red).

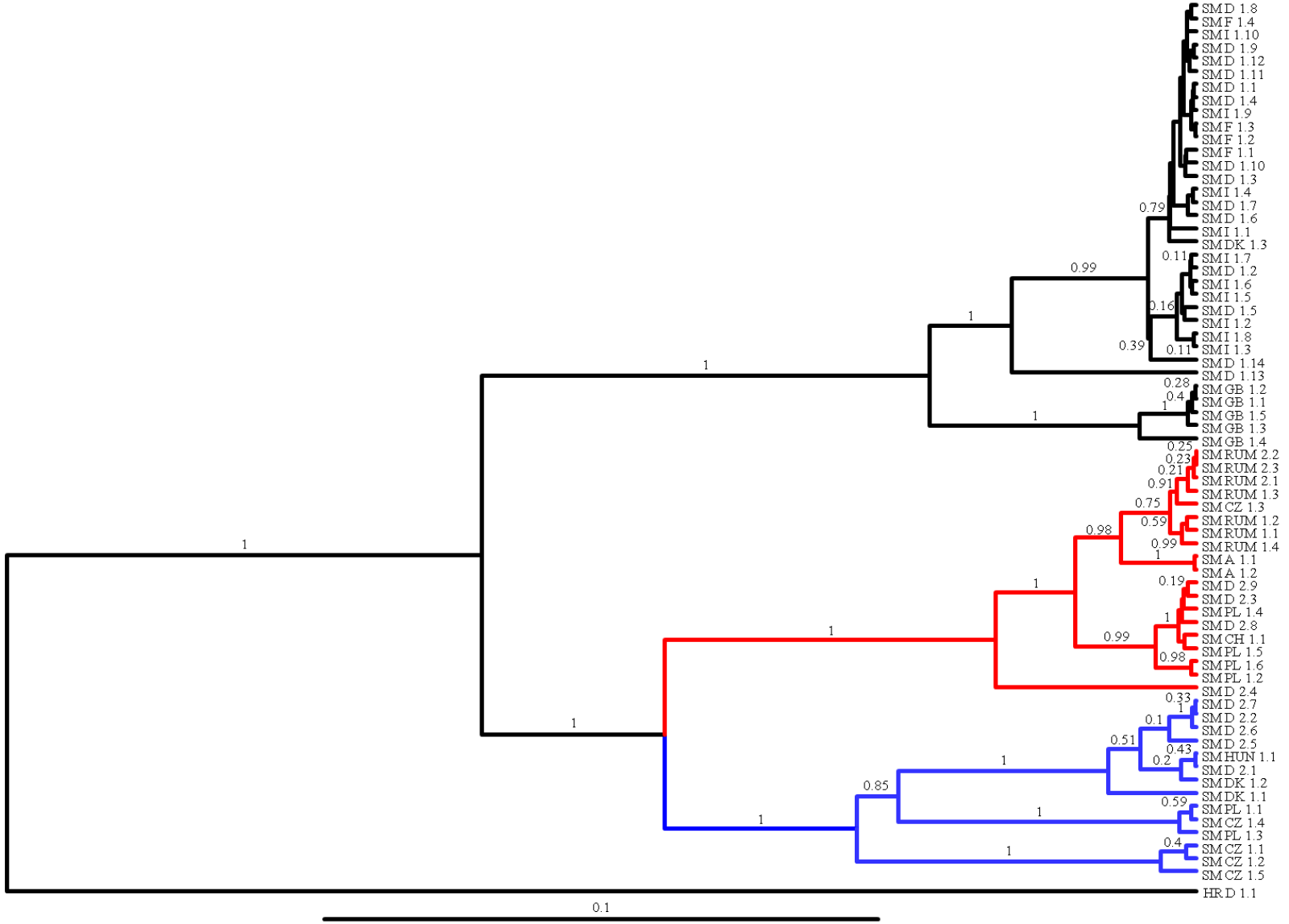

Figure A14: Bayesian phylogeny after $10x10^6$ generations from the 67 *COI* protein sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are posterior probabilities from BEAST. Branch colours show the different clades (black, blue, red).

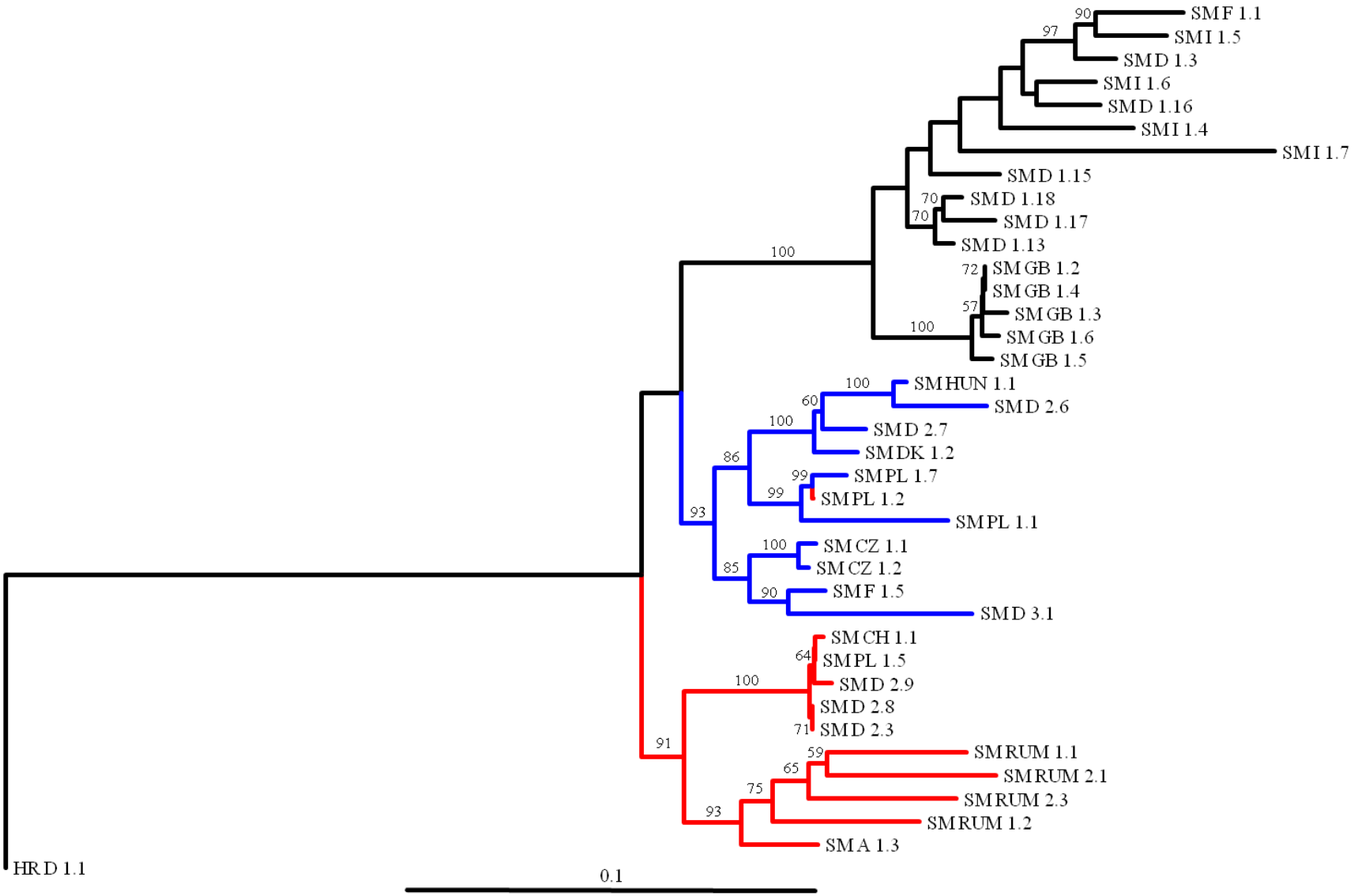

Figure A15: Neighbor-Joining tree of the *ef 1α* nucleotide sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are bootstrap values after 100,000 pseudo-replicates computed in PAUP*. Branch colours show the different clades (black, blue, red).

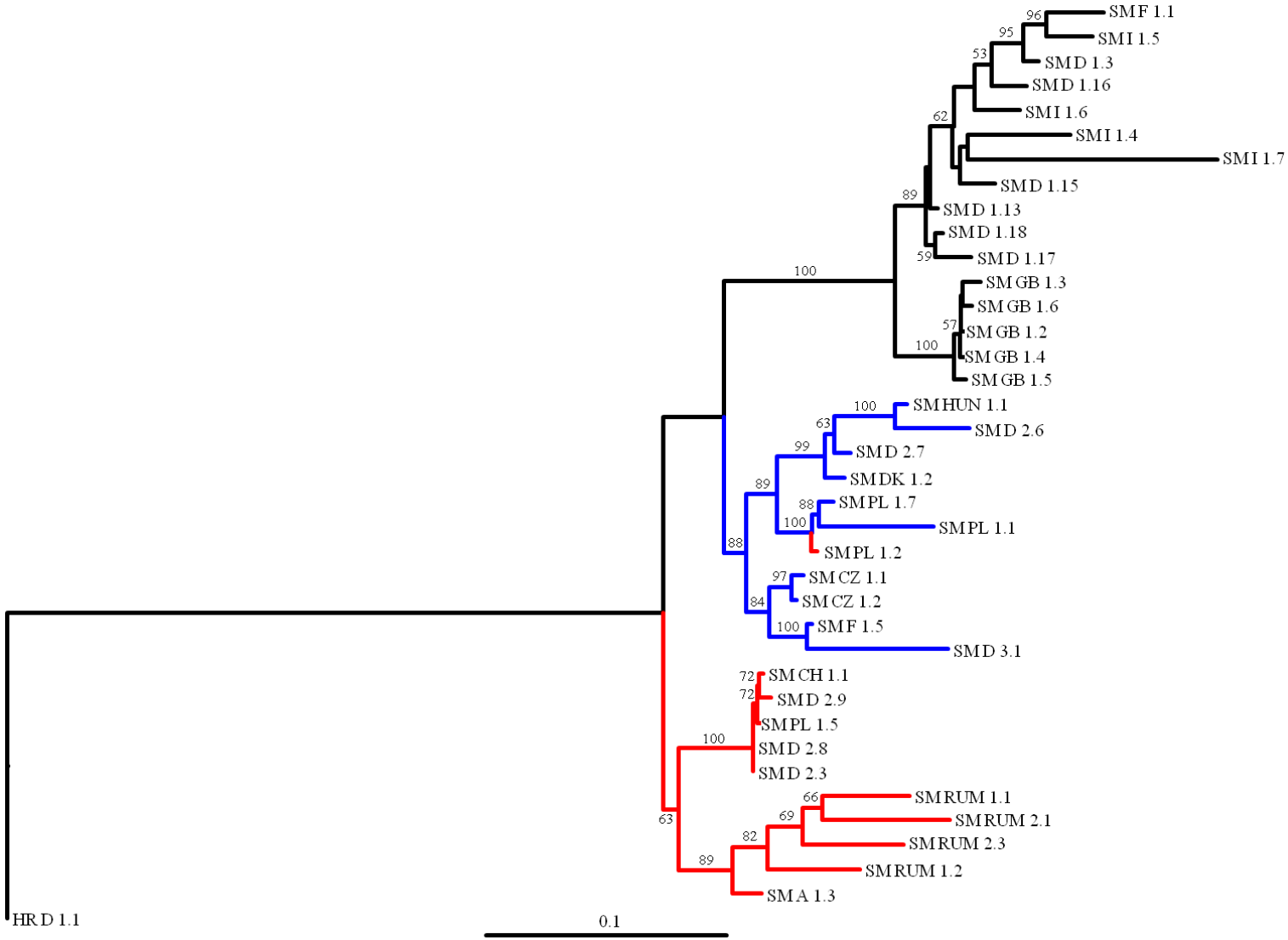

Figure A16: Neighbor-Joining tree with model of sequence evolution (TrNef+G) of the *ef 1α* nucleotide sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1).. Numbers on the branches are bootstrap values after 100,000 pseudo-replicates computed in PAUP*. Branch colours show the different clades (black, blue, red).

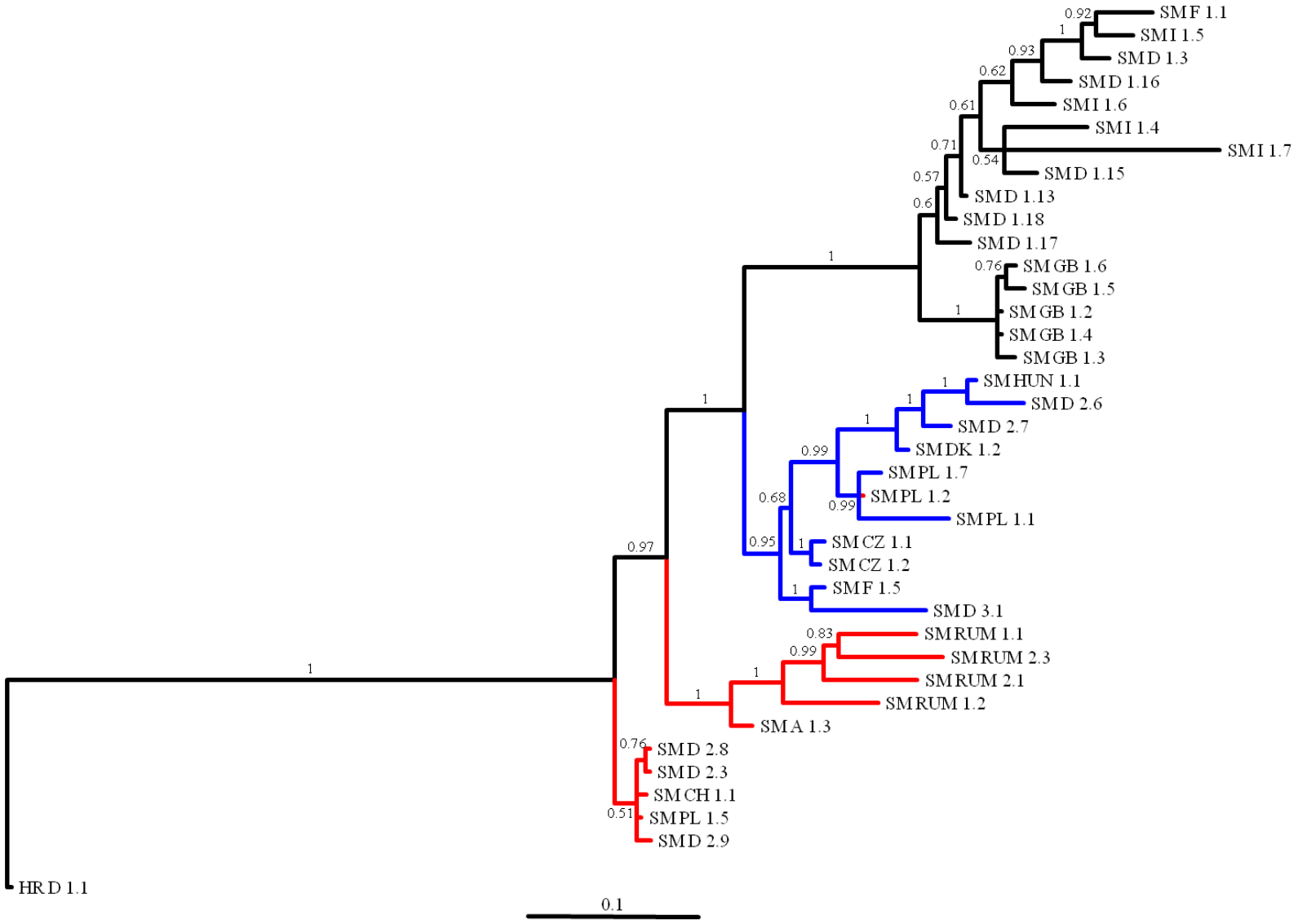

Figure A17: Bayesian phylogeny after $10x10^6$ generations from the *ef 1α* nucleotide sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1).. Numbers on the branches are posterior probabilities from MrBayes. Branch colours show the different clades (black, blue, red).

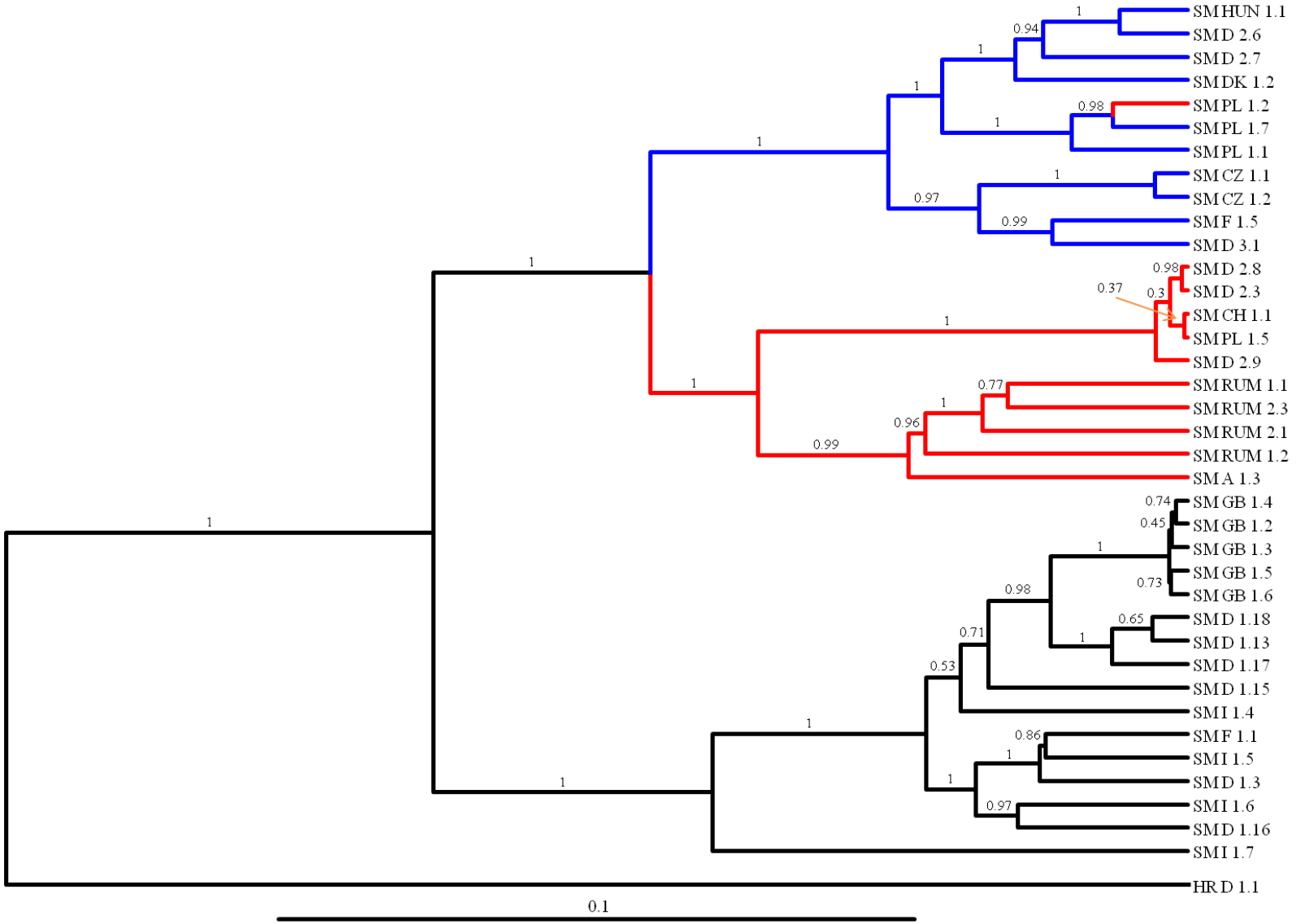

Figure A18: Bayesian phylogeny after $10x10^6$ generations from the *ef 1α* nucleotide sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are posterior probabilities from BEAST. Branch colours show the different clades (black, blue, red).

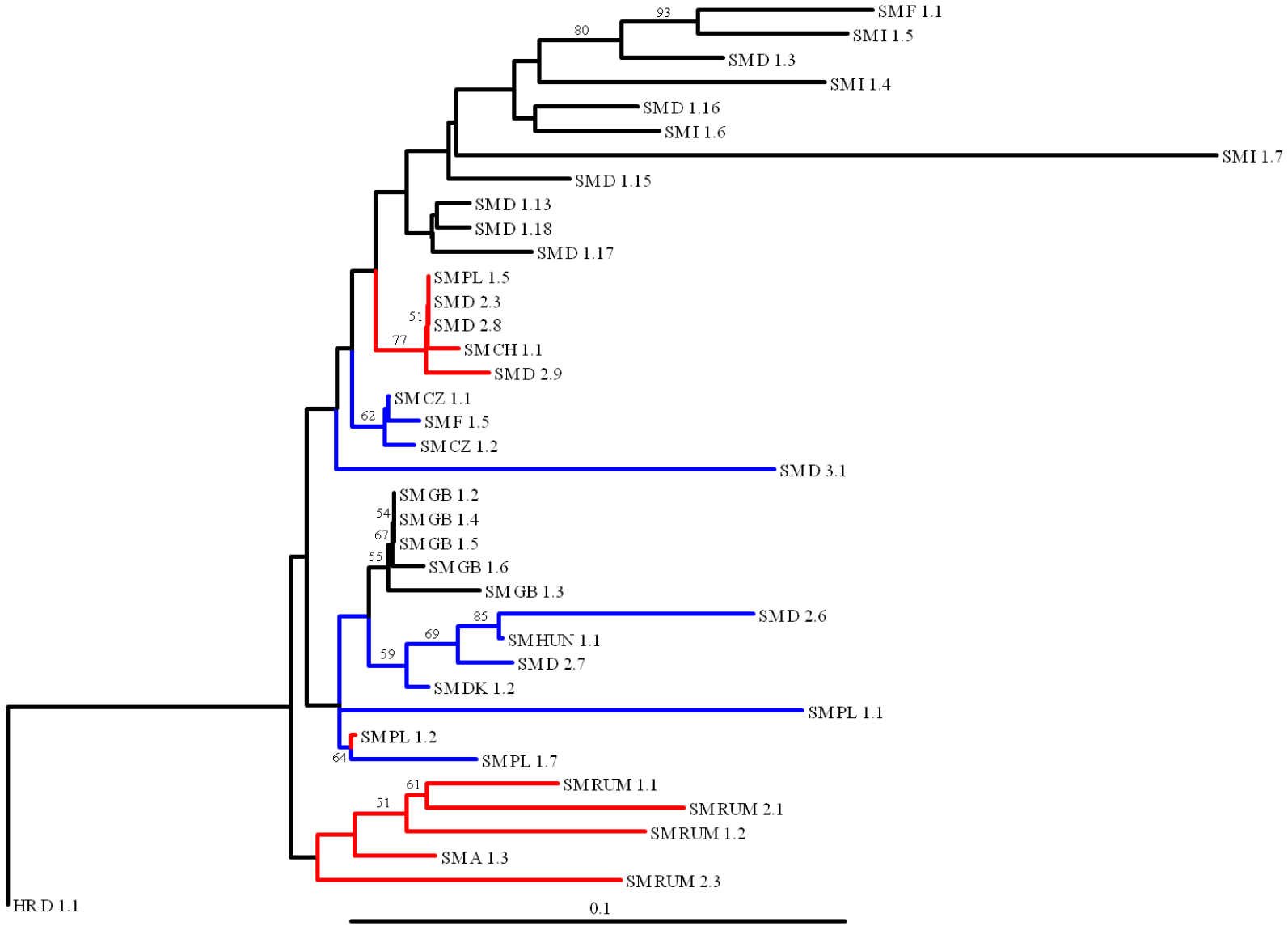

Figure A19: Neighbor-Joining tree of the *ef 1α* protein sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are bootstrap values after 100,000 pseudo-replicates computed in PAUP*. Branch colours show the different clades (black, blue, red).

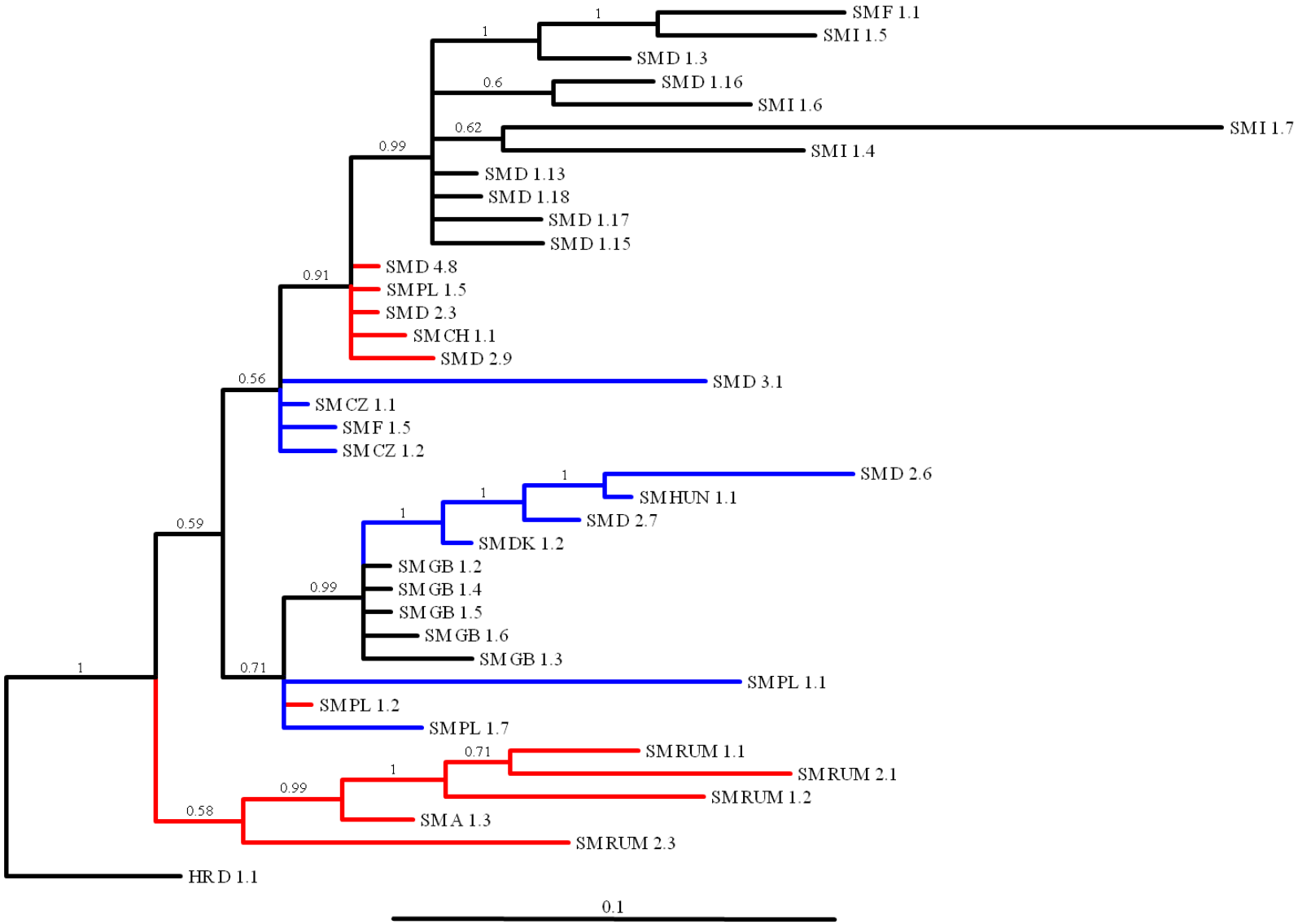

Figure A20: Bayesian phylogeny after $10x10^6$ generations from the *ef 1α* protein sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are posterior probabilities from MrBayes. Branch colours show the different clades (black, blue, red).

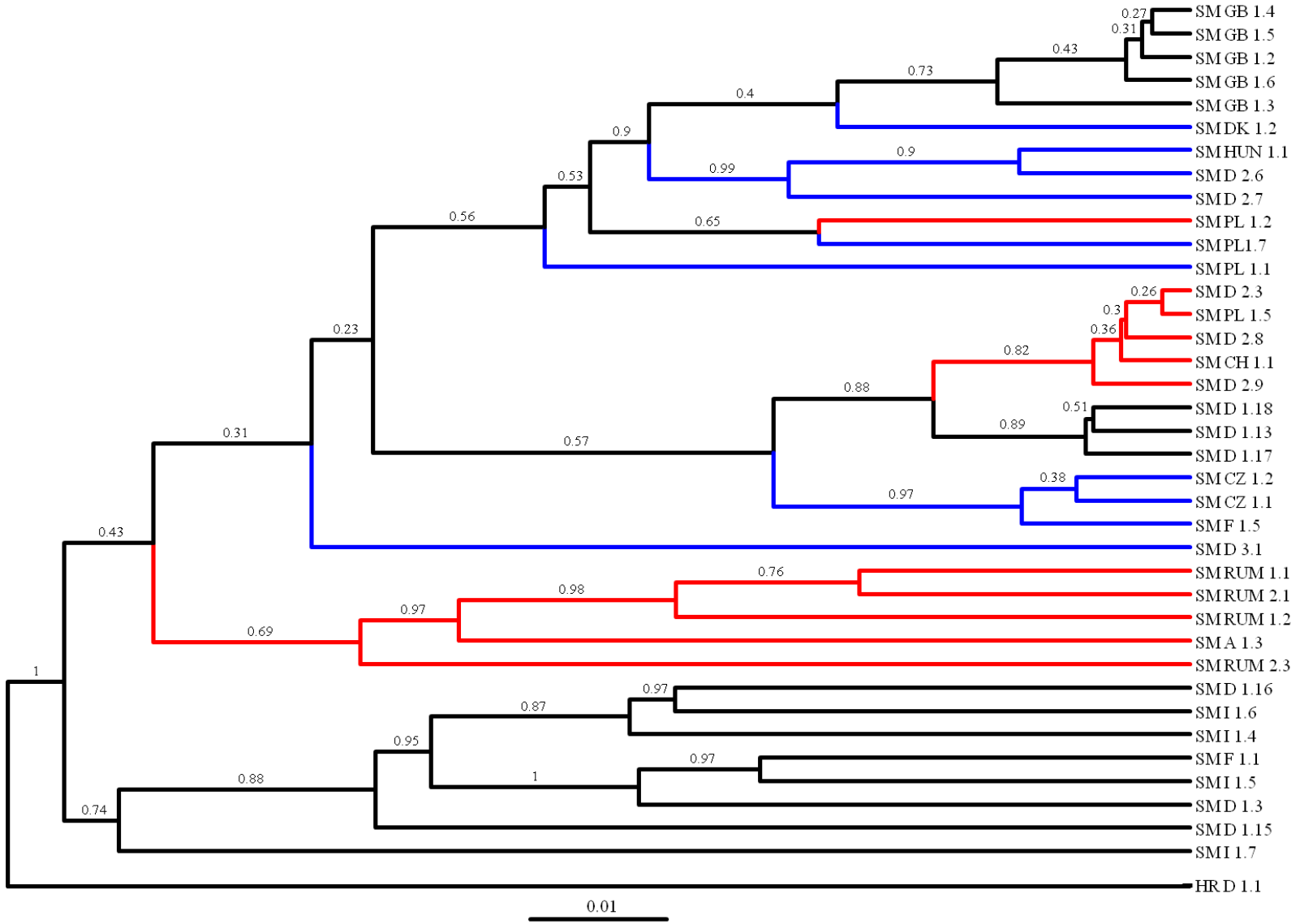

Figure A21: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 from the *ef 1α* protein sequences of *Steganacarus magnus*. Outgroup is *Hypochthonius rufulus* (HD_D_1.1). Numbers on the branches are posterior probabilities from Beast. Branch colours show the different clades (black, blue, red).

Table A9: Mean pairwise percentage differences of uncorrected p-distances for the *COI* protein of *Steganacarus magnus* from eleven locations. The diagonal is the within population differences (bold) and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Locations with less than two individuals were excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9	10	11
1	**A_1**	**0**										
2	**CZ_1**	1.6	**1**									
3	**D_1**	**3.1**	2.1	**0.3**								
4	**D_2**	1.5	1.1	2.5	**1.1**							
5	**DK_1**	2.5	1.5	1.8	1.5	**1.7**						
6	**F_1**	3	1.9	0.2	2.4	1.7	**0**					
7	**GB_1**	3	2.7	1.1	3.1	2.5	1	**0**				
8	**I_1**	3.1	2.1	0.4	2.5	1.6	0.3	1.1	**0.3**			
9	**PL_1**	0.8	1.1	2.6	1.1	2	2.5	2.9	2.6	**0.8**		
10	**RUM_1**	0.1	1.7	**3.1**	1.4	2.5	3	3	**3.1**	0.9	**0.3**	
11	**RUM_2**	**0**	1.6	**3.1**	1.4	2.5	3	3	**3.1**	0.9	0.1	**0**

Table A10: Mean pairwise percentage differences of uncorrected p-distances for the *ef 1α* nucleotide of *Steganacarus magnus* from nine locations. The diagonal is the within population differences (bold) and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Locations with less than two individuals were excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9
1	**CZ_1**	**0**								
2	**D_1**	0.9	**0.4**							
3	**D_2**	**0.5**	1.7	**0.9**						
4	**F_1**	0.7	0.8	1.3	**1.3**					
5	**GB_1**	1.6	1.1	1.4	1.6	**0.5**				
6	**I_1**	2.6	2.1	3.2	2.1	3	**3.1**			
7	**PL_1**	1.1	2.1	1.2	1.9	1.7	**3.7**	**1.6**		
8	**RUM_1**	1	1.7	1.2	1.3	1.8	3	1.6	**1.3**	
9	**RUM_2**	0.7	1.4	0.7	1	0.9	2.8	0.8	1	**0**

Table A11: Tajima's D and Fu's FS neutrality tests of *Steganacarus magnus COI* nucleotide sequences from eleven locations. Bold red letters show significance (P<0.05).

Neutrality tests	A_1	CZ_1	D_1	D_4	DK_1	F_2	GB_1	I_1	PL_1	RUM_3	RUM_4	Mean	s.d.
Tajima's D test													
Samplesize	2	5	14	9	3	4	5	10	6	4	3	5.909	3.646
S	0	193	35	215	159	2	76	14	160	20	10	80.364	84.270
Pi	0	94.4	8.703	95.75	108.66667	1	31.6	6.644	85.2	10	6.667	40.785	44.848
Tajima's D	0	0.145	-0.905	1.094	6409109.877	-0.710	-1.014	1.569	1.4	-0.854	0	582646.418	1932419.315
Tajima's D p-value	1	0.584	0.185	0.906	0.999	0.284	0.137	0.958	0.954	0.07	0.72	0.618	0.38
Fu's FS test													
Real no. of alleles	1	5	9	5	3	3	4	5	6	3	3	4.273	2.102
Orig. no. of alleles	1	5	9	5	3	3	4	5	6	3	3	4.273	2.102
Theta_pi	0	94.4	8.703	95.75	108.667	1	31.6	6.644	85.2	10	6.667	40.785	44.848
Exp. no. of alleles	0	4.897	8.661	8.645	2.973	2.083	4.711	6.412	5.831	3.512	2.639	4.579	2.7
FS	0	2.208	0.171	12.039	3.584	-0.887	3.581	2.352	1.671	2.624	0.703	2.55	3.471
FS p-value	N.A.	0.54	0.534	1	0.583	0.089	0.895	0.866	0.495	0.84	0.414	N.A.	N.A.

Table A12: Tajima's D and Fu's FS neutrality tests of *Steganacarus magnus COI* protein sequences from eleven locations. Bold red letters show significance (P<0.05).

Neutrality tests	A_1	CZ_1	D_1	D_4	DK_1	F_2	GB_1	I_1	PL_1	RUM_3	RUM_4	Mean	s.d.
Tajima's D test													
Samplesize	2	5	14	9	3	4	5	10	6	4	3	5.909	3.646
S	0	5	4	4	5	0	0	2	3	1	0	2.182	2.089
Pi	0	1.9	0.67	2.111	3.333	0	0	0.644	1.6	0.5	0	0.978	1.11
Tajima's D	0	-1.405	-1.539	1.766	0	0	0	-0.285	1.124	-0.612	0	-0.086	0.954
Tajima's D p-value	1	0	**0.043**	0.977	0.809	1	1	0.325	0.881	0.386	1	0.675	0.404
Fu's FS test													
Real no. of alleles	1	5	4	4	3	1	3	5	5	3	1	3.182	1.601
Orig. no. of alleles	1	5	4	4	3	1	3	5	5	3	1	3.182	1.601
Theta_pi	0	1.9	0.67	2.111	3.333	0	0	0.644	1.6	0.5	0	0.978	1.11
Exp. no. of alleles	0	2.852	2.653	3.948	2.394	0	3	2.388	2.936	1.676	0	1.986	1.387
FS	0	0.558	-1.29	1.919	-0.077	0	0	-0.332	2.506	0.172	0	0.314	1.049
FS p-value	N.A.	0.54	0.072	0.848	0.235	N.A.	N.A.	0.29	0.865	0.34	N.A.	N.A.	N.A.

Table A13: Tajima's D and Fu's FS neutrality tests of *Steganacarus magnus ef 1α* nucleotide sequences from eleven locations. Bold red letters show significance (P<0.05).

Neutrality tests	CZ_1	D_1	D_4	F_2	GB_1	I_1	PL_1	RUM_3	RUM_4	Mean	s.d.
Tajima's D test											
Samplesize	2	6	5	2	5	4	4	2	2	3.556	1.590
S	3	38	61	65	8	77	61	35	39	43	25.539
Pi	3	17.133	32.8	65	3.4	42	30.5	35	39	29.759	19.636
Tajima's D	0	0.188	0.910	0	-0.807	0	-0.869	0	0	-0.064	0.528
Tajima's D p-value	1	0.581	0.808	1	0.304	0.662	**0.027**	1	1	0.709	0.353
Fu's FS test											
Real no. of alleles	2	6	4	2	4	4	4	2	2	3.333	1.414
Orig. no. of alleles	2	6	4	2	4	4	4	2	2	3.333	1.414
Theta_pi	3	17.133	32.8	65	3.4	42	30.5	35	39	29.759	19.636
Exp. no. of alleles	1.75	5.276	4.720	1.985	3.393	3.865	3.817	1.972	1.975	3.195	1.326
FS	1.099	-0.193	3.648	4.174	-0.128	1.903	1.567	3.555	3.664	2.143	1.688
FS p-value	0.434	0.275	0.9	0.611	0.343	0.516	0.492	0.612	0.608	0.532	0.183

Table A14: Tajima's D and Fu's FS neutrality tests of *Steganacarus magnus ef 1α* protein sequences from eleven locations. Bold red letters show significance (P<0.05).

Neutrality tests	CZ_1	D_1	D_4	F_2	GB_1	I_1	PL_1	RUM_3	RUM_4	Mean	s.d.
Tajima's D test											
Samplesize	2	6	5	2	5	4	4	2	2	3.556	1.590
S	0	2	3	2	2	9	5	2	0	2.778	2.774
Pi	0	0.667	1.3	2	0.8	4.667	2.5	2	0	1.548	1.468
Tajima's D	0	-1.132	-0.612	0	-0.973	-0.492	-0.797	0	0	-0.445	0.460
Tajima's D p-value	1	0.154	0.388	1	0.191	0.463	0.170	1	1	0.596	0.396
Fu's FS test											
Real no. of alleles	2	6	4	2	3	4	4	2	2	3.222	1.394
Orig. no. of alleles	2	6	4	2	3	4	4	2	2	3.222	1.394
Theta_pi	0	0.667	1.3	2	0.8	4.667	2.5	2	0	1.548	1.468
Exp. no. of alleles	0	2.092	2.507	1.667	2.107	3.132	2.724	1.667	0	1.766	1.108
FS	0	0.952	-1.786	0.693	-0.829	-0.615	-1.514	0.693	0	-0.267	0.986
FS p-value	N.A.	0.613	**0.021**	0.361	0.089	0.188	0.056	0.366	N.A.	N.A.	N.A.

Table A15: Results of the McDonald-Kreitman test for *COI* of *Steganacarus magnus*. The differences between seven populations are significant (*0.01<P<0.05), between other two populations high significant (**0.001<P<0.01) and between seven populations extremely high significant (***P<0.001). Number of fixed and polymorph synonymous and non-synonymous mutations are shown. Locations with less than two individuals were excluded.

Population		A 1			CZ 1			D 1			D 2			DK 1			F 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
CZ 1	**fixed**	55	3	0.0203*															
Decin	**poly**	169	35																
D 1	**fixed**	112	46	0.1653 ns	38	19	0.0531 ns												
Kranichstein	**poly**	21	15		185	48		.											
D 2	**fixed**	44	5	0.6424 ns	7	0	0.5996 ns	32	19	0.0038**									
Meckl. Seenpl.	**poly**	19	30		284	46		203	43										
DK 1	**fixed**	82	24	0.2064 ns	9	2	1 ns	0	0	- ns	1	0	1 ns						
Copenhagen	**poly**	112	49		234	66		128	62		223	55							
F 1	**fixed**	11	2	1 ns	42	19	0.0282*	1	0	1 ns	34	19	0.0005***	5	0	0.3228 ns			
Loire	**poly**	2	0		171	35		24	14		191	30		114	48				
GB 1	**fixed**	94	47	0.0000***	32	21	0.0002***	73	10	0.316 ns	25	20	0.0000***	40	6	0.0866 ns	76	12	0.3175 ns
Bedford	**poly**	70	6		215	39		89	20		234	35		164	54		72	6	
I 1	**fixed**	11	47	0.7704 ns	40	19	0.0308*	0	0	- ns	32	19	0.0007***	2	0	0.5739 ns	1	0	1 ns
Grosetto	**poly**	10	5		177	40		22	18		195	35		119	53		12	5	
PL 1	**fixed**	71	8	0.0148*	1	0	1 ns	48	23	0.4414 ns	0	0	- ns	17	3	0.3009 ns	51	23	0.2003 ns
Krakau	**poly**	126	38		224	43		142	52		240	44		201	74		128	38	
RUM 1	**fixed**	100	5	0.0359*	29	1	0.0577 ns	99	44	0.866 ns	34	3	0.4381 ns	62	23	0.7706 ns	105	44	0.4395 ns
Bagau	**poly**	16	4		177	37		38	18		197	31		123	52		17	4	
RUM 2	**fixed**	104	5	1 ns	31	1	0.0379*	105	45	1 ns	33	3	0.5895 ns	64	23	0.6634 ns	110	45	0.0375*
Cluj	**poly**	10	0		173	35		31	14		196	30		119	49		12	0	

Table A15: continue

Population		GB 1			I 1			PL 1			RUM 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
I 1	**fixed**	76	11	1 ns									
Grosetto	**poly**	78	11										
PL 1	**fixed**	47	26	0.0062**	49	23	0.2686 ns						
Krakau	**poly**	182	43		132	43							
RUM 1	**fixed**	75	46	0.0000***	101	44	0.6822 ns	58	8	0.071 ns			
Bagau	**poly**	85	10		26	9		134	40				
RUM 2	**fixed**	82	47	0.0000***	107	45	0.3534 ns	60	9	0.1087 ns	5	0	1 ns
Cluj	**poly**	75	6		20	5		131	38		24	4	

Table A16: Results of the McDonald-Kreitman test for *ef 1α* from *Steganacarus magnus*. The differences between two populations are significant (*0.01<P<0.05), between other three populations high significant (**0.001<P<0.01) and between 14 populations extremely high significant (***P<0.001). Number of fixed and polymorph synonymous and non-synonymous mutations are shown. Locations with less than two individuals are excluded.

Population		CZ 1			D 1			D 2			F 1			GB 1			I 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 1	**fixed**	35	4	0.0000***															
Kranichstein	**poly**	19	24																
D 2	**fixed**	6	0	0.1684 ns	27	2	0.0003***												
Meckl. Seenpl.	**poly**	43	21		58	42													
F 1	**fixed**	6	0	0.1701 ns	2	0	0.5204 ns	6	0	0.176 ns									
Loire	**poly**	46	22		52	34		80	38										
GB 1	**fixed**	46	4	0.0067**	7	5	0.5196 ns	33	1	0.0002***	13	2	0.1322 ns						
Bedford	**poly**	6	5		21	27		44	24		47	25							
I 1	**fixed**	32	4	0.0000***	0	0	- ns	23	2	0.0000***	1	0	1 ns	8	5	0.1434 ns			
Grosetto	**poly**	32	51		35	62		69	66		64	61		34	53				
PL 1	**fixed**	5	0	0.1487 ns	27	2	0.0001***	0	0	- ns	5	0	0.1582 ns	34	2	0.0000***	22	2	0.0000***
Krakau	**poly**	38	26		51	46		51	39		73	44		38	29		64	71	
RUM 1	**fixed**	28	10	0.4596 ns	41	9	0.0005***	11	7	0.7934 ns	23	8	0.3861 ns	45	12	0.0491*	34	10	0.0003***
Bagau	**poly**	24	14		37	36		56	31		60	32		26	17		52	63	
RUM 2	**fixed**	37	8	0.0006***	48	7	0.0000***	16	2	0.0091**	28	4	0.0027 **	52	8	0.0000***	43	7	0.0000***
Cluj	**poly**	19	22		34	44		55	42		61	43		21	26		47	72	

Population		PL 1			RUM 1		
		syn.	nons.	sign.	syn.	nons.	sign.
RUM 1	**fixed**	12	7	1 ns			
Bagau	**poly**	54	36				
RUM 2	**fixed**	15	4	0.0422*	4	2	0.6773 ns
Cluj	**poly**	51	47		36	34	

Table A17: Neutrality indices of *COI* from *Steganacarus magnus* computed in the McDonald-Kreitman test with DnaSP v5 of eleven locations. Locations with less than two individuals were excluded.

Population	A 1	CZ 1	D 1	D 2	DK 1	F 1	GB 1	I 1	PL 1	RUM 1
CZ 1	3.797									
D 1	1.739	0.519								
D 2	1.389	-	0.357							
DK 1	1.495	1.269	-	-						
F 1	0	0.452	-	0.281	-					
GB 1	0.171	0.276	1.64	0.187	2.195	0.528				
I 1	1.213	0.476	-	0.302	-	-	0.974			
PL 1	2.677	-	0.764	-	2.086	0.658	0.427	0.694		
RUM 1	5	6.062	1.066	1.783	1.14	0.561	0.192	0.795	2.164	
RUM 2	0	6.272	1.054	1.684	1.146	0	0.14	0.594	1.934	-

Table A18: Neutrality indices of *ef 1α* from *Steganacarus magnus* computed in the McDonald-Kreitman test with DnaSP v5 of nine locations. Locations with less than two individuals were excluded.

Population	CZ 1	D 1	D 2	F 1	GB 1	I 1	PL 1	RUM 1
D 1	11.053							
D 2	-	9.776						
F 1	-	-	-					
GB 1	9.583	1.8	18	3.457				
I 1	12.75	-	11	-	2.494			
PL 1	-	12.176	-	-	12.974	12.203		
RUM 1	1.633	4.432	0.87	1.533	2.452	4.119	1.143	
RUM 2	5.355	8.874	6.109	4.934	8.048	9.41	3.456	1.889

Table A19: Standard diversity measures of *Nothrus silvestris* with DnaSP v5. Populations with less than two individuals were excluded.

population	sample size n	invarible sites N_{is}	variable sites N_{vs}	parsimony inform. sites N_{pars}	number of singeltons N_s	number of haplotypes N_h	haplotype diversity H_d	variance	nucleotide diversity Π_n
CH_2	3	572	9	0	9	2	0.67	0.099	0.01
CH_3	2	579	2	0	2	2	1	0.25	0.003
D_1	4	581	0	0	0	1	0	0	0
D_2	4	570	11	1	10	4	1	0.031	0.010
D_4	8	561	20	10	10	6	0.93	0.007	0.014
D_6	2	571	10	0	10	2	1	0.25	0.017
D_7	4	581	0	0	0	1	0	0	0
D_8	3	581	0	0	0	1	0	0	0
D_9	4	581	0	0	0	1	0	0	0
D_11	4	578	3	0	3	2	0.5	0.070	0.003
D_16	4	580	1	0	1	2	0.5	0.070	0.001
D_17	3	568	13	0	13	3	1	0.074	0.015
D_18	4	581	0	0	0	1	0	0	0
F_3	4	572	9	0	9	3	0.83	0.049	0.008
FIN_1	4	581	0	0	0	1	0	0	0
GB_1	4	571	10	0	10	3	0.83	0.049	0.009
GB_2	4	568	13	11	2	3	0.83	0.049	0.014
NL_1	3	581	0	0	0	1	0	0	0
NL_2	2	572	9	0	9	2	1	0.25	0.015
PL_2	13	575	6	0	6	2	0.15	0.016	0.002
RUM_1	3	578	3	0	3	2	0.67	0.099	0.003
RUM_2	4	581	0	0	0	1	0	0	0
RUM_5	4	581	0	0	0	1	0	0	0
S_3	4	571	10	0	10	3	0.83	0.049	0.009
all	98	536	45	21	24	25	0.67	0.003	0.009

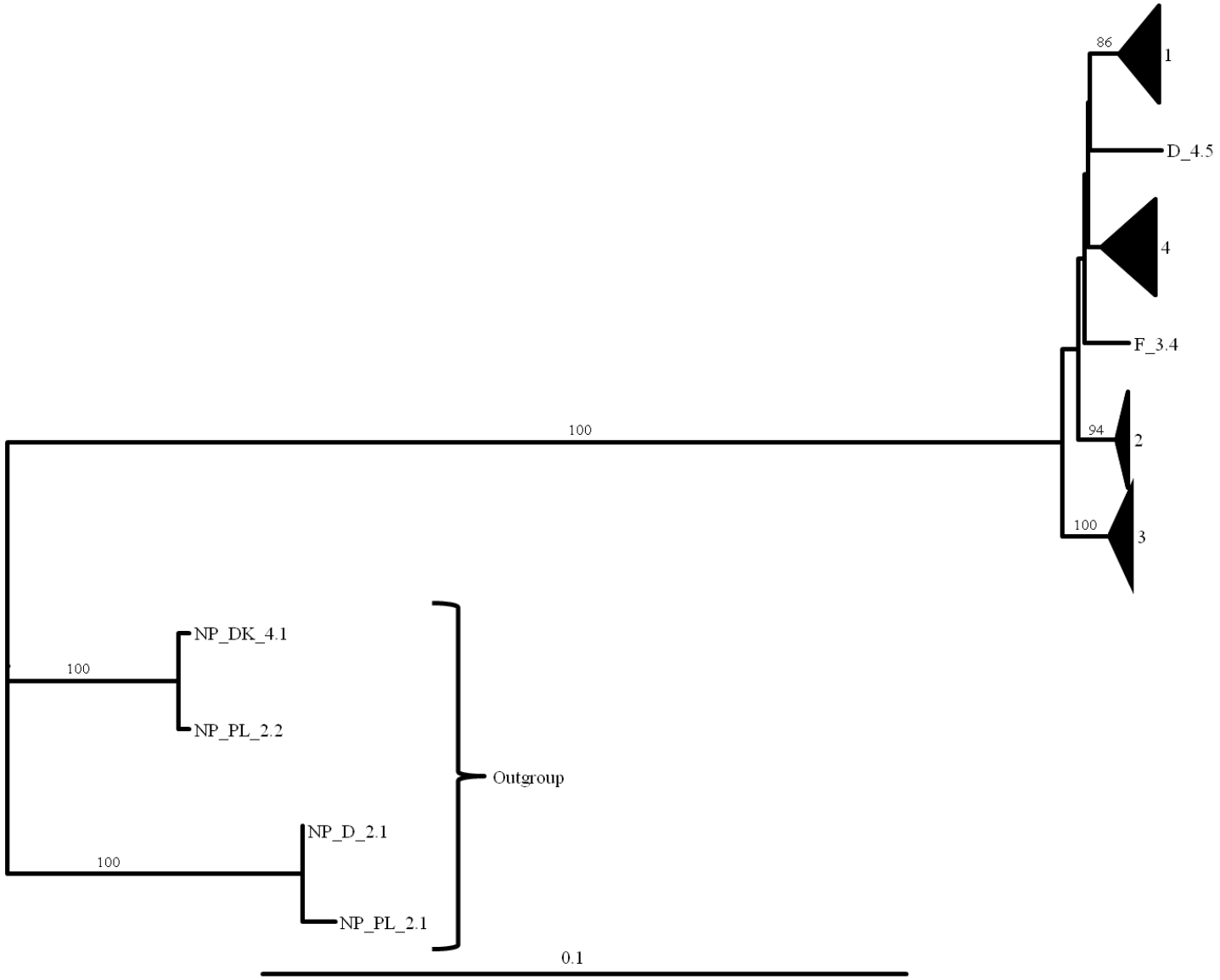

Figure A22: Neighbor-Joining tree of 100 *COI* nucleotide sequences of *Nothrus silvestris*. Outgroup are four individuals of *Nothrus palustris* from different locations. Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Tip numbers are the different subclades and explain in Table 15.

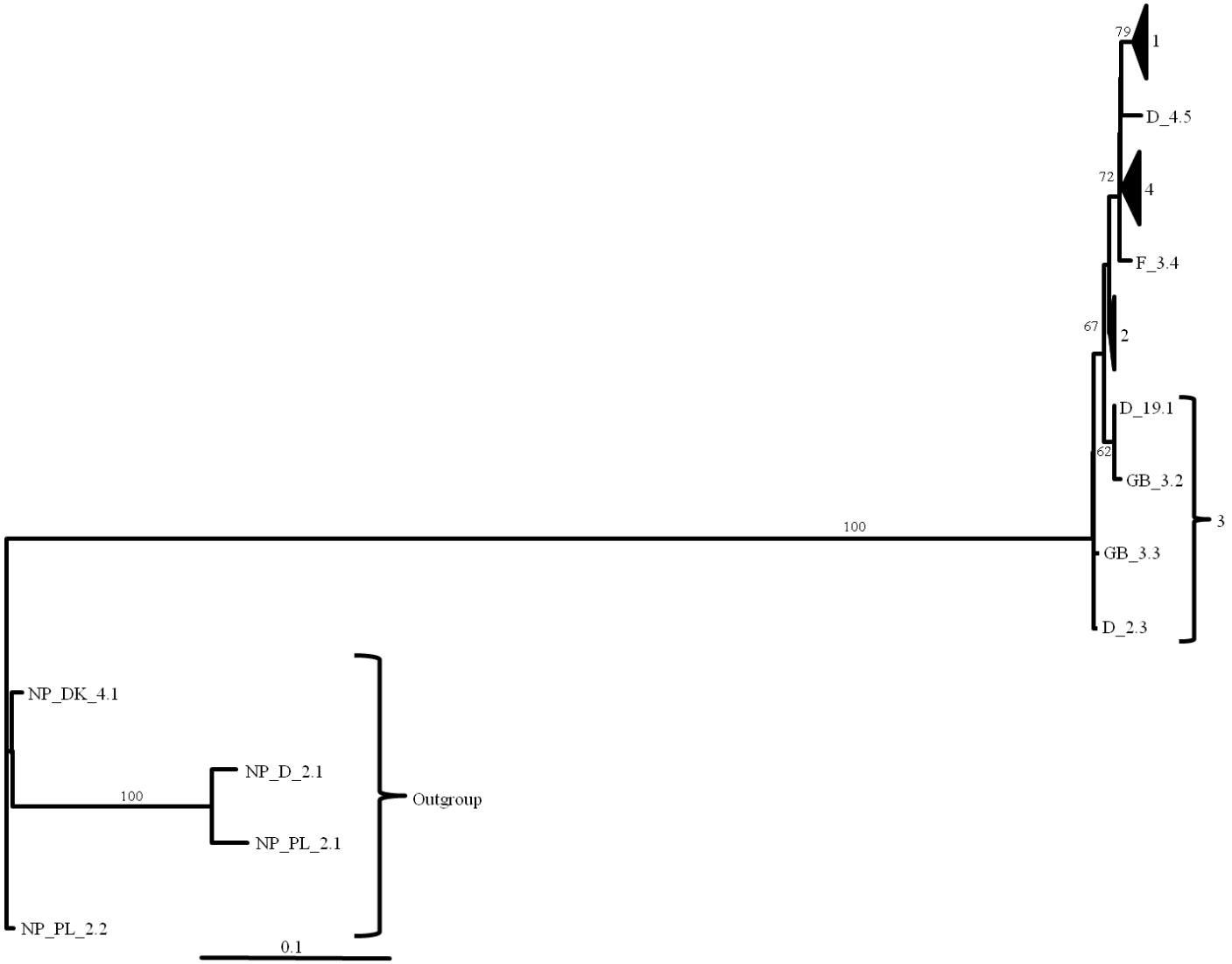

Figure A23: Neighbor-Joining tree of 100 *COI* nucleotide sequences of *Nothrus silvestris* with model of sequence evolution HKY+G. Outgroup are four individuals of *Nothrus palustris* from different locations. Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Tip numbers are the different subclades and explain in Table 15.

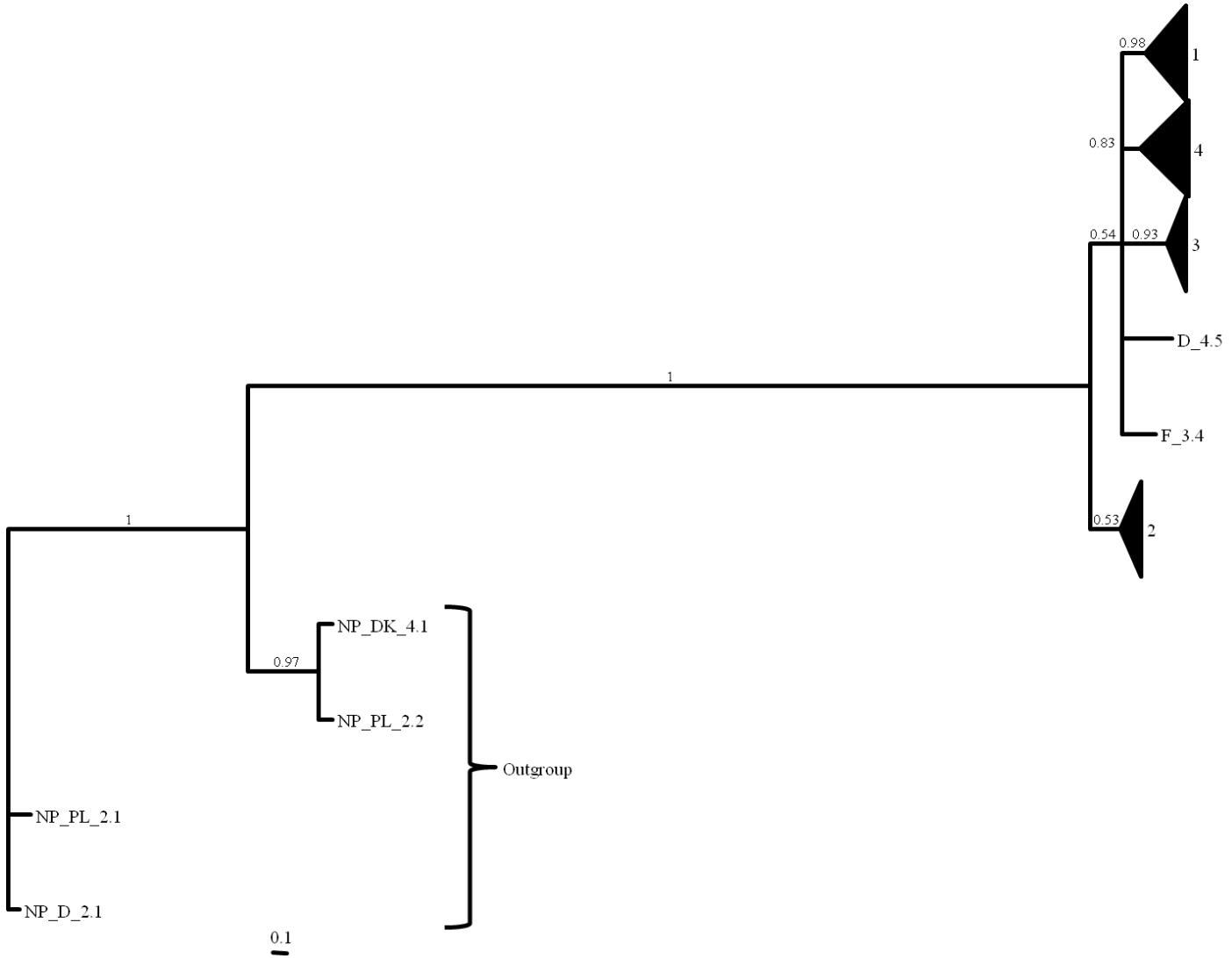

Figure A24: Bayesian tree after 10x10^6 generations from 100 *COI* nucleotide sequences of *Nothrus silvestris* with MrBayes. Split frequencies of 0.007767 and burnin of 25%. Outgroup four individuals of *Nothrus palustris* from different locations. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 15.

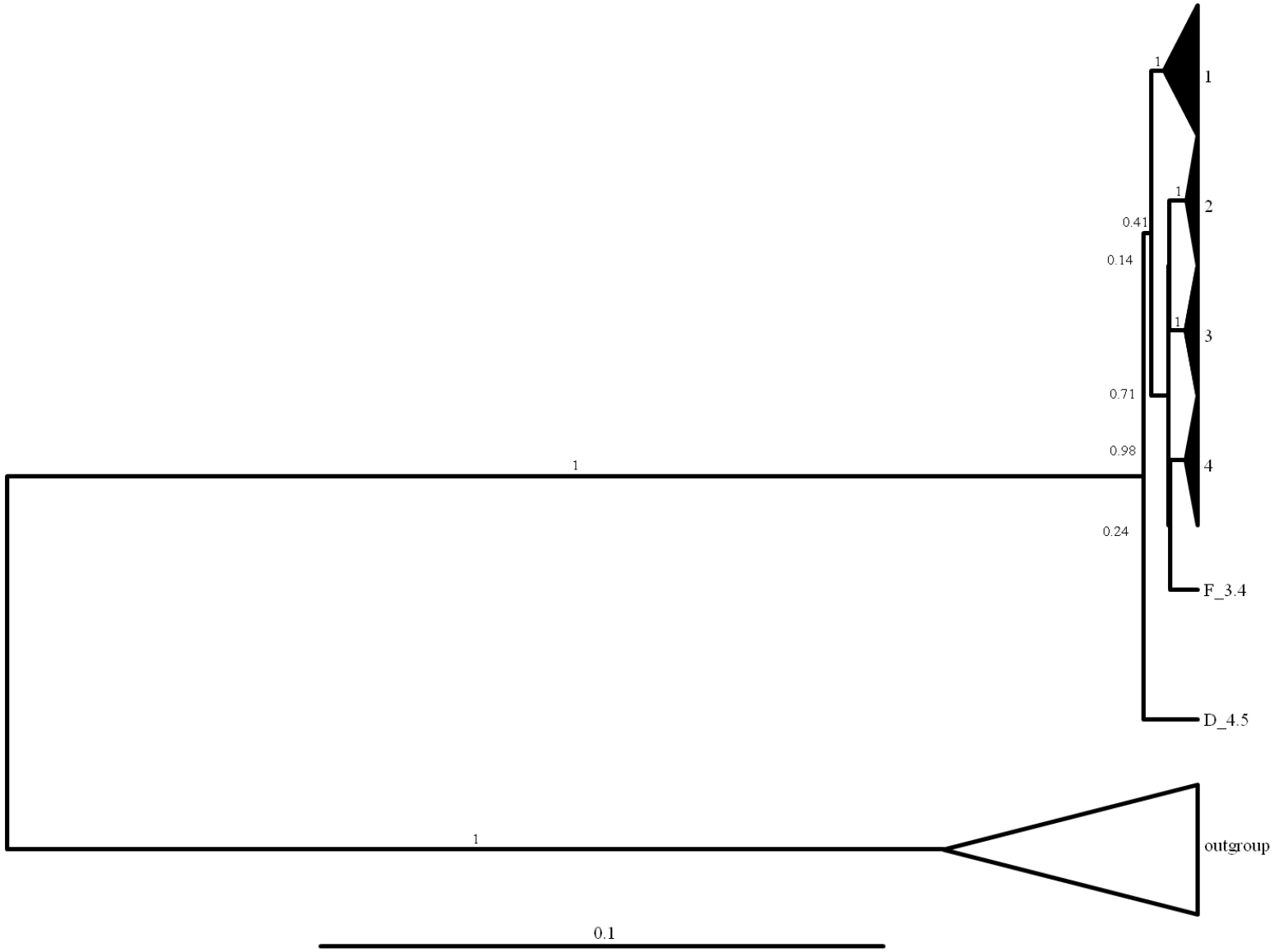

Figure A25: Bayesian phylogeny after $10x10^6$ generations from 100 *COI* nucleotide sequences of *Nothrus silvestris* with Beast v1.5.4. Outgroup are four individuals of *Nothrus palustris* from different locations. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 15.

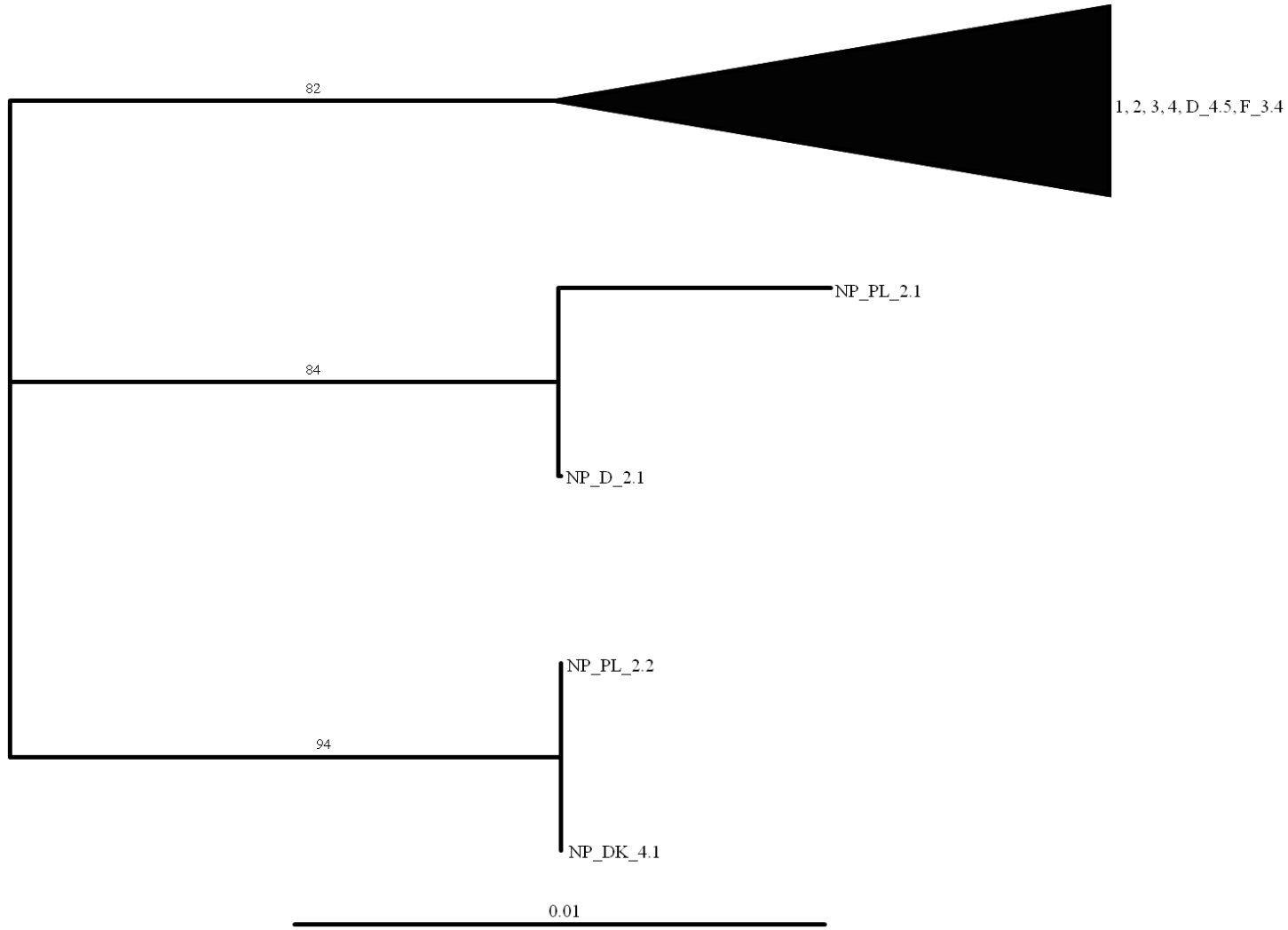

Figure A26: Neighbor-Joining tree of 100 *COI* protein sequences of *Nothrus silvestris*. Outgroup are four individuals of *Nothrus palustris* from different locations. Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Tip numbers are the different subclades and explain in Table 15.

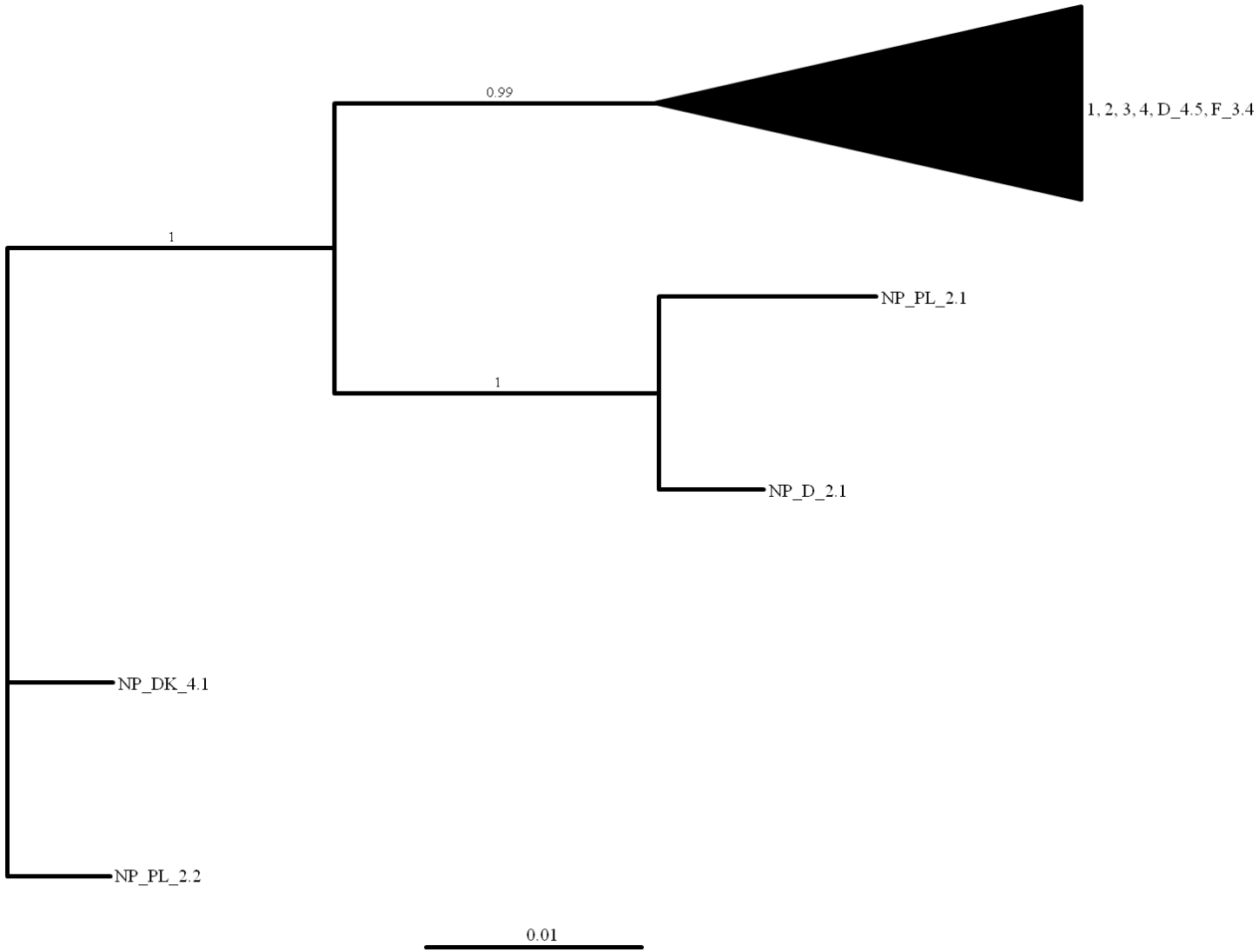

Figure A27: Bayesian tree after $10x10^6$ generations from 100 *COI* protein sequences of *Nothrus silvestris*. Split frequencies of 0.042324 and burnin of 25%. Outgroup are four individuals of *Nothrus palustris* from different locations. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 15.

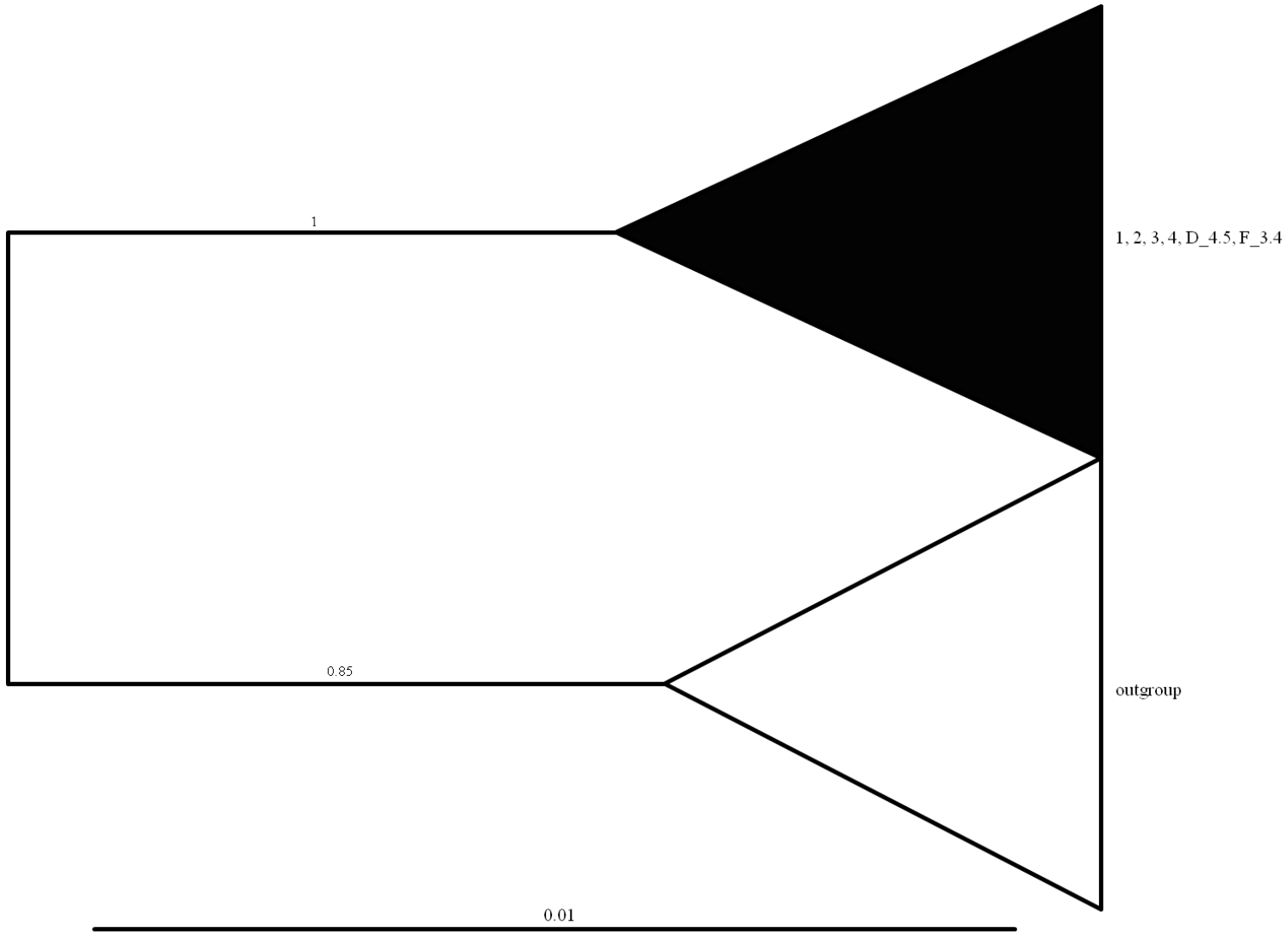

Figure A28: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 from the 100 *COI* protein sequences of *Nothrus silvestris*. Outgroup are four individuals of *Nothrus palustris* from different locations. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 15.

Appendix

Table A20: Mean pairwise differences of uncorrected p-distances of the protein of *Nothrus silvestris* from 24 locations in percent. The diagonal is the within population differences (bold) and below the diagonal among populations. Bold letters in red are the minimum and maximum differences within and among the populations. Locations with less than two individuals were excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	CH 2	0																							
2	CH 3	0	0																						
3	D 1	0	0	0																					
4	D 2	0	0	0	0																				
5	D 4	0	0	0	0	0																			
6	D 6	0	0	0	0	0	0																		
7	D 7	0	0	0	0	0	0	0																	
8	D 8	0	0	0	0	0	0	0	0																
9	D 9	0	0	0	0	0	0	0	0	0															
10	D 11	0	0	0	0	0	0	0	0	0	0														
11	D 16	0	0	0	0	0	0	0	0	0	0	0													
12	D 17	0	0	0	0	0	0	0	0	0	0	0	0												
13	D 18	0	0	0	0	0	0	0	0	0	0	0	0	0											
14	F 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0										
15	FIN 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
16	GB 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
17	GB 2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3							
18	NL 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0						
19	NL 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0					
20	PL 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0				
21	RUM 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0			
22	RUM 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0		
23	RUM 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	
24	S 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0

Table A21: Tajima's D and Fu's FS neutrality tests of *Nothrus silvestris COI* nucleotide sequences from 24 locations. Bold red letters show significance (P<0.05).

Neutrality tests	CH 2	CH 3	D 1	D 2	D 4	D 6	D 7	D 8	D 9	D 11	D 16	D 17	D 18	F 3	FIN 1	GB 1	GB 2	NL 1	NL 2	PL 2
Tajima's D test																				
Samplesize	3	2	4	4	8	2	4	3	4	4	4	3	4	4	4	3	4	3	2	13
S	9	2	0	11	20	10	0	0	0	3	1	13	0	9	0	10	13	0	9	6
Pi	6	2	0	5.67	7.93	10	0	0	0	1.5	0.5	8.67	0	4.5	0	6.67	8.33	0	9	0.92
Tajima's D	0	0	0	-0.56	0.15	0	0	0	0	-0.75	-0.61	0	0	-0.83	0	0	1.77	0	0	-1.93
Tajima's D p-value	0.73	1	1	0.43	0.57	1	1	1	1	0.23	0.38	0.70	1	0.12	1	0.72	0.94	1	1	0.01
Fu's FS test																				
Real no. of alleles	2	2	1	4	6	2	1	1	1	2	2	3	1	3	1	3	3	1	2	2
Orig. no. of alleles	2	2	1	4	6	2	1	1	1	2	2	3	1	3	1	3	3	1	2	2
Theta_pi	6	2	0	5.67	7.93	10	0	0	0	1.5	0.5	8.67	0	4.5	0	6.67	8.33	0	9	0.92
Exp. no. of alleles	2.61	1.67	0	3.24	5.79	1.91	0	0	0	2.36	1.68	2.71	0	3.11	0	2.64	3.43	0	1.9	3.05
FS	3.30	0.69	0	-0.36	0.46	2.3	0	0	0	1.72	0.17	0.99	0	1.34	0	0.7	2.32	0	2.2	2.3
FS p-value	0.89	0.37	N.A.	0.22	0.54	0.56	N.A.	N.A.	N.A.	0.75	0.34	0.45	N.A.	0.69	N.A.	0.42	0.81	N.A.	0.55	0.84

Neutrality tests	RUM 1	RUM 2	RUM 5	S 3	mean	s.d.
Tajima's D test						
Samplesize	3	4	4	4	4.04	2.24
S	3	0	0	10	5.38	5.78
Pi	2	0	0	5	3.28	3.61
Tajima's D	0	0	0	-0.83	-0.15	0.63
Tajima's D p-value	0.88	1	1	0.11	0.74	0.34
Fu's FS test						
Real no. of alleles	2	1	1	3	2.08	1.21
Orig. no. of alleles	2	1	1	3	2.08	1.21
Theta_pi	2	0	0	5	3.28	3.61
Exp. no. of alleles	2.17	0	0	3.17	1.73	1.58
FS	1.61	0	0	1.51	0.89	1.04
FS p-value	0.69	N.A.	N.A.	0.71	N.A.	N.A.

Table A22: Tajima's D and Fu's FS neutrality tests of *Nothrus silvestris COI* protein sequences from 24 locations. Bold red letters show significance (P<0.05).

Neutrality tests	CH 2	CH 3	D 1	D 2	D 4	D 6	D 7	D 8	D 9	D 11	D 16	D 17	D 18	F 3	FIN 1	GB 1	GB 2	NL 1	NL 2	PL 2
Tajima's D test																				
Samplesize	3	2	4	4	8	2	4	3	4	4	4	3	4	4	4	3	4	3	2	13
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Pi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0
Tajima's D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-0.61	0	0	0
Tajima's D p-value	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.38	1	1	1
Fu's FS test																				
Real no. of alleles	2	1	1	2	4	2	1	1	1	2	2	2	1	1	1	3	2	1	2	1
Orig. no. of alleles	2	1	1	2	4	2	1	1	1	2	2	2	1	1	1	3	2	1	2	1
Theta_pi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0
Exp. no. of alleles	2	0	0	2	4	0	0	0	0	2	2	2	0	0	0	0	1.68	0	0	0
FS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.17	0	0	0
FS p-value	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.34	N.A.	N.A.	N.A.

Neutrality tests	RUM 1	RUM 2	RUM 5	S 3	mean	s.d.
Tajima's D test						
Samplesize	3	4	4	4	4.04	2.24
S	0	0	0	0	0.04	0.20
Pi	0	0	0	0	0.02	0.10
Tajima's D	0	0	0	0	-0.03	0.13
Tajima's D p-value	1	1	1	1	0.97	0.13
Fu's FS test						
Real no. of alleles	1	1	1	2	1.58	0.78
Orig. no. of alleles	1	1	1	2	1.58	0.78
Theta_pi	0	0	0	0	0.02	0.10
Exp. no. of alleles	0	0	0	2	0.74	1.14
FS	0	0	0	0	0.01	0.04
FS p-value	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

Table A23: Results of the McDonald-Kreitman test for *Nothrus silvestris*. The differences between the populations are not significant. Locations with less than two individuals were excluded.

Population		CH 2			CH 3			D 1			D 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
CH 3	fixed	0	0	-									
Rohrschach	poly	10	1										
D 1	fixed	0	0	-	0	0	-						
Kranichstein	poly	8	1		2	0							
D 2	fixed	0	0	-	5	0	1 ns	5	0	1 ns			
Goettingen	poly	18	2		12	0		10	1				
D 4	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Meckl. Seenpl.	poly	17	3		18	3		17	3		26	3	
D 6	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Black Forest	poly	9	1		11	1		9	1		19	2	
D 7	fixed	0	0	-	8	1	1 ns	8	1	-	3	1	0.48 ns
Uelzen	poly	8	1		2	0		0	0		10	1	
D 8	fixed	0	0	-	0	0	-	0	0	-	5	0	1 ns
Cuxhaven	poly	8	1		2	0		0	0		10	1	
D 9	fixed	0	0	-	0	0	-	0	0	-	5	0	1 ns
Bonn	poly	8	1		2	0		0	0		10	1	
D 11	fixed	3	0	1 ns	8	0	0.38 ns	8	2	0.27 ns	0	0	-
Wittmoor	poly	10	2		4	1		2	1		12	1	
D 16	fixed	0	0	-	0	0	-	0	0	-	5	0	1 ns
Solling	poly	8	2		2	1		0	1		10	1	
D 17	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Rubi	poly	15	2		14	1		12	1		16	2	
D 18	fixed	0	0	-	0	0	-	0	0	-	5	0	1 ns
Steinh. Meer	poly	8	1		2	0		0	0		10	1	
F 3	fixed	0	0	-	4	0	-	4	0	-	0	0	-
Saint Isidore	poly	16	1		11	0		9	0		17	1	
FIN 1	fixed	0	0	-	0	0	-	0	0	-	5	0	1 ns
Lahti	poly	8	1		2	0		0	0		10	1	
GB 1	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Ascot	poly	8	2		10	2		8	2		18	3	
GB 2	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Braemar	poly	14	3		13	2		11	2		15	3	
NL 1	fixed	0	0	-	0	0	-	0	0	-	5	0	1 ns
Wageningen	poly	8	1		2	0		0	0		10	1	
NL 2	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Hoge Veluwe	poly	8	1		10	1		8	1		18	2	
PL 2	fixed	0	0	-	0	0	-	0	0	-	1	0	1 ns
Warsaw	poly	10	1		8	0		6	0		15	1	
RUM 1	fixed	0	0	-	0	0	-	0	0	-	3	0	1 ns
Sibiu 1	poly	9	1		5	0		3	0		13	1	
RUM 2	fixed	0	0	-	0	0	-	0	0	-	5	0	1 ns
Sibiu 2	poly	8	1		2	0		0	0		10	1	
RUM 5	fixed	2	0	1 ns	4	0	-	4	0	-	5	0	1 ns
Busteni	poly	8	1		2	0		0	0		10	1	
S 3	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Malmoe	poly	9	1		11	1		9	1		19	2	

Population		D 4			D 6			D 7			D 8		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 6	fixed	0	0	-									
Black Forest	poly	18	3										
D 7	fixed	0	0	-	0	0	-						
Uelzen	poly	17	3		9	1							
D 8	fixed	0	0	-	0	0	-	8	1	-			
Cuxhaven	poly	17	3		9	1		0	0				
D 9	fixed	0	0	-	0	0	-	8	1	-	0	0	-
Bonn	poly	17	3		9	1		0	0		0	0	

Table A23 continue:

Population		D 4			D 6			D 7			D 8		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 11	**fixed**	2	0	1 ns	3	0	1 ns	6	1	1 ns	8	0	0.27 ns
Wittmoor	**poly**	19	3		11	2		2	1		2	1	
D 16	**fixed**	0	0	-	0	0	-	8	1	0.2 ns	0	0	-
Solling	**poly**	17	3		9	2		0	1		0	1	
D 17	**fixed**	0	0	-	0	0	-	3	1	0.43 ns	0	0	-
Rubi	**poly**	23	4		16	2		12	1		12	1	
D 18	**fixed**	0	0	-	0	0	-	8	1	-	0	0	-
Steinh. Meer	**poly**	17	3		9	1		0	0		0	0	
F 3	**fixed**	0	0	-	0	0	-	3	1	0.31 ns	4	0	-
Saint isidore	**poly**	24	3		17	1		9	0		9	0	
FIN 1	**fixed**	0	0	-	0	0	-	8	1	-	0	0	-
Lahti	**poly**	17	3		9	1		0	0		0	0	
GB 1	**fixed**	0	0	-	0	0	-	0	0	-	0	0	-
Ascot	**poly**	17	4		9	2		8	2		8	2	
GB 2	**fixed**	0	0	-	0	0	-	3	1	1 ns	0	0	-
Braemar	**poly**	23	5		15	3		11	2		11	2	
NL 1	**fixed**	0	0	-	0	0	-	0	0	-	0	0	-
Wageningen	**poly**	17	3		9	1		2	0		0	0	
NL 2	**fixed**	0	0	-	0	0	-	0	0	-	0	0	-
Hoge Veluwe	**poly**	17	3		9	1		8	1		8	1	
PL 2	**fixed**	0	0	-	0	0	-	4	1	0.45 ns	0	0	-
Warsaw	**poly**	18	3		11	1		6	0		6	0	
RUM 1	**fixed**	0	0	-	0	0	-	6	1	1 ns	0	0	-
Sibiu 1	**poly**	18	3		10	1		3	0		3	0	
RUM 2	**fixed**	0	0	-	0	0	-	8	1	-	0	0	-
Sibiu 2	**poly**	17	3		9	1		0	0		0	0	
RUM 5	**fixed**	2	0	1 ns	2	0	1 ns	8	1	-	4	0	-
Busteni	**poly**	17	3		9	1		0	0		0	0	
S 3	**fixed**	0	0	-	0	0	-	0	0	-	0	0	-
Malmoe	**poly**	18	3		10	1		9	1		9	1	

Population		D 9			D 11			D 16			D 17		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons	sign.
D 11	fixed	8	0	0.27 ns									
Wittmoor	poly	2	1										
D 16	fixed	0	0	-	8	0	0.27 ns						
Solling	poly	0	1		2	1							
D 17	fixed	0	0	-	3	0	1 ns	0	0	-			
Rubi	poly	12	1		14	2		12	2				
D 18	fixed	0	0	-	8	0	0.27 ns	0	0	-	0	0	-
Steinh. Meer	poly	0	0		2	1		0	1		12	1	
F 3	fixed	4	0	-	1	0	1 ns	4	0	1 ns	0	0	-
Saint Isidore	poly	9	0		11	1		9	1		20	1	
FIN 1	fixed	0	0	-	8	0	0.27 ns	0	0	-	0	0	-
Lahti	poly	0	0		2	1		0	1		12	1	
GB 1	fixed	0	0	-	3	0	1 ns	0	0	-	0	0	-
Ascot	poly	10	2		10	3		8	3		15	3	
GB 2	fixed	0	0	-	3	0	1 ns	0	0	-	0	0	-
Braemar	poly	11	2		13	3		11	3		12	3	
NL 1	fixed	0	0	-	8	0	0.27 ns	0	0	-	0	0	-
Wageningen	poly	0	0		2	1		0	1		12	1	
NL 2	fixed	0	0	-	3	0	1 ns	0	0	-	0	0	-
Hoge Veluwe	poly	8	1		10	2		8	2		11	2	
PL 2	fixed	0	0	-	3	0	1 ns	0	0	-	0	0	-
Warsaw	poly	6	0		8	1		6	1		12	1	
RUM 1	fixed	0	0	-	6	0	1 ns	0	0	-	0	0	-
Sibiu 1	poly	3	0		5	1		3	1		13	1	

Table A23 continue:

Population		D 9			D 11			D 16			D 17		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons	sign.
RUM 2	fixed	0	0	-	8	0	0.27 ns	0	0	-	0	0	-
Sibiu 2	poly	0	0		2	1		0	1		12	1	
RUM 5	fixed	4	0	-	8	0	0.27 ns	4	0	0.2 ns	2	0	1 ns
Busteni	poly	0	0		2	1		0	1		12	1	
S 3	fixed	0	0	-	3	0	1 ns	0	0	-	0	0	-
Malmoe	poly	9	1		11	2		9	2		16	2	

Population		D 18			F 3			FIN 1			GB 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
F 3	fixed	4	0	-									
Saint Isidore	poly	9	0										
FIN 1	fixed	0	0	-	4	0	-						
Lahti	poly	0	0		9	0							
GB 1	fixed	0	0	-	0	0	-	0	0	-			
Ascot	poly	8	2		16	2		8	2				
GB 2	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Braemar	poly	11	2		19	2		11	2		14	4	
NL 1	fixed	0	0	-	4	0	-	0	0	-	0	0	-
Wageningen	poly	0	0		9	0		0	0		8	2	
NL 2	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Hoge Veluwe	poly	8	1		16	1		8	1		8	2	
PL 2	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Warsaw	poly	6	0		13	0		6	0		10	2	
RUM 1	fixed	0	0	-	3	0	-	0	0	-	0	0	-
Sibiu 1	poly	3	0		11	0		3	0		9	2	
RUM 2	fixed	0	0	-	4	0	-	0	0	-	0	0	-
Sibiu 2	poly	0	0		9	0		0	0		8	2	
RUM 5	fixed	4	0	-	5	0	-	4	0	-	2	0	1 ns
Busteni	poly	0	0		9	0		0	0		8	2	
S 3	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Malmoe	poly	9	1		17	1		9	1		9	2	

Population		GB 2			NL 1			NL 2			PL 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
NL 1	fixed	0	0	-									
Wageningen	poly	11	2										
NL 2	fixed	0	0	-	0	0	-						
Hoge Veluwe	poly	14	3		8	1							
PL 2	fixed	0	0	-	0	0	-	0	0	-			
Warsaw	poly	13	2		6	0		10	1				
RUM 1	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Sibiu 1	poly	12	2		3	0		9	1		8	0	
RUM 2	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Sibiu 2	poly	11	2		0	0		8	1		6	0	
RUM 5	fixed	2	0	1 ns	4	0	-	2	0	1 ns	3	0	-
Busteni	poly	11	2		0	0		8	1		6	0	
S 3	fixed	0	0	-	0	0	-	0	0	-	0	0	-
Malmoe	poly	15	3		9	1		9	1		11	1	

Population		RUM 1			RUM 2			RUM 5		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM 2	fixed	0	0	-						
Sibiu 2	poly	3	0							
RUM 5	fixed	1	0	-	4	0	-			
Busteni	poly	3	0		0	0				
S 3	fixed	0	0	-	0	0	-	2	0	1 ns
Malmoe	poly	10	1		9	1		9	1	

Table A24: Neutrality indices of *Nothrus silvestris* computed in the McDonald-Kreitman test with DnaSP v5. Locations with less than two individuals were excluded.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	**CH 2**																							
2	**CH 3**	-																						
3	**D 1**	-	-																					
4	**D 2**	-	-	-																				
5	**D 4**	-	-	-	-																			
6	**D 6**	-	-	-	-	-																		
7	**D 7**	-	0	-	0.3	-	-																	
8	**D 8**	-	-	-	-	-	-	-																
9	**D 9**	-	-	-	-	-	-	-	-															
10	**D 11**	-	-	-	-	-	-	3	-	-														
11	**D 16**	-	-	-	-	-	-	-	-	-	-													
12	**D 17**	-	-	-	-	-	-	0.25	-	-	-	-												
13	**D 18**	-	-	-	-	-	-	-	-	-	-	-	-											
14	**F 3**	-	-	-	-	-	-	0	-	-	-	-	-	-										
15	**FIN 1**	-	-	-	-	-	-	-	-	-	-	-	-	-	-									
16	**GB 1**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
17	**GB 2**	-	-	-	-	-	-	0.55	-	-	-	-	-	-	-	-	-							
18	**NL 1**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
19	**NL 2**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
20	**PL 2**	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-				
21	**RUM 1**	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-			
22	**RUM 2**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
23	**RUM 5**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
24	**S 3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

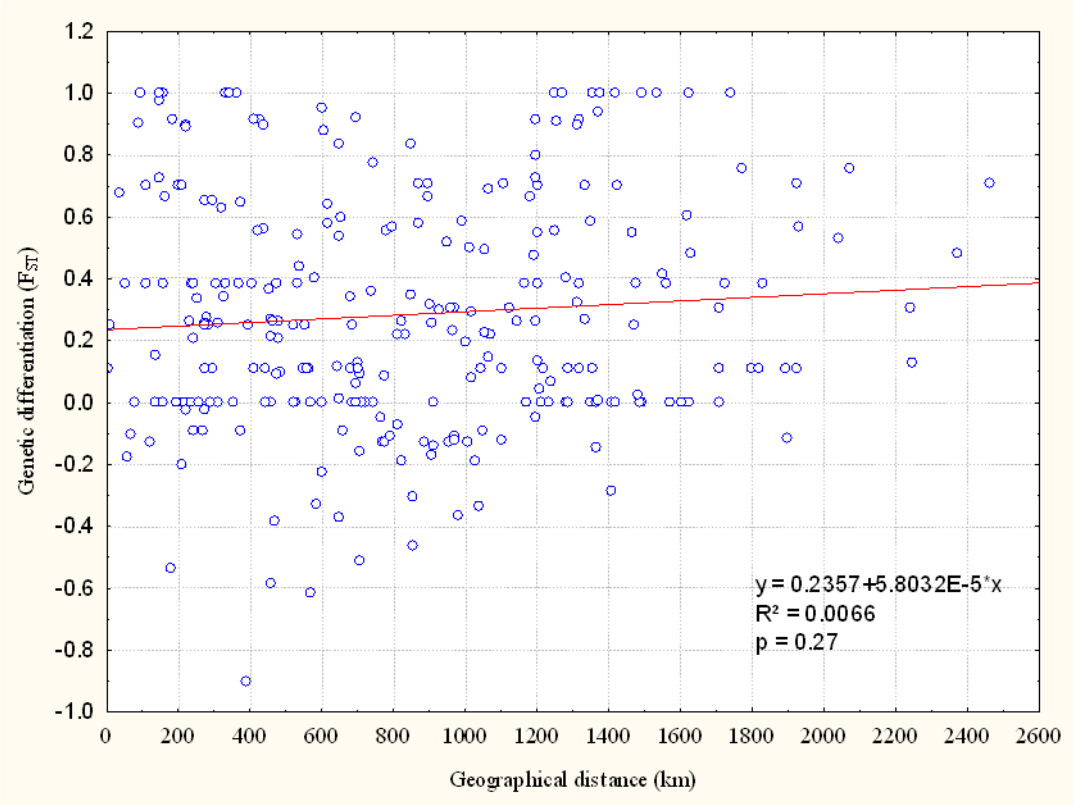

Figure A29: Linear regression of geographical distances (kilometers) vs. genetic differentiations (F_{ST}) based on *COI* of *Nothrus silvestris*. Regression is not significant (p=0.27; (*0.01<P<0.05; **0.001<P<0.01; ***P<0.001)) using 1000 randomizations.

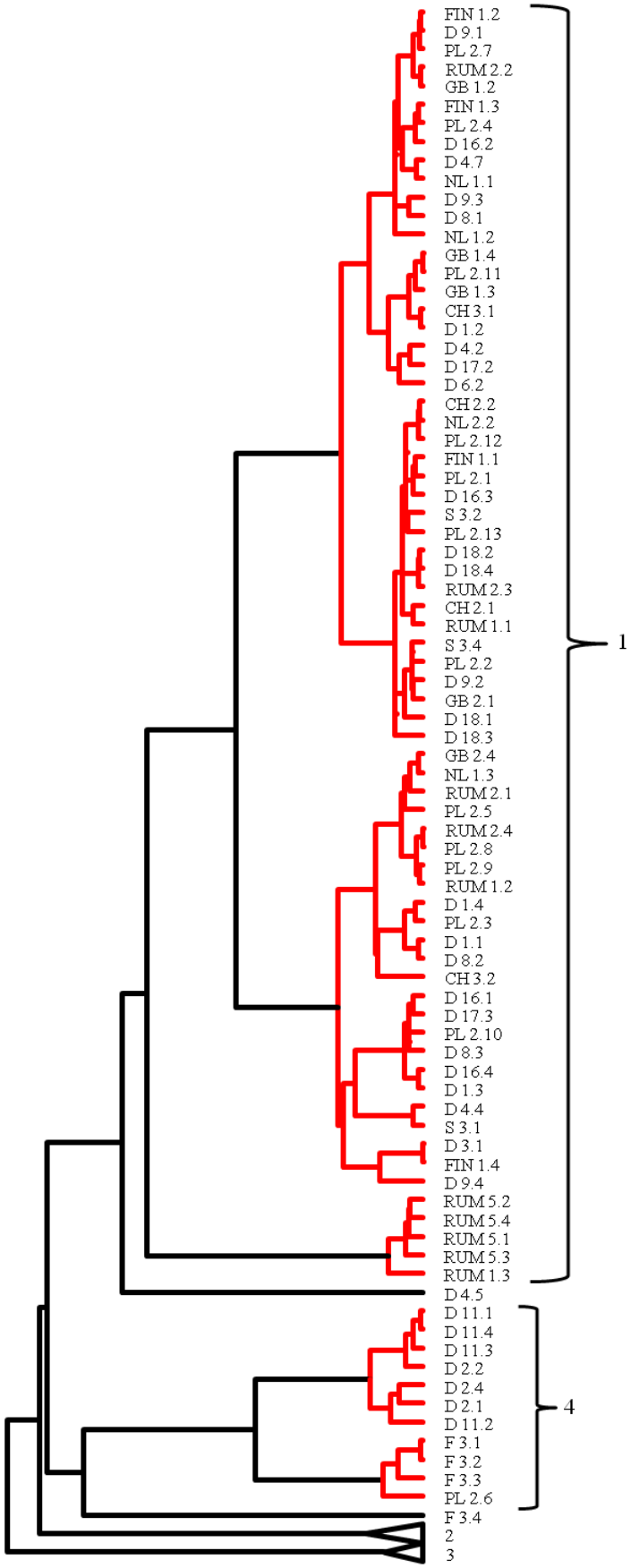

Figure A30: Cluster delimitation of *COI* nucleotide sequences from *Nothrus silvestris* after the single method of the generalized mixed Yule coalescent method (GMYC). Red branches are the different distinct clusters. Tip labels are explained in Table 15.

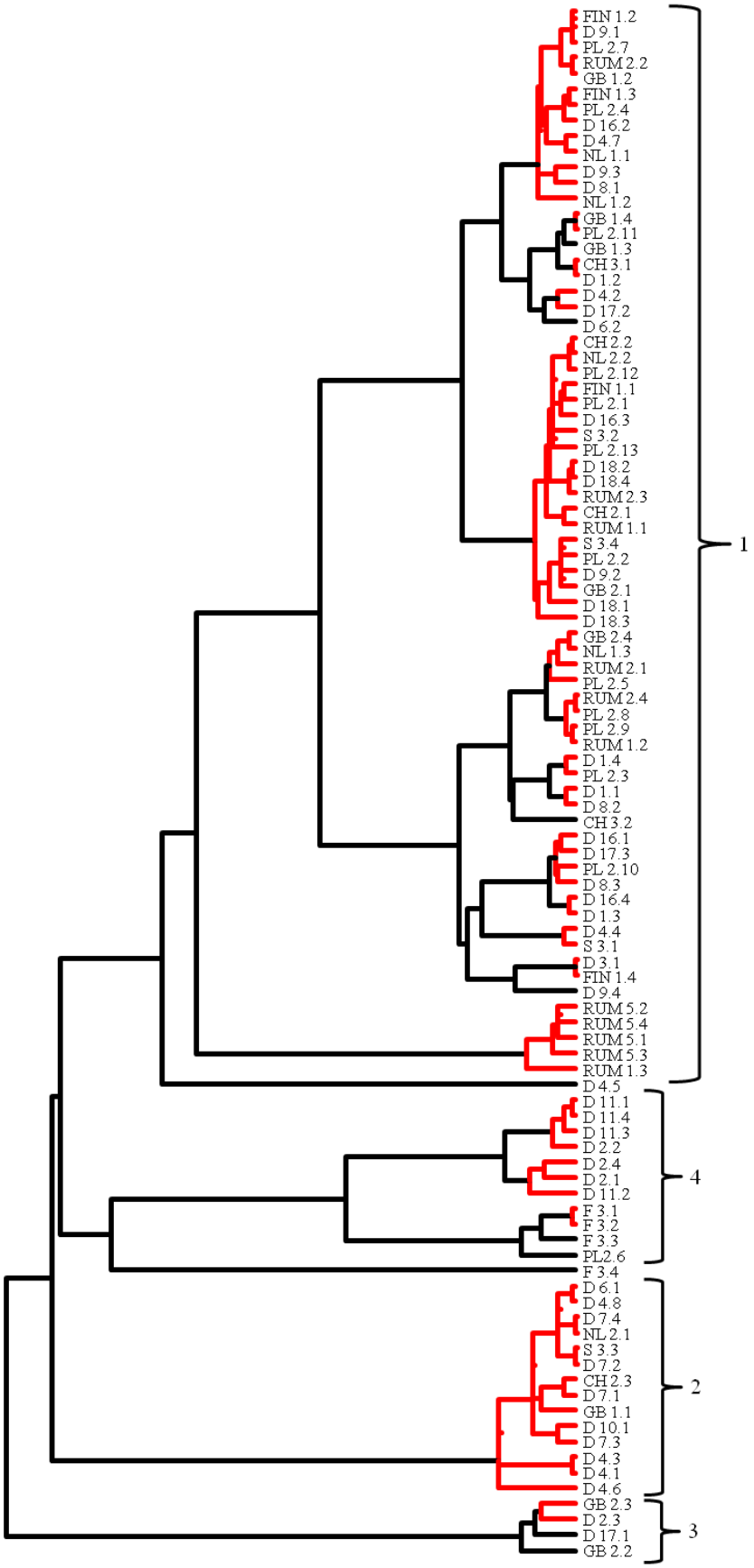

Figure A31 Cluster delimitation of *COI* nucleotide sequences from *Nothrus silvestris* after the multiple method of the generalized mixed Yule coalescent method (GMYC). Red branches are the different distinct clusters. Tip labels are explained in Table 15.

Table A25: Standard diversity measures of *Platynothrus peltifer*. Populations with less than two individuals were excluded.

population	sample size n	invarible sites N_{is}	variable sites N_{vs}	parsimony inform. sites N_{pars}	number of singeltons N_s	number of haplotypes N_h	haplotype diversity H_d	variance	nucleotide diversity Π_n
A_1	2	555	1	0	1	2	1	0.25	0.002
A_2	2	556	0	0	0	1	0	0	0
A_3	3	553	3	0	3	2	0.67	0.099	0.004
A_4	2	556	0	0	0	1	0	0	0
A_5	4	413	143	32	111	4	1	0.031	0.144
A_9	5	553	3	1	2	4	0.9	0.026	0.003
A_10	5	556	0	0	0	1	0	0	0
CH_3	5	554	2	0	2	3	0.7	0.048	0.001
D_1	4	456	100	6	94	4	1	0.031	0.093
D_2	2	556	0	0	0	1	0	0	0
D_3	4	549	7	2	5	4	1	0.031	0.007
D_4	8	455	101	99	2	4	0.64	0.034	0.078
D_5	4	546	10	1	9	4	1	0.031	0.009
D_6	5	555	1	0	1	2	0.4	0.056	0.001
D_7	3	556	0	0	0	1	0	0	0
D_8	4	443	113	104	9	4	1	0.031	0.132
D_9	3	456	100	0	100	2	0.67	0.099	0.12
D_11	2	454	102	0	102	2	1	0.25	0.183
D_12	5	551	5	2	3	3	0.7	0.048	0.004
D_15	3	444	112	0	112	3	1	0.074	0.134
D_17	3	446	110	0	110	3	1	0.074	0.132
D_18	7	527	29	25	4	4	0.71	0.033	0.024
DK_2	6	529	27	27	0	3	0.73	0.024	0.030
DK_3	6	556	0	0	0	1	0	0	0
F_1	4	412	144	62	82	3	0.83	0.049	0.157
F_3	2	556	0	0	0	1	0	0	0
F_5	3	553	3	0	3	3	1	0.074	0.004
FIN_1	4	555	1	0	1	2	0.5	0.07	0.001
GB_1	3	445	111	0	111	2	0.67	0.099	0.133
GB_2	4	530	26	0	26	2	0.5	0.07	0.024
N_1	2	554	2	0	2	2	1	0.25	0.004
NL_1	3	473	83	0	83	2	0.67	0.099	0.1
NL_2	4	553	3	1	2	3	0.83	0.049	0.003
PL_2	9	511	45	27	18	8	0.97	0.004	0.027
RUM_2	2	458	98	0	98	2	1	0.25	0.176
S_1	3	554	2	0	2	3	1	0.074	0.002
S_2	9	467	89	82	7	8	0.97	0.004	0.063
S_4	4	537	19	0	19	3	0.83	0.049	0.017
S_5	4	552	4	1	3	3	0.83	0.049	0.004
all	157	360	196	172	24	74	0.98	0	0.113

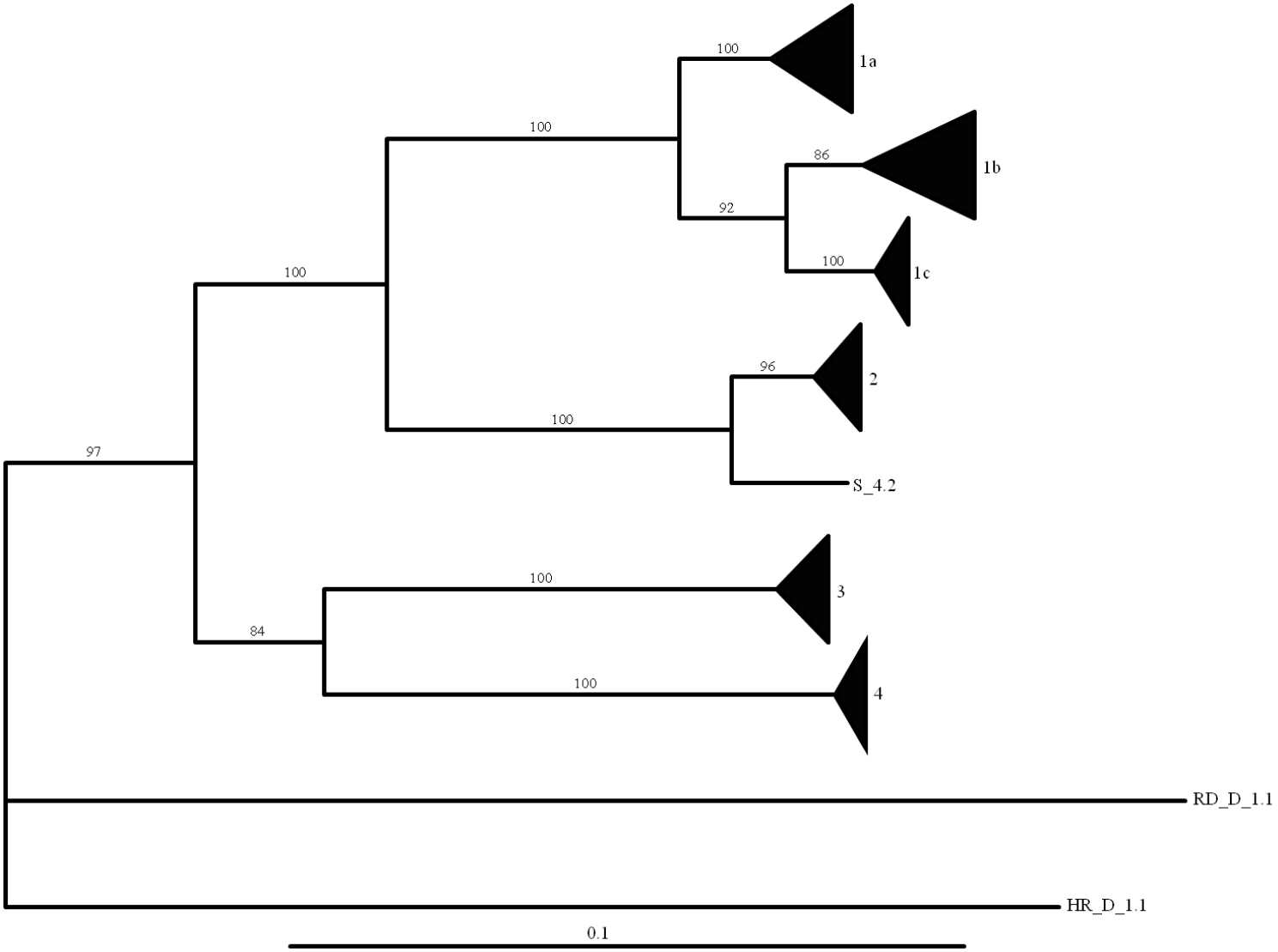

Figure A32: Neighbor-Joining tree of 160 *COI* nucleotide sequences of *Platynothrus peltifer*. Outgroups are one individual of *Hypochthonius rufulus* and *Rhysotritia duplicata*, respectively. Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Tip numbers are the different subclades and explain in Table 20.

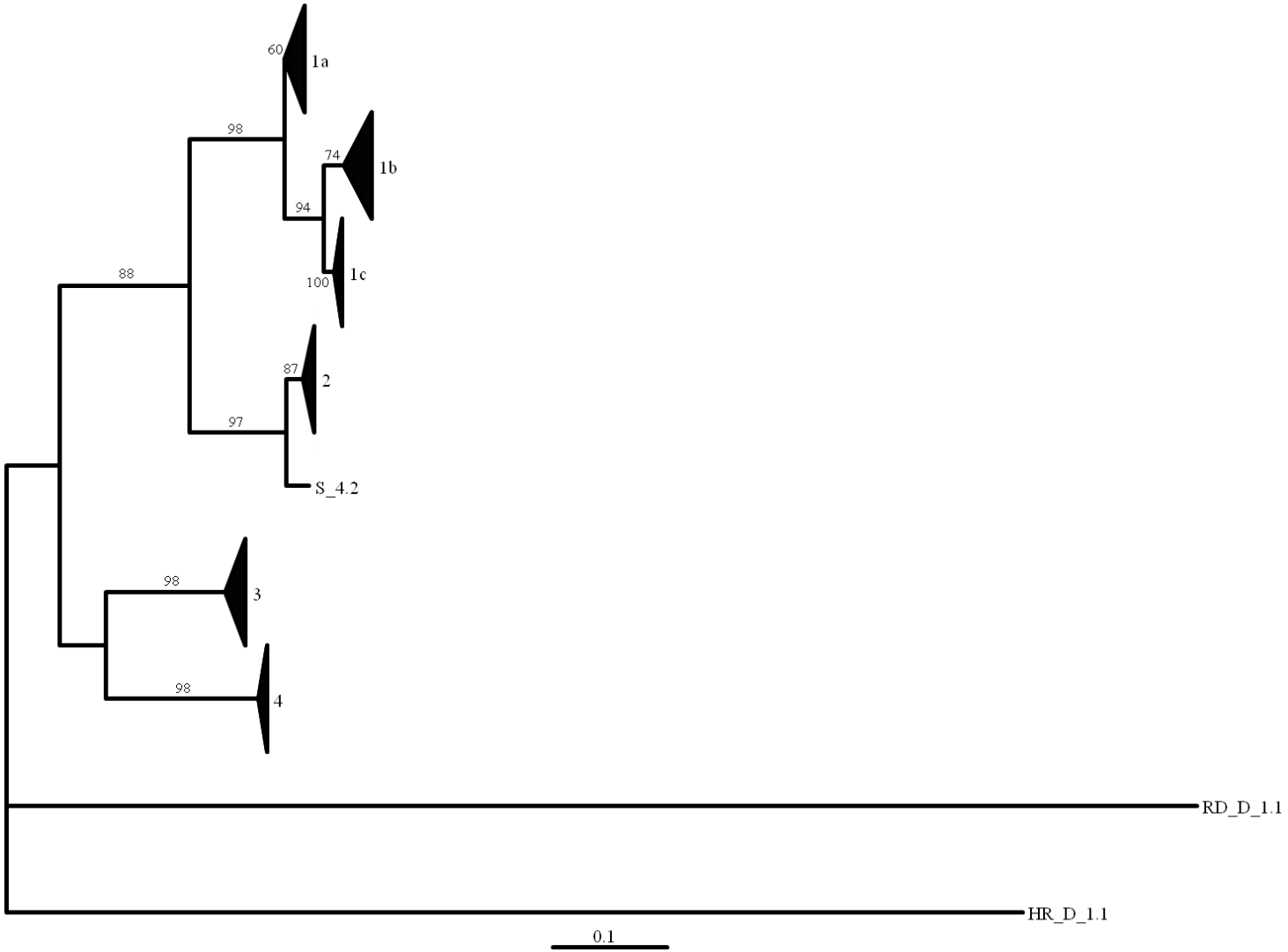

Figure A33: Neighbor-Joining tree of 160 *COI* nucleotide sequences of *Platynothrus peltifer* with model of sequence evolution HKY+G. Outgroups are one individual of *Hypochthonius rufulus* and *Rhysotritia duplicata*, respectively. Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Tip numbers are the different subclades and explain in Table 20.

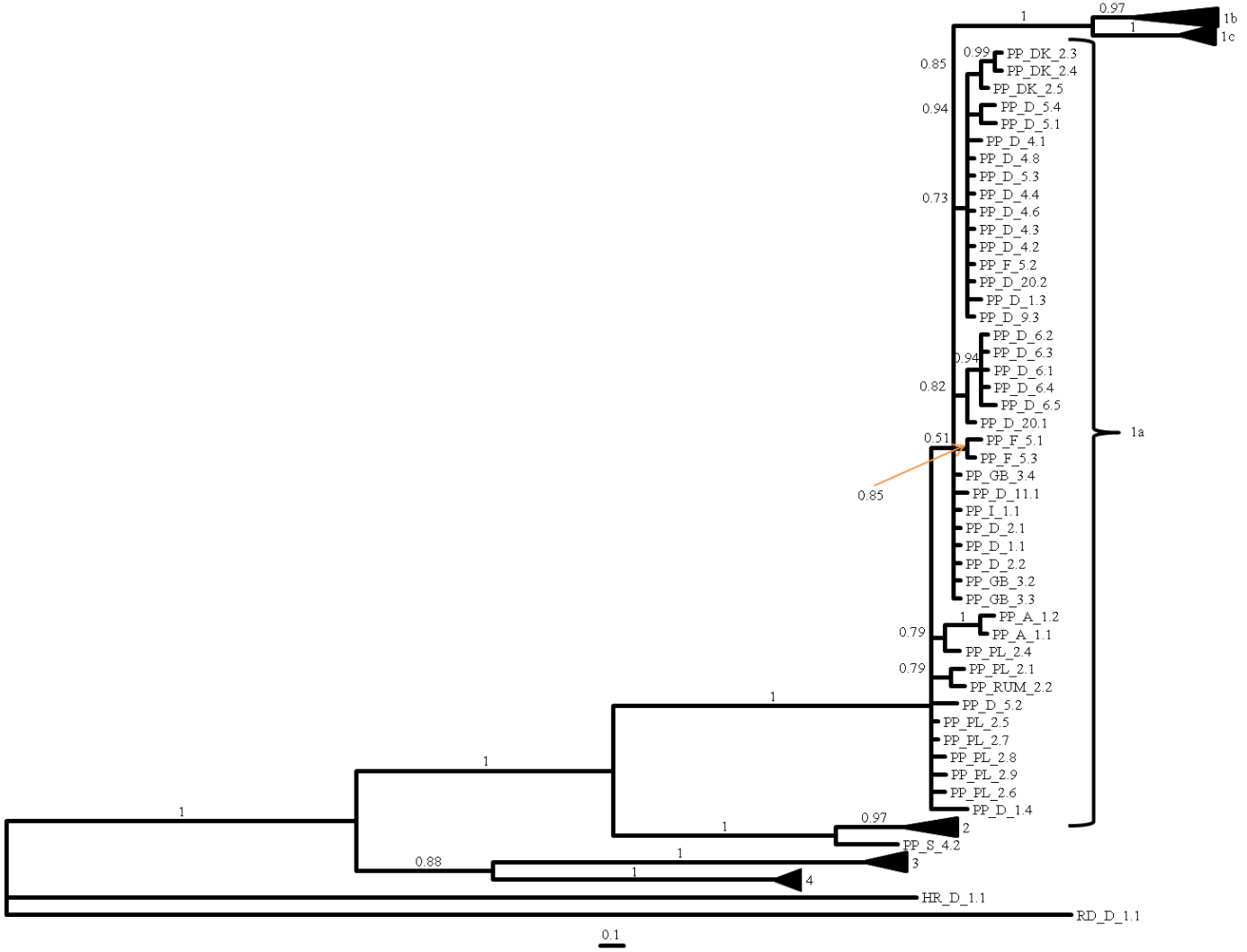

Figure A34: Bayesian tree after $10x10^6$ generations from 160 *COI* nucleotide sequences from *Platynothrus peltifer* with MrBayes. Split frequencies of 0.01069 and burnin of 25%. Outgroups are one individual of *Hypochthonius rufulus* and *Rhysotritia duplicata*, respectively. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 20.

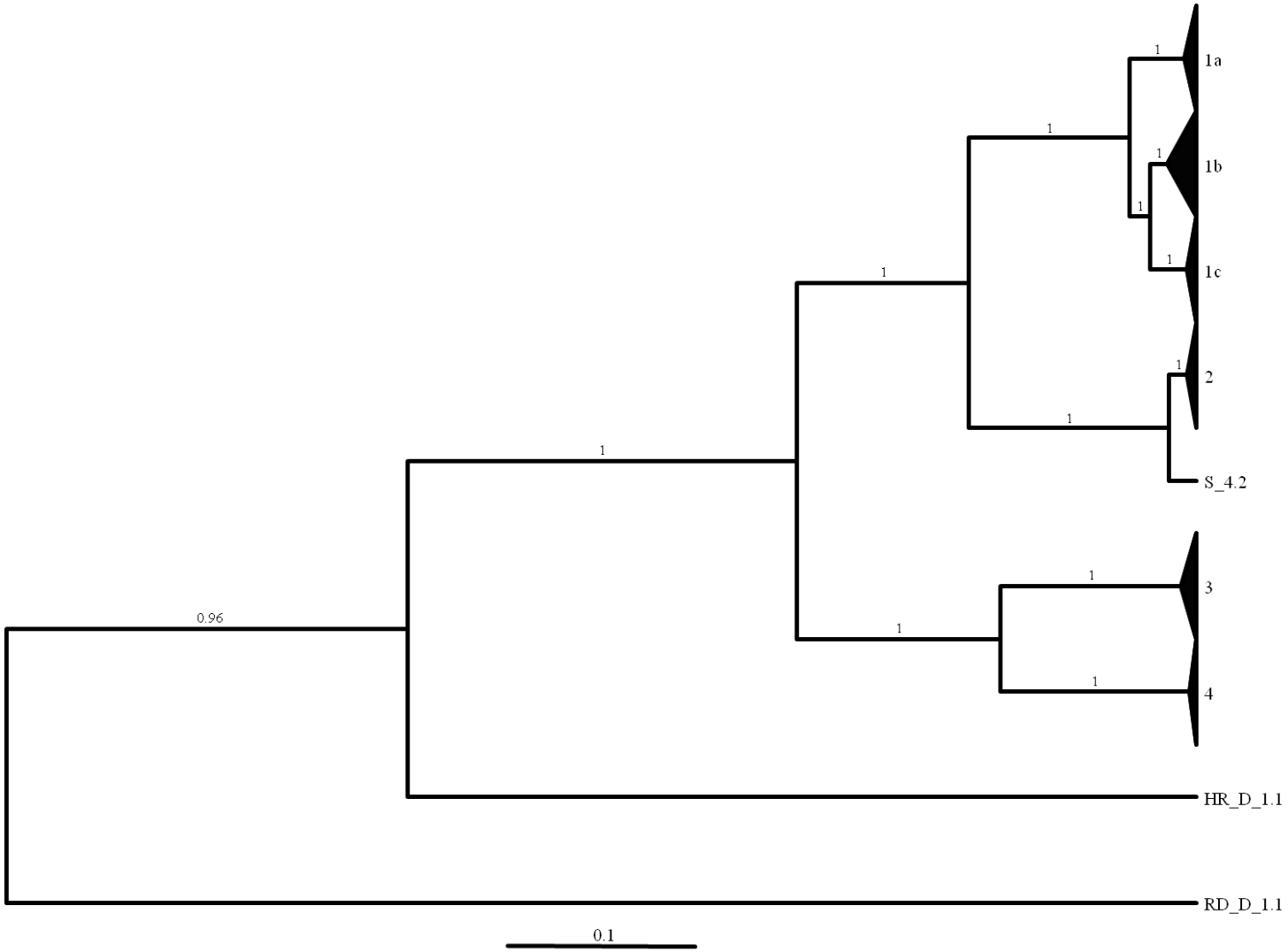

Figure A35: Bayesian phylogeny after $10x10^6$ generations from the 160 *COI* nucleotide sequences of *Platynothrus peltifer* with Beast v1.5.4. Outgroups are one individual of *Hypochthonius rufulus* and *Rhysotritia duplicata,* respectively. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 20.

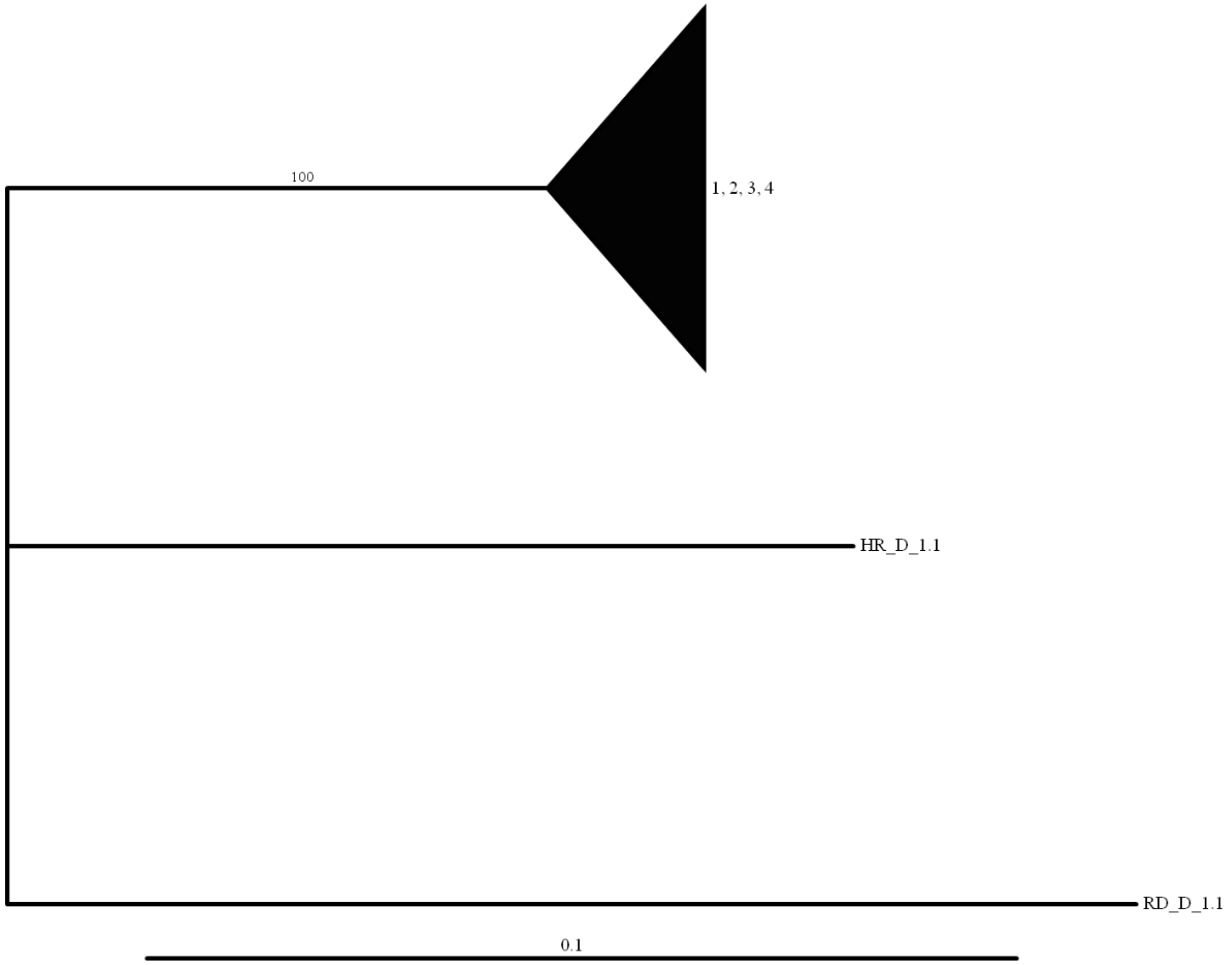

Figure A36: Neighbor-Joining tree of 160 *COI* protein sequences of *Platynothrus peltifer*. Outgroups are one individual of *Hypochthonius rufulus* and *Rhysotritia duplicata*, respectively. Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Tip numbers are the different subclades and explain in Table 20.

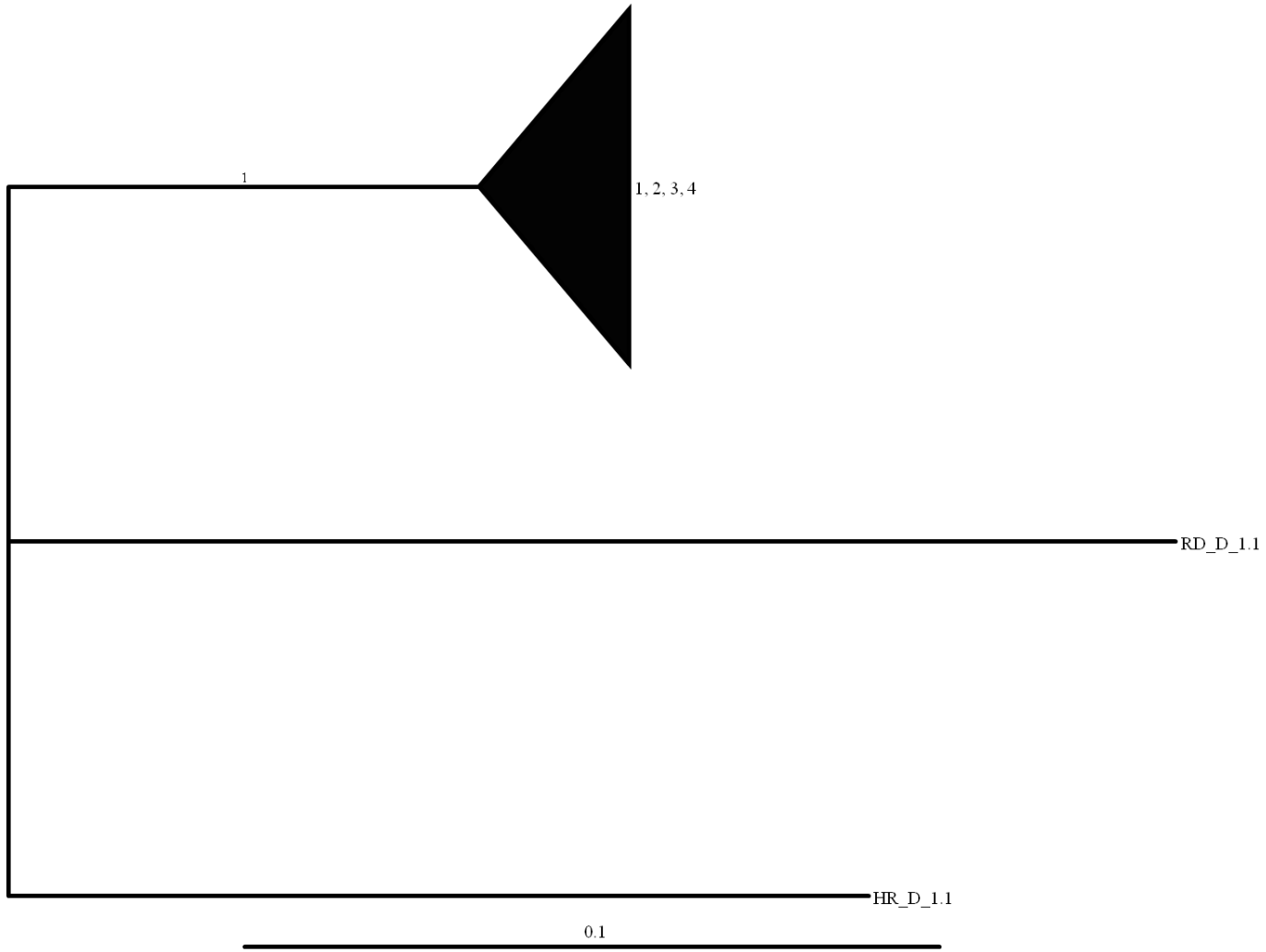

Figure A37: Bayesian tree after $10x10^6$ generations from 160 *COI* protein sequences of *Platynothrus peltifer*. Split frequencies of 0.028819 and burnin of 25%. Outgroups are one individual of *Hypochthonius rufulus* and *Rhysotritia duplicata*, respectively. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 20.

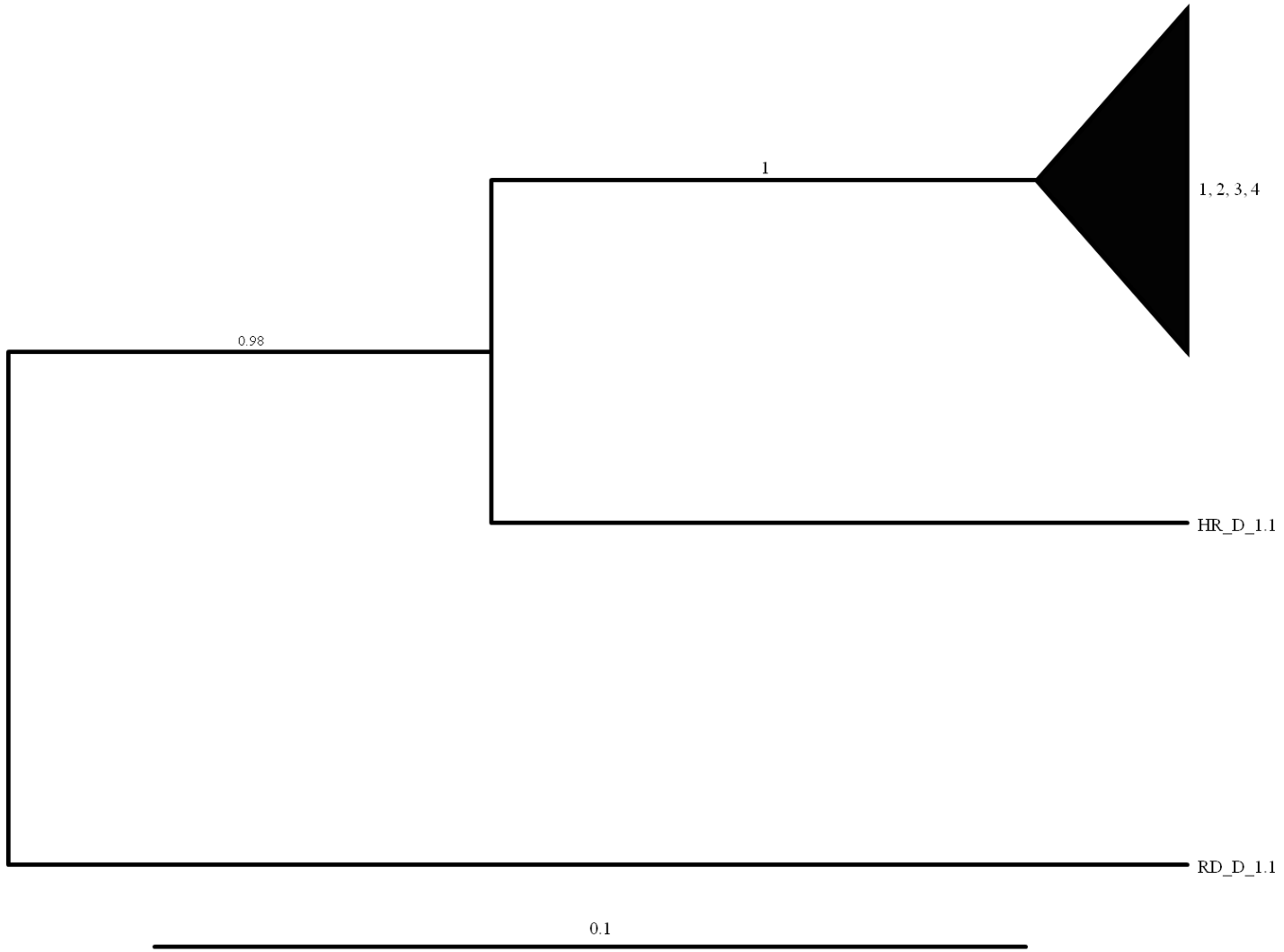

Figure A38: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 from the 160 *COI* protein sequences of *Platynothrus peltifer*. Outgroups are one individual of *Hypochthonius rufulus* and *Rhysotritia duplicata,* respectively. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 20.

Table A26: Mean pairwise percentage differences of uncorrected p-distances of the protein of *Platynothrus peltifer* from 39 locations. The diagonal is the within population differences (bold) and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Locations with less than two individuals were excluded from the analysis.

Population	A 1	A 2	A 3	A 4	A 5	A 9	A 10	CH 3	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 11	D 12	D 15	D 17	D 18	DK 2	DK 3
A 1	0																							
A 2	0	0																						
A 3	0	0	0																					
A 4	0	0	0	0																				
A 5	0	0	0	0	0																			
A 9	0	0	0	0	0	0																		
A 10	0	0	0	0	0	0	0																	
CH 3	0	0	0	0	0	0	0	0																
D 1	0	0	0	0	0	0	0	0	0															
D 2	0	0	0	0	0	0	0	0	0	0														
D 3	0	0	0	0	0	0	0	0	0	0	0													
D 4	0	0	0	0	0	0	0	0	0	0	0	0												
D 5	0	0	0	0	0	0	0	0	0	0	0	0	0											
D 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0										
D 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
D 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
D 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							
D 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
D 12	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	**0.2**					
D 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0				
D 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0			
D 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0		
DK 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	
DK 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
F 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
F 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
F 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
FIN 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
GB 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
GB 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
N 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
NL 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
NL 2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
PL 2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
RUM 2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
S 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
S 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
S 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
S 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0

Table A26 continue:

Population	F 1	F 3	F 5	FIN 1	GB 1	GB 2	N 1	NL 1	NL 2	PL 2	RUM 2	S 1	S 2	S 4	S 5
F 1	**0**														
F 3	**0**	**0**													
F 5	**0**	**0**	**0**												
FIN 1	**0**	**0**	**0**	**0**											
GB 1	**0**	**0**	**0**	**0**	**0**										
GB 2	**0**	**0**	**0**	**0**	**0**	**0**									
N 1	**0**	**0**	**0**	**0**	**0**	**0**	**0**								
NL 1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							
NL 2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	**0.3**						
PL 2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	**0.1**					
RUM 2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	**0.4**	0.3	**0.5**				
S 1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	0.1	0.1	0.3	**0**			
S 2	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	0.1	0.1	0.3	**0**	**0**		
S 4	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	0.1	0.1	0.3	**0**	**0**	**0**	
S 5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	0.1	0.1	0.3	**0**	**0**	**0**	**0**

Table A27: Tajima's D and Fu's FS neutrality tests of *Platynothrus peltifer COI* nucleotide sequences from 39 locations. Bold red letters show significance (P<0.05).

Neutrality tests	A 1	A 2	A 3	A 4	A 5	A 9	A 10	CH 3	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 11	D 12	D 15	D 17	D 18	DK 2
Tajima's D test																							
Samplesize	2	2	3	2	4	5	5	5	4	2	4	8	4	5	3	4	3	2	5	3	3	7	6
S	1	0	3	0	143	3	0	2	101	0	7	102	10	1	0	113	101	103	5	112	110	30	28
Pi	1	0	2	0	80.5	1.4	0	0.8	51.83	0	3.83	43.36	5.17	0.4	0	73.83	67.33	103	2.4	74.67	73.33	13.62	16.73
Tajima's D	0	0	0	0	0.34	-0.17	0	-0.97	-0.62	0	0.04	0.56	-0.53	-0.82	0	2.07	0	0	0	0	0	0.64	2.3
Tajima's D p-value	1	1	0.87	1	0.73	0.48	1	0.19	0.34	1	0.67	0.77	0.44	0.3	1	0.98	**0.04**	1	0.58	0.92	0.08	0.77	1
Fu's FS test																							
Real no. of alleles	2	1	2	1	4	4	1	3	4	1	4	4	4	2	1	4	2	2	3	3	3	4	3
Orig. no. of alleles	2	1	2	1	4	4	1	3	4	1	4	4	4	2	1	4	2	2	3	3	3	4	3
Theta_pi	1	0	2	0	80.5	1.4	0	0.8	51.83	0	3.83	43.36	5.17	0.4	0	73.83	67.33	103	2.4	74.67	73.33	13.62	16.73
Exp. no. of alleles	1.5	0	2.17	0	3.93	2.57	0	2.11	3.89	0	3.01	7.42	3.19	1.66	0	3.92	2.96	1.99	3.07	2.96	2.96	5.82	5.26
FS	0	0	1.61	0	2.57	-1.65	0	-0.83	2.12	0	-0.88	9.95	-0.48	0.09	0	2.49	7.77	4.63	0.95	3.21	3.19	4.3	6.44
FS p-value	0.26	N.A.	0.7	N.A.	0.55	0.05	N.A.	0.09	0.53	N.A.	0.14	1	0.21	0.3	N.A.	0.54	0.99	0.63	0.67	0.57	0.58	0.96	0.99

Table A27 continue:

Neutrality tests	DK 3	F 1	F 3	F 5	FIN 1	GB 1	GB 2	N 1	NL 1	NL 2	PL 2	RUM 2	S 1	S 2	S 4	S 5	mean	s.d.
Tajima's D test																		
Samplesize	6	4	2	3	4	3	4	2	3	4	9	2	3	9	4	4	4.03	1.84
S	0	145	0	3	1	111	27	3	84	3	46	99	2	89	19	4	41.31	50.26
Pi	0	87.83	0	2	0.5	74	13.5	3	56	1.67	15	99	1.33	35.33	9.5	2.17	26.05	34.24
Tajima's D	0	1.16	0	0	-0.61	0	-0.86	0	0	0.17	-0.58	0	0	0.41	-0.85	-0.07	0.04	0.66
Tajima's D p-value	1	0.85	1	0.87	0.38	0.67	0.05	1	0.66	0.73	0.29	1	0.93	0.71	0.07	0.58	0.69	0.32
Fu's FS test																		
Real no. of alleles	1	3	1	3	2	2	2	2	2	3	8	2	3	8	3	3	2.82	1.59
Orig. no. of alleles	1	3	1	3	2	2	2	2	2	3	8	2	3	8	3	3	2.82	1.59
Theta_pi	0	87.83	0	2	0.5	74	13.5	3	56	1.67	15	99	1.33	35.33	9.5	2.17	26.05	34.24
Exp. no. of alleles	0	3.93	0	2.17	1.68	2.96	3.62	1.75	2.95	2.44	7.24	1.99	1.97	8.12	3.49	2.62	2.75	2.02
FS	0	6.61	0	-0.69	0.17	7.95	6.42	1.1	7.41	-0.13	-0.24	4.6	-1.22	1.29	2.54	0.25	2.09	3.04
FS p-value	N.A.	0.98	N.A.	0.13	0.35	0.99	0.99	0.43	0.99	0.26	0.37	0.62	0.07	0.66	0.83	0.46	N.A.	N.A.

Table A28: Tajima's D and Fu's FS neutrality tests of *Platynothrus peltifer COI* protein sequences from 39 locations. Bold red letters show significance (P<0.05).

Neutrality tests	A 1	A 2	A 3	A 4	A 5	A 9	A 10	CH 3	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 11	D 12	D 15	D 17	D 18	DK 2
Tajima's D test																							
Samplesize	2	2	3	2	4	5	5	5	4	2	4	8	4	5	3	4	3	2	5	3	3	7	6
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Pi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0	0	0	0
Tajima's D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-0.82	0	0	0	0
Tajima's D p-value	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.31	1	1	1	1
Fu's FS test																							
Real no. of alleles	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	2	1	2	2	1	1	1	2
Orig. no. of alleles	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	2	1	2	2	1	1	1	2
Theta_pi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0	0	0	0
Exp. no. of alleles	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	2	0	0	1.66	0	0	0	2
FS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.09	0	0	0	0
FS p-value	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.29	N.A.	N.A.	N.A.	N.A.

Table A28 continue:

Neutrality tests	DK 3	F 1	F 3	F 5	FIN 1	GB 1	GB 2	N 1	NL 1	NL 2	PL 2	RUM 2	S 1	S 2	S 4	S 5	mean	s.d.
Tajima's D test																		
Samplesize	6	4	2	3	4	3	4	2	3	4	9	2	3	9	4	4	4.03	1.84
S	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0.10	0.31
Pi	0	0	0	0	0	0	0	0	0	0.5	0.22	1	0	0	0	0	0.05	0.19
Tajima's D	0	0	0	0	0	0	0	0	0	-0.61	-1.09	0	0	0	0	0	-0.06	0.23
Tajima's D p-value	1	1	1	1	1	1	1	1	1	0.38	0.20	1	1	1	1	1	0.95	0.19
Fu's FS test																		
Real no. of alleles	1	1	1	1	1	1	1	2	1	2	3	2	1	5	2	1	1.41	0.79
Orig. no. of alleles	1	1	1	1	1	1	1	2	1	2	3	2	1	5	2	1	1.41	0.79
Theta_pi	0	0	0	0	0	0	0	0	0	0.5	0.22	1	0	0	0	0	0.05	0.19
Exp. no. of alleles	0	0	0	0	0	0	0	0	0	1.68	1.54	1.5	0	5	2	0	0.55	1.07
FS	0	0	0	0	0	0	0	0	0	0.17	-0.26	0	0	0	0	0	0.00	0.05
FS p-value	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.35	0.17	0.24	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

Table A29: Results of the McDonald-Kreitman test of *Platynothrus peltifer*. The differences between 64 populations are significant (*0.01<P<0.05), between other 17 populations high significant (**0.001<P<0.01) and between 3 populations extremely high significant (***P<0.001). Locations with less than two individuals were excluded.

Population		A 1			A 2			A 3			A 4		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
A 2	fixed	99	1	1 ns									
Bregenz	poly	1	0										
A 3	fixed	98	1	1 ns	0	0	- ns						
Hittisau	poly	4	0		3	0							
A 4	fixed	99	1	1 ns	2	0	- ns	0	0	- ns			
Holzgau	poly	1	0		0	0		3	0				
A 5	fixed	8	1	0.1084 ns	0	0	- ns	0	0	- ns	0	0	- ns
Memm. Huette	poly	152	1		152	1		153	1		152	1	
A 9	fixed	98	1	1 ns	1	0	- ns	0	0	- ns	1	0	- ns
Glacier	poly	4	0		3	0		6	0		3	0	
A 10	fixed	101	1	1 ns	2	0	- ns	1	0	- ns	2	0	- ns
Stuiben	poly	1	0		0	0		3	0		0	0	
CH 3	fixed	98	1	1 ns	1	0	- ns	0	0	- ns	0	0	- ns
Rohrschach	poly	3	0		2	0		4	0		2	0	
D 1	fixed	4	1	0.0472*	2	0	- ns	2	0	- ns	2	0	- ns
Kranichstein	poly	101	0		101	0		102	0		101	0	
D 2	fixed	7	1	1 ns	99	1	- ns	98	0	- ns	99	0	- ns
Goettingen	poly	1	0		0	0		3	0		0	0	
D 3	fixed	97	1	0.0145*	0	0	- ns	0	0	- ns	1	0	1 ns
Lake Constance	poly	6	2		5	2		7	2		5	2	
D 4	fixed	5	1	0.0561 ns	0	0	- ns	0	0	- ns	0	0	- ns
Meckl. Seenpl.	poly	101	0		101	0		102	0		101	0	
D 5	fixed	4	1	0.3125 ns	96	0	- ns	95	0	- ns	96	0	- ns
Moerfelden	poly	11	0		10	0		13	0		10	0	
D 6	fixed	9	1	0.3182 ns	97	0	0.0102*	96	0	0.04*	97	0	0.0102*
Black Forest	poly	1	1		0	1		3	1		0	1	
D 7	fixed	28	1	1 ns	109	0	- ns	108	0	- ns	109	0	- ns
Uelzen	poly	1	0		0	0		3	0		0	0	
D 8	fixed	12	1	0.2827 ns	0	0	- ns	0	0	- ns	0	0	- ns
Cuxhaven	poly	110	2		110	2		111	2		110	2	
D 9	fixed	5	1	0.0566 ns	0	0	- ns	0	0	- ns	0	0	- ns
Bonn	poly	100	0		100	0		101	0		100	0	
D 11	fixed	4	1	0.0917 ns	1	0	1 ns	1	0	1 ns	1	0	1 ns
Wittmoor	poly	101	1		101	1		102	1		101	1	
D 12	fixed	98	1	0.1115 ns	1	0	1 ns	0	0	- ns	0	0	- ns
Sonthofen	poly	5	1		4	1		5	1		4	1	
D 15	fixed	12	1	0.1048 ns	0	0	- ns	0	0	- ns	0	0	- ns
Langenwang	poly	111	0		111	0		112	0		111	0	
D 17	fixed	13	1	0.1138 ns	0	0	- ns	0	0	- ns	0	0	- ns
Rubi	poly	109	0		109	0		110	0		109	0	
D 18	fixed	6	1	0.1944 ns	92	1	- ns	91	0	- ns	92	0	- ns
Steinh. Meer	poly	29	0		28	0		31	0		28	0	
DK 2	fixed	5	1	0.3352 ns	94	0	0.2167 ns	93	0	0.2377 ns	94	0	0.2167 ns
Hjorring	poly	26	1		25	1		28	1		25	1	
DK 3	fixed	100	1	1 ns	3	0	- ns	2	0	- ns	3	0	- ns
Arhus	poly	1	0		0	0		3	0		0	0	
F 1	fixed	10	1	0.0651 ns	0	0	- ns	0	0	- ns	0	0	- ns
Mont Blanc	poly	158	0		158	0		158	0		158	0	
F 3	fixed	28	1	1 ns	109	1	- ns	108	1	1 ns	109	1	- ns
Saint Isidore	poly	1	0		0	0		3	0		0	0	
F 5	fixed	7	1	1 ns	98	0	- ns	97	0	- ns	98	0	- ns
Bruenstatt	poly	4	0		3	0		6	0		3	0	
FIN 1	fixed	29	1	1 ns	108	0	- ns	107	0	- ns	108	0	- ns
Lahti	poly	2	0		1	0		4	0		1	0	
GB 1	fixed	12	1	0.1057 ns	0	0	- ns	0	0	- ns	0	0	- ns
Ascot	poly	110	0		110	0		111	0		110	0	

Table A29 continue:

Population		A 1			A 2			A 3			A 4		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
GB 2	fixed	6	1	0.2121 ns	95	1	- ns	94	0	- ns	95	0	- ns
Braemar	poly	26	0		25	0		28	0		25	0	
N 1	fixed	71	1	0.0789 ns	102	0	0.0192*	101	0	0.0472*	102	0	0.0192*
Narvik	poly	2	1		1	1		4	1		1	1	
NL 1	fixed	71	1	0.4645 ns	2	0	- ns	1	0	- ns	1	0	- ns
Wageningen	poly	83	0		82	0		83	0		82	0	
NL 2	fixed	28	1	0.2311 ns	108	0	0.027*	107	0	0.0531 ns	108	0	0.027*
Hoge Veluwe	poly	3	1		2	1		5	1		2	1	
PL 2	fixed	2	1	0.2865 ns	88	0	0.0112*	87	0	0.0139*	88	0	0.0112*
Warsaw	poly	41	4		40	4		43	4		40	4	
RUM 2	fixed	5	1	0.1126 ns	1	0	1 ns	1	0	1 ns	1	0	1 ns
Sibiu 2	poly	97	1		97	1		98	1		97	1	
S 1	fixed	71	1	1 ns	102	0	- ns	101	0	- ns	102	0	- ns
Umea	poly	3	0		2	0		5	0		2	0	
S 2	fixed	10	1	1 ns	67	0	0.0709 ns	66	0	0.0755 ns	67	0	0.0709 ns
Stroemstad	poly	85	5		84	5		87	5		84	5	
S 4	fixed	64	1	0.1366 ns	97	0	0.0256*	96	0	0.0335*	97	0	0.0256*
Abisko	poly	18	2		17	2		20	2		17	2	
S 5	fixed	96	1	1 ns	1	0	- ns	0	0	- ns	1	0	- ns
Gothenburg	poly	5	0		4	0		7	0		4	0	

Population		A 5			A 9			A 10			CH 3		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
A 9	fixed	0	0	- ns									
Glacier	poly	154	1										
A 10	fixed	1	0	1 ns	1	0	- ns						
Stuiben	poly	152	1		3	0							
CH 3	fixed	0	0	- ns	0	0	- ns	1	0	- ns			
Rohrschach	poly	152	1		5	0		2	0				
D 1	fixed	1	0	1 ns	2	0	- ns	3	0	- ns	2	0	- ns
Kranichstein	poly	159	1		102	0		101	0		101	0	
D 2	fixed	7	0	1 ns	98	0	- ns	101	1	- ns	98	0	- ns
Goettingen	poly	152	1		3	0		0	0		2	0	
D 3	fixed	0	0	- ns	0	0	- ns	1	0	1 ns	0	0	- ns
Lake Constance	poly	154	3		8	2		5	2		6	2	
D 4	fixed	0	0	- ns	0	0	- ns	1	0	- ns	0	0	- ns
Meckl. Seenpl.	poly	159	1		102	0		101	0		101	0	
D 5	fixed	7	0	1 ns	95	0	- ns	98	0	- ns	95	0	- ns
Moerfelden	poly	155	1		13	0		10	0		12	0	
D 6	fixed	7	0	1 ns	96	0	0.04*	99	0	0.01*	96	0	0.0303*
Black Forest	poly	152	2		3	1		0	1		2	1	
D 7	fixed	0	0	- ns	109	0	- ns	111	0	- ns	108	0	- ns
Uelzen	poly	152	1		3	0		0	0		2	0	
D 8	fixed	0	0	- ns	0	0	- ns	1	0	1 ns	0	0	- ns
Cuxhaven	poly	152	3		112	2		110	2		110	2	
D 9	fixed	0	0	- ns	0	0	- ns	1	0	- ns	0	0	- ns
Bonn	poly	159	1		101	0		100	0		100	0	
D 11	fixed	0	0	- ns	1	0	1 ns	2	0	1 ns	1	0	1 ns
Wittmoor	poly	160	2		102	1		101	1		101	1	
D 12	fixed	0	0	- ns	0	0	- ns	1	0	1 ns	0	0	- ns
Sonthofen	poly	154	2		7	1		4	1		5	1	
D 15	fixed	0	0	- ns	0	0	- ns	1	0	- ns	0	0	- ns
Langenwang	poly	153	1		113	0		111	0		111	0	
D 17	fixed	0	0	- ns	0	0	- ns	1	0	- ns	0	0	- ns
Rubi	poly	152	1		111	0		109	0		109	0	

Table A29 continue:

Population		A 5			A 9			A 10			CH 3		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 18	fixed	0	0	- ns	91	0	- ns	94	0	- ns	91	0	- ns
Steinh. Meer	poly	160	1		31	0		28	0		30	0	
DK 2	fixed	0	0	- ns	93	0	0.2377 ns	96	0	0.2131 ns	93	0	0.2314 ns
Hjorring	poly	159	2		28	1		25	1		27	1	
DK 3	fixed	1	0	1 ns	2	0	- ns	3	0	- ns	2	0	- ns
Arhus	poly	152	1		3	0		0	0		2	0	
F 1	fixed	0	0	- ns	0	0	- ns	0	0	- ns	0	0	- ns
Mont Blanc	poly	185	1		160	0		158	0		158	0	
F 3	fixed	0	1	0.013*	109	1	1 ns	111	1	- ns	108	1	1 ns
Saint Isidore	poly	152	1		3	0		0	0		2	0	
F 5	fixed	6	0	1 ns	97	0	- ns	100	0	- ns	97	0	- ns
Bruenstatt	poly	154	1		6	0		3	0		5	0	
FIN 1	fixed	0	0	- ns	108	0	- ns	110	0	- ns	107	0	- ns
Lahti	poly	152	1		4	0		1	0		3	0	
GB 1	fixed	0	0	- ns	0	0	- ns	1	0	- ns	0	0	- ns
Ascot	poly	152	1		112	0		110	0		110	0	
GB 2	fixed	0	0	- ns	94	0	- ns	97	0	- ns	94	0	- ns
Braemar	poly	159	1		28	0		25	0		27	0	
N 1	fixed	0	0	- ns	103	0	0.0463*	103	0	0.0191*	101	0	0.0381*
Narvik	poly	152	2		4	1		1	1		3	1	
NL 1	fixed	1	0	1 ns	1	0	- ns	1	0	- ns	1	0	- ns
Wageningen	poly	184	1		84	0		82	0		82	0	
NL 2	fixed	0	0	- ns	108	0	0.0526*	110	0	0.0266*	107	0	0.0446*
Hoge Veluwe	poly	152	2		5	1		2	1		4	1	
PL 2	fixed	0	0	- ns	87	0	0.0139*	90	0	0.0106*	87	0	0.0131*
Warsaw	poly	165	5		43	4		40	4		42	4	
RUM 2	fixed	0	0	- ns	1	0	1 ns	2	0	1 ns	0	0	- ns
Sibiu 2	poly	160	2		98	1		97	1		98	1	
S 1	fixed	0	0	- ns	103	0	- ns	103	0	- ns	101	0	- ns
Umea	poly	152	1		5	0		2	0		4	0	
S 2	fixed	0	0	- ns	68	0	0.0716 ns	69	0	0.0685 ns	66	0	0.0744 ns
Stroemstad	poly	154	6		86	5		84	5		86	5	
S 4	fixed	0	0	- ns	98	0	0.0324*	98	0	0.0252*	96	0	0.031*
Abisko	poly	155	2		20	2		17	2		19	2	
S 5	fixed	0	0	- ns	0	0	- ns	1	0	- ns	0	0	- ns
Gothenburg	poly	152	2		6	0		4	0		5	0	

Population		D 1			D 2			D 3			D 4		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 2	fixed	97	0	0.0039**									
Goettingen	poly	5	2										
D 3	fixed	1	0	- ns	1	0	- ns						
Lake Constance	poly	101	0		103	2							
D 4	fixed	0	0	- ns	1	0	- ns	1	0	- ns			
Meckl. Seenpl.	poly	104	0		101	0		103	2				
D 5	fixed	0	0	- ns	0	0	- ns	94	0	0.0223*	0	0	- ns
Moerfelden	poly	108	0		10	0		15	2		108	0	
D 6	fixed	0	0	- ns	2	0	0.3333 ns	95	0	0.0003***	1	0	1 ns
Black Forest	poly	101	1		0	1		5	3		101	1	
D 7	fixed	20	0	- ns	23	0	- ns	107	0	0.0033**	19	0	- ns
Uelzen	poly	101	0		0	0		5	2		101	0	
D 8	fixed	1	0	1 ns	9	0	1 ns	0	0	- ns	0	0	- ns
Cuxhaven	poly	121	2		110	2		112	4		120	2	
D 9	fixed	0	0	- ns	1	0	- ns	0	0	- ns	0	0	- ns
Bonn	poly	103	0		100	0		102	2		101	0	

Table A29 continue:

Population		D 1			D 2			D 3			D 4		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 11	fixed	0	0	- ns	0	0	- ns	1	0	1 ns	0	0	- ns
Wittmoor	poly	105	1		101	1		103	3		104	1	
D 12	fixed	1	0	1 ns	98	0	0.0485*	0	0	- ns	0	0	- ns
Sonthofen	poly	103	1		4	1		9	3		103	1	
D 15	fixed	0	0	- ns	9	0	- ns	0	0	- ns	0	0	- ns
Langenwang	poly	122	0		111	0		113	2		121	0	
D 17	fixed	1	0	- ns	10	0	- ns	0	0	- ns	0	0	- ns
Rubi	poly	121	0		109	0		111	2		120	0	
D 18	fixed	0	0	- ns	0	0	- ns	90	0	0.0768ns	0	0	- ns
Steinh. Meer	poly	122	0		28	0		33	2		121	0	
DK 2	fixed	0	0	- ns	0	0	- ns	92	0	0.0172*	0	0	- ns
Hjørring	poly	121	1		25	1		30	3		120	1	
DK 3	fixed	1	0	- ns	100	0	- ns	2	0	1 ns	1	0	- ns
Arhus	poly	101	0		0	0		5	2		101	0	
F 1	fixed	0	0	- ns	9	0	- ns	0	0	- ns	0	0	- ns
Mont Blanc	poly	168	0		158	0		159	2		167	0	
F 3	fixed	20	1	0.1721 ns	23	1	- ns	107	1	0.0093**	19	1	0.1653 ns
Saint Isidore	poly	101	0		0	0		5	2		101	0	
F 5	fixed	0	0	- ns	0	0	- ns	96	0	0.0081**	0	0	- ns
Bruenstatt	poly	103	1		3	0		8	2		103	0	
FIN 1	fixed	20	1	- ns	24	0	- ns	106	0	0.0044**	19	0	- ns
Lahti	poly	102	0		1	0		6	2		102	0	
GB 1	fixed	1	0	- ns	9	0	- ns	0	0	- ns	0	0	- ns
Ascot	poly	121	0		110	0		112	2		120	0	
GB 2	fixed	0	0	- ns	0	0	- ns	93	0	0.064 ns	1	0	- ns
Braemar	poly	123	0		25	0		30	2		121	0	
N 1	fixed	44	0	1 ns	74	0	0.0263*	100	0	0.0004***	44	0	1 ns
Narvik	poly	101	1		1	1		6	3		101	1	
NL 1	fixed	3	0	- ns	71	0	- ns	1	0	1 ns	1	0	- ns
Wageningen	poly	152	0		82	0		85	2		153	0	
NL 2	fixed	20	1	- ns	23	0	0.1154 ns	106	0	0.0005***	19	0	1 ns
Hoge Veluwe	poly	102	0		2	1		7	3		102	1	
PL 2	fixed	0	0	- ns	0	0	- ns	86	0	0.0022**	1	0	1 ns
Warsaw	poly	131	4		40	4		45	6		129	4	
RUM 2	fixed	0	0	- ns	4	0	1 ns	0	0	- ns	0	0	- ns
Sibiu 2	poly	105	1		97	1		100	3		104	1	
S 1	fixed	44	0	- ns	74	0	- ns	100	0	0.0061**	44	0	- ns
Umea	poly	102	0		2	0		7	2		102	0	
S 2	fixed	6	0	1 ns	9	0	1 ns	65	0	0.0422*	5	0	1 ns
Stroemstad	poly	153	5		84	5		89	7		153	5	
S 4	fixed	38	0	1 ns	66	0	0.0479*	95	0	0.0018**	38	0	1 ns
Abisko	poly	114	2		17	2		22	4		113	2	
S 5	fixed	2	0	- ns	96	0	- ns	0	0	- ns	0	0	- ns
Gothenburg	poly	101	0		4	0		8	2		101	0	

Population		D 5			D 6			D 7			D 8		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 6	fixed	2	0	1 ns									
Black Forest	poly	10	1										
D 7	fixed	23	0	- ns	25	0	0.0385*						
Uelzen	poly	10	0		0	1							
D 8	fixed	9	0	1 ns	9	0	1 ns	0	0	- ns			
Cuxhaven	poly	117	2		110	3		110	2				
D 9	fixed	0	0	- ns	1	0	1 ns	20	0	- ns	0	0	- ns
Bonn	poly	107	0		100	1		100	0		120	2	

Table A29 continue:

Population		D 5			D 6			D 7			D 8		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 11	fixed	0	0	- ns	0	0	- ns	19	0	1 ns	0	0	- ns
Wittmoor	poly	108	1		101	2		101	1		121	3	
D 12	fixed	95	0	0.1364 ns	96	0	0.0029**	109	0	0.0439*	0	0	- ns
Sonthofen	poly	14	1		4	2		4	1		113	3	
D 15	fixed	9	0	- ns	9	0	1 ns	1	0	- ns	0	0	- ns
Langenwang	poly	118	0		111	1		111	0		112	2	
D 17	fixed	10	0	- ns	10	0	1 ns	1	0	- ns	0	0	- ns
Rubi	poly	116	0		109	1		109	0		110	2	
D 18	fixed	0	0	- ns	1	0	1 ns	0	0	- ns	0	0	- ns
Steinh. Meer	poly	37	0		28	1		28	0		121	2	
DK 2	fixed	0	0	- ns	2	0	1 ns	0	0	- ns	0	0	- ns
Hjørring	poly	33	1		25	2		25	1		120	3	
DK 3	fixed	97	0	- ns	98	0	0.0101*	112	0	- ns	2	0	1 ns
Arhus	poly	10	0		0	1		0	0		110	2	
F 1	fixed	9	0	- ns	9	0	1 ns	2	0	- ns	0	0	- ns
Mont Blanc	poly	162	0		158	1		158	0		160	2	
F 3	fixed	23	1	1 ns	25	1	0.0741 ns	0	1	- ns	0	1	0.0266*
Saint Isidore	poly	10	0		0	1		0	0		110	2	
F 5	fixed	0	0	- ns	2	0	1 ns	22	0	- ns	8	0	1 ns
Bruenstatt	poly	12	0		3	1		3	0		113	2	
FIN 1	fixed	24	0	- ns	26	1	0.0714 ns	2	0	- ns	1	0	1 ns
Lahti	poly	11	0		1	1		1	0		110	2	
GB 1	fixed	9	0	- ns	9	0	1 ns	0	0	- ns	0	0	- ns
Ascot	poly	117	0		110	1		110	0		110	2	
GB 2	fixed	0	0	- ns	2	0	1 ns	0	0	- ns	0	0	- ns
Braemar	poly	35	0		25	1		25	0		121	2	
N 1	fixed	69	0	0.1482 ns	72	0	0.0011**	76	0	0.0256*	40	0	0.5675 ns
Narvik	poly	11	1		1	2		1	1		110	3	
NL 1	fixed	67	0	- ns	69	0	1 ns	77	0	- ns	1	0	1 ns
Wageningen	poly	90	0		82	1		82	0		159	2	
NL 2	fixed	23	0	0.3611 ns	25	0	0.0148*	0	0	- ns	0	0	- ns
Hoge Veluwe	poly	12	1		2	2		2	1		111	3	
PL 2	fixed	0	0	- ns	2	0	1 ns	0	0	- ns	0	0	- ns
Warsaw	poly	46	4		40	5		40	4		129	6	
RUM 2	fixed	2	0	1 ns	4	0	1 ns	23	0	1 ns	0	0	- ns
Sibiu 2	poly	105	1		97	2		97	1		121	3	
S 1	fixed	69	0	- ns	72	0	0.04*	75	0	- ns	40	0	1 ns
Umea	poly	12	0		2	1		2	0		110	2	
S 2	fixed	9	0	1 ns	9	0	1 ns	0	0	- ns	0	0	- ns
Stroemstad	poly	88	5		84	6		84	5		152	6	
S 4	fixed	61	0	0.1014 ns	64	0	0.012*	67	0	0.0468*	35	0	0.5766 ns
Abisko	poly	27	2		17	3		17	2		120	4	
S 5	fixed	93	0	- ns	95	0	0.05 ns	106	0	- ns	0	0	- ns
Gothenburg	poly	14	0		4	1		4	0		110	2	

Population		D 9			D 11			D 12			D15		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 11	fixed	0	0	- ns									
Wittmoor	poly	103	1										
D 12	fixed	0	0	- ns	1	0	1 ns						
Sonthofen	poly	102	1		103	2							
D 15	fixed	0	0	- ns	0	0	- ns	0	0	- ns			
Langenwang	poly	121	0		122	1		112	1				
D 17	fixed	0	0	- ns	0	0	- ns	0	0	- ns	0	0	- ns
Rubi	poly	120	0		121	1		112	1		111	0	

Table A29 continue:

Population		D 9			D 11			D 12			D15		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 18	fixed	0	0	- ns	0	0	- ns	92	0	0.2581 ns	1	0	- ns
Steinh. Meer	poly	121	0		122	1		31	1		121	0	
DK 2	fixed	0	0	- ns	0	0	- ns	94	0	0.057 ns	1	0	1 ns
Hjorring	poly	120	1		121	2		28	2		120	1	
DK 3	fixed	1	0	- ns	2	0	1 ns	0	0	- ns	0	0	- ns
Arhus	poly	100	0		101	1		4	1		111	0	
F 1	fixed	0	0	- ns	0	0	- ns	0	0	- ns	0	0	- ns
Mont Blanc	poly	167	0		167	1		158	1		160	0	
F 3	fixed	20	1	0.1736 ns	19	1	0.3021 ns	109	0	0.0854 ns	1	1	0.0177*
Saint Isidore	poly	100	0		101	1		4	1		111	0	
F 5	fixed	0	0	- ns	0	0	- ns	98	0	0.0667 ns	9	0	- ns
Bruenstatt	poly	102	1		103	1		6	1		113	0	
FIN 1	fixed	20	0	- ns	19	0	1 ns	108	0	0.0526 ns	1	0	- ns
Lahti	poly	101	0		102	1		5	1		111	0	
GB 1	fixed	0	0	- ns	0	0	- ns	0	0	- ns	0	0	- ns
Ascot	poly	120	0		121	1		113	1		112	0	
GB 2	fixed	1	0	- ns	0	0	- ns	95	0	0.2339 ns	1	0	- ns
Braemar	poly	121	0		122	1		28	1		121	0	
N 1	fixed	44	0	1 ns	44	0	0.5778 ns	101	0	0.0036**	40	0	1 ns
Narvik	poly	100	1		101	2		5	2		111	1	
NL 1	fixed	1	0	- ns	0	0	- ns	1	0	1 ns	1	0	- ns
Wageningen	poly	152	0		153	1		83	1		159	0	
NL 2	fixed	20	0	1 ns	19	0	1 ns	108	0	0.0042**	1	0	1 ns
Hoge Veluwe	poly	101	1		102	2		6	2		112	1	
PL 2	fixed	1	0	1 ns	0	0	- ns	88	0	0.0048**	0	0	- ns
Warsaw	poly	129	4		130	5		43	5		130	4	
RUM 2	fixed	0	0	- ns	0	0	- ns	1	0	1 ns	0	0	- ns
Sibiu 2	poly	103	1		104	2		99	2		122	1	
S 1	fixed	44	0	- ns	44	0	1 ns	101	0	0.0648 ns	40	0	- ns
Umea	poly	101	0		102	1		6	1		111	0	
S 2	fixed	5	0	1 ns	5	0	1 ns	67	0	0.0407*	1	0	1 ns
Stroemstad	poly	153	5		153	6		87	6		152	5	
S 4	fixed	38	0	1 ns	38	0	0.5764 ns	96	0	0.0072**	35	0	1 ns
Abisko	poly	113	2		114	3		21	3		121	2	
S 5	fixed	0	0	- ns	1	0	1 ns	0	0	- ns	0	0	- ns
Gothenburg	poly	100	0		101	1		8	1		111	0	

Population		D 17			D 18			DK 2			DK 3		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 18	fixed	1	0	- ns									
Steinh. Meer	poly	120	0										
DK 2	fixed	1	0	1 ns	0	0	- ns						
Hjorring	poly	119	1		29	1							
DK 3	fixed	2	0	- ns	94	0	- ns	96	0	0.2131 ns			
Arhus	poly	109	0		28	0		25	1				
F 1	fixed	0	0	- ns	2	0	- ns	2	0	1 ns	0	0	- ns
Mont Blanc	poly	160	0		166	0		165	1		158	0	
F 3	fixed	1	1	0.018*	0	1	0.0345*	1	0	0.0741 ns	112	1	- ns
Saint Isidore	poly	109	0		28	0		25	1		0	0	
F 5	fixed	9	0	- ns	0	0	- ns	0	0	- ns	100	0	- ns
Bruenstatt	poly	112	0		29	0		26	1		3	0	
FIN 1	fixed	1	0	- ns	2	0	- ns	2	0	1 ns	1	0	1 ns
Lahti	poly	109	0		28	0		25	1		152	1	
GB 1	fixed	0	0	- ns	0	0	- ns	0	0	- ns	2	0	- ns
Ascot	poly	110	0		121	0		120	1		110	0	

Table A29 continue:

Population		D 17			D 18			DK 2			DK 3		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
GB 2	fixed	1	0	- ns	0	0	- ns	0	0	- ns	97	0	- ns
Braemar	poly	120	0		30	0		27	1		25	0	
N 1	fixed	41	0	1 ns	62	0	0.3261 ns	62	0	0.0944 ns	103	0	0.0191*
Narvik	poly	109	1		29	1		26	2		1	1	
NL 1	fixed	1	0	- ns	64	0	- ns	66	0	1 ns	2	0	- ns
Wageningen	poly	158	0		104	0		101	1		82	0	
NL 2	fixed	1	0	1 ns	0	0	- ns	0	0	- ns	111	0	0.0263*
Hoge Veluwe	poly	110	1		29	1		27	2		2	1	
PL 2	fixed	0	0	- ns	0	0	- ns	0	0	- ns	90	0	0.0106*
Warsaw	poly	129	4		45	4		41	5		40	4	
RUM 2	fixed	0	0	- ns	4	0	1 ns	3	0	1 ns	2	0	1 ns
Sibiu 2	poly	121	1		118	1		118	2		97	1	
S 1	fixed	41	0	- ns	62	0	- ns	62	0	0.3034 ns	103	0	- ns
Umea	poly	109	0		29	0		26	1		2	0	
S 2	fixed	1	0	1 ns	0	0	- ns	0	0	- ns	69	0	0.0685 ns
Stroemstad	poly	151	5		96	5		93	6		84	5	
S 4	fixed	36	0	1 ns	55	0	0.1951 ns	55	0	0.0779 ns	98	0	0.0252*
Abisko	poly	119	2		42	2		39	3		17	2	
S 5	fixed	0	0	- ns	89	0	- ns	91	0	0.2479 ns	2	0	- ns
Gothenburg	poly	109	0		32	0		29	1		4	0	

Population		F 1			F 3			F 5			FIN 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
F 3	fixed	2	1	0.0186*									
Saint Isidore	poly	158	0										
F 5	fixed	9	0	- ns	22	1	1 ns						
Bruenstatt	poly	159	0		3	0							
FIN 1	fixed	2	0	- ns	2	1	1 ns	23	0	- ns			
Lahti	poly	158	0		1	0		4	0				
GB 1	fixed	0	1	0.009**	0	1	0.009**	8	0	- ns	1	0	- ns
Ascot	poly	110	0		110	0		113	0		110	0	
GB 2	fixed	2	0	- ns	0	1	0.0385*	0	0	- ns	2	0	- ns
Braemar	poly	165	0		25	0		27	0		25	0	
N 1	fixed	24	0	1 ns	76	1	0.0503 ns	72	0	0.0649 ns	75	0	0.0385*
Narvik	poly	158	1		1	1		4	1		2	1	
NL 1	fixed	0	0	- ns	77	1	0.4875 ns	69	0	- ns	75	0	- ns
Wageningen	poly	161	0		82	0		85	0		83	0	
NL 2	fixed	2	0	1 ns	0	1	1 ns	22	0	0.2143 ns	2	0	1 ns
Hoge Veluwe	poly	158	1		2	1		5	1		3	1	
PL 2	fixed	2	0	1 ns	0	1	0.1111 ns	0	0	- ns	0	0	- ns
Warsaw	poly	172	4		40	4		42	4		40	4	
RUM 2	fixed	0	0	- ns	23	1	0.3561 ns	4	0	1 ns	23	0	1 ns
Sibiu 2	poly	168	1		97	1		99	1		98	1	
S 1	fixed	24	0	- ns	75	1	1 ns	72	0	- ns	74	0	- ns
Umea	poly	158	0		2	0		5	0		3	0	
S 2	fixed	0	1	0.0667 ns	0	1	0.0667 ns	8	0	1 ns	1	0	1 ns
Stroemstad	poly	84	5		84	5		86	5		84	5	
S 4	fixed	20	0	1 ns	67	1	0.1189 ns	64	0	0.0632 ns	66	0	0.052 ns
Abisko	poly	167	2		17	2		20	2		18	2	
S 5	fixed	0	0	- ns	106	1	1 ns	95	0	- ns	105	0	- ns
Gothenburg	poly	158	0		4	0		7	0		5	0	

Table A29 continue:

Population		GB 1			GB 2			N 1			NL 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
GB 2	fixed	0	0	- ns									
Braemar	poly	121	0										
N 1	fixed	40	0	1 ns	62	0	0.3034 ns						
Narvik	poly	110	1		26	1							
NL 1	fixed	1	0	- ns	67	0	- ns	63	0	1 ns			
Wageningen	poly	159	0		100	0		83	1				
NL 2	fixed	0	0	- ns	0	0	- ns	75	0	0.0032**	77	0	1 ns
Hoge Veluwe	poly	111	1		26	1		3	2		82	1	
PL 2	fixed	0	0	- ns	0	0	- ns	57	0	0.0157*	64	0	0.2982 ns
Warsaw	poly	129	4		42	4		41	5		110	4	
RUM 2	fixed	0	0	- ns	4	0	1 ns	45	0	0.5673 ns	1	0	1 ns
Sibiu 2	poly	121	1		118	1		97	2		151	1	
S 1	fixed	40	0	- ns	62	0	- ns	0	0	- ns	63	0	- ns
Umea	poly	110	0		26	0		2	1		84	0	
S 2	fixed	0	0	- ns	0	0	- ns	0	0	- ns	42	0	0.3529 ns
Stroemstad	poly	152	5		93	5		84	6		141	5	
S 4	fixed	35	0	1 ns	55	0	0.1798 ns	0	0	- ns	59	0	0.5266 ns
Abisko	poly	120	2		39	2		18	3		95	2	
S 5	fixed	0	0	- ns	92	0	- ns	101	0	0.0561 ns	1	0	- ns
Gothenburg	poly	110	0		29	0		5	1		84	0	

Population		NL 2			PL 2			RUM 2			S 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
PL 2	fixed	0	0	- ns									
Warsaw	poly	42	5										
RUM 2	fixed	23	0	1 ns	0	0	- ns						
Sibiu 2	poly	98	2		128	5							
S 1	fixed	74	0	0.0633 ns	57	0	0.0351*	45	0	1 ns			
Umea	poly	4	1		41	4		98	1				
S 2	fixed	0	0	- ns	0	0	- ns	6	0	1 ns	0	0	- ns
Stroemstad	poly	85	6		103	8		152	6		84	5	
S 4	fixed	66	0	0.014*	50	0	0.0308*	39	0	0.5699 ns	0	0	- ns
Abisko	poly	19	3		54	6		110	3		19	2	
S 5	fixed	105	0	0.0625 ns	85	0	0.0156*	0	0	- ns	101	0	- ns
Gothenburg	poly	6	1		44	4		98	1		6	0	

Population		S 2			S 4		
		syn.	nons.	sign.	syn.	nons.	sign.
S 4	fixed	0	0	- ns			
Abisko	poly	92	7				
S 5	fixed	66	0	0.0744 ns	96	0	0.036*
Gothenburg	poly	86	5		21	2	

Table A30: Neutrality indices of *Platynothrus peltifer* computed in the McDonald-Kreitman test with DnaSP v5. Locations with less than two individuals were excluded.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	**A 1**																											
2	**A 2**	0																										
3	**A 3**	0	-																									
4	**A 4**	0	-	-																								
5	**A 5**	0.1	-	-	-																							
6	**A 9**	0	-	-	-	-																						
7	**A 10**	0	-	-	-	-	-																					
8	**CH 3**	0	-	-	-	-	-	-																				
9	**D 1**	0	-	-	-	-	-	-	-																			
10	**D 2**	0	-	-	-	-	-	-	-	-																		
11	**D 3**	32.3	-	-	-	-	-	-	-	-	-																	
12	**D 4**	0	-	-	-	-	-	-	-	-	-	-																
13	**D 5**	0	-	-	-	-	-	-	-	-	-	-	-															
14	**D 6**	9	-	-	-	-	-	-	-	-	-	-	-	-														
15	**D 7**	0	-	-	-	-	-	-	-	-	-	-	-	-	-													
16	**D 8**	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-												
17	**D 9**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-											
18	**D 11**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
19	**D 12**	19.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-									
20	**D 15**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
21	**D 17**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
22	**D 18**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
23	**DK 2**	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
24	**DK 3**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
25	**F 1**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
26	**F 3**	0	-	0	-	0	0	-	0	0	-	42.8	0	0	-	-	0	0	0.2	27.3	0	0	0	0	-	0		
27	**F 5**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
28	**FIN 1**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-
29	**GB 1**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	-
30	**GB 2**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-
31	**N 1**	35.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	76	-
32	**NL 1**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-
33	**NL 2**	9.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-
34	**PL 2**	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-
35	**RUM 2**	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
36	**S 1**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-
37	**S 2**	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	-
38	**S 4**	7.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.9	-
39	**S 5**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-

Table A30 continue:

Population		28	29	30	31	32	33	34	35	36	37	38
29	GB 1	-										
30	GB 2	-	-									
31	N 1	-	-	-								
32	NL 1	-	-	-	-							
33	NL 2	-	-	-	-	-						
34	PL 2	-	-	-	-	-	-					
35	RUM 2	-	-	-	-	-	-	-				
36	S 1	-	-	-	-	-	-	-	-			
37	S 2	-	-	-	-	-	-	-	-	-		
38	S 4	-	-	-	-	-	-	-	-	-	-	
39	S 5	-	-	-	-	-	-	-	-	-	-	-

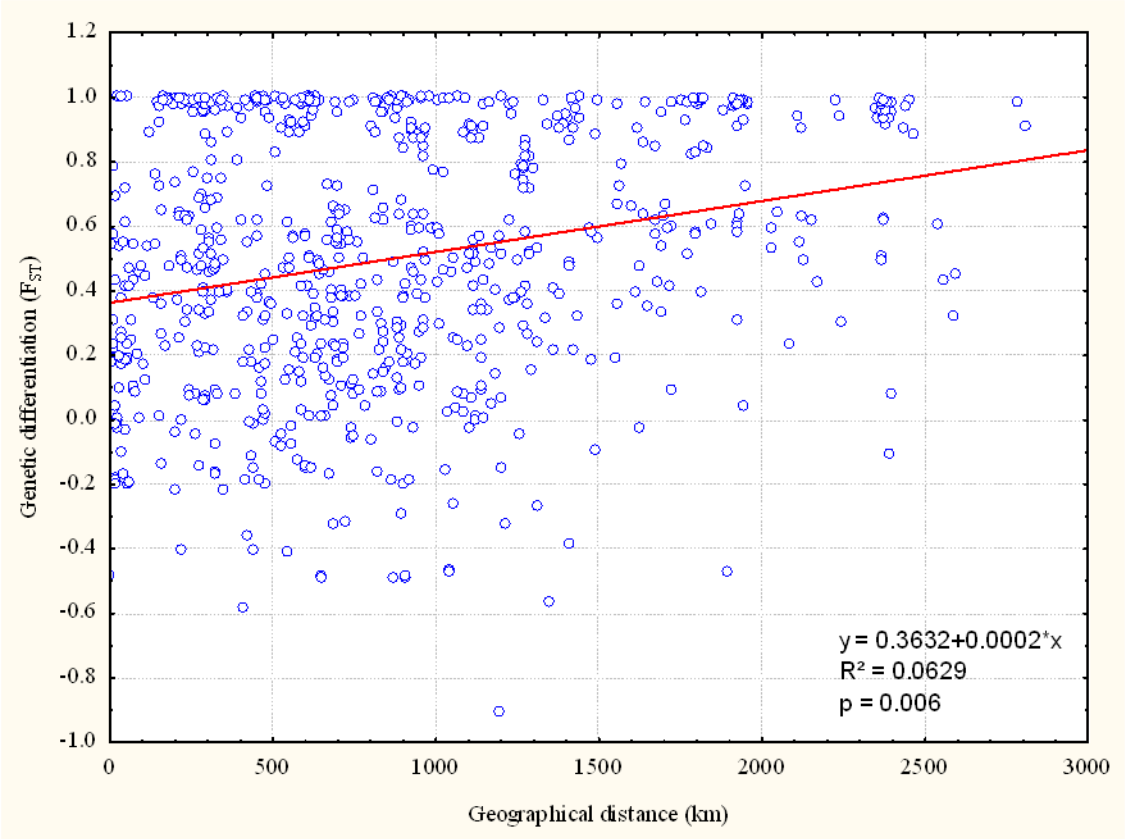

Figure A39: Linear regression of geographical distances (kilometers) vs. genetic differentiations (F_{ST}) based on *COI* of *Platynothrus peltifer*. Regression is significant (p=0.006**; (*0.01<P<0.05; **0.001<P<0.01; ***P<0.001)) using 1000 randomizations.

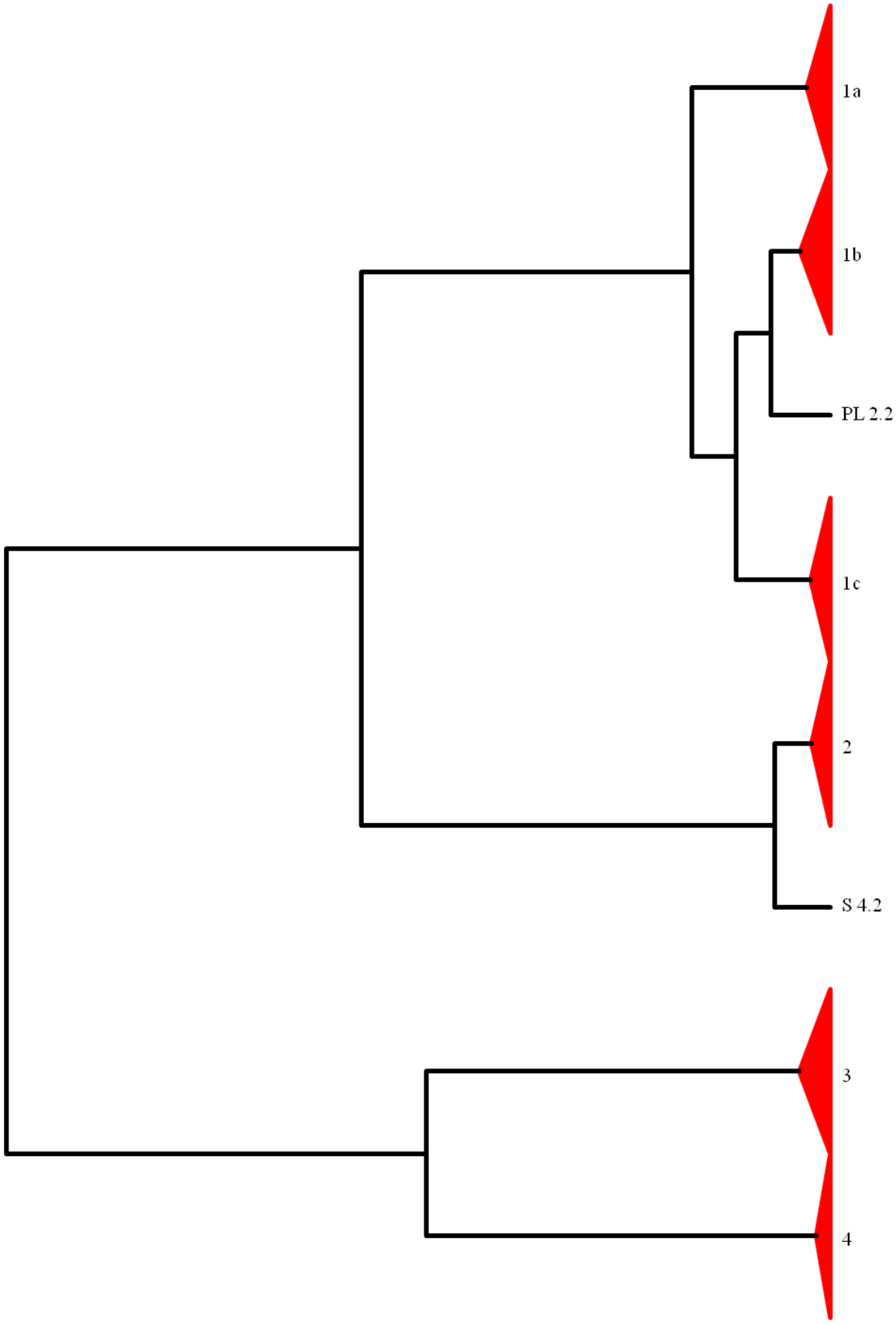

Figure A40: Cluster delimitation of *COI* nucleotide sequences from *Platynothrus peltifer* after the single method of the generalized mixed Yule coalescent method (GMYC). Red branches are the different distinct clusters. Tip labels are explained in Table 20.

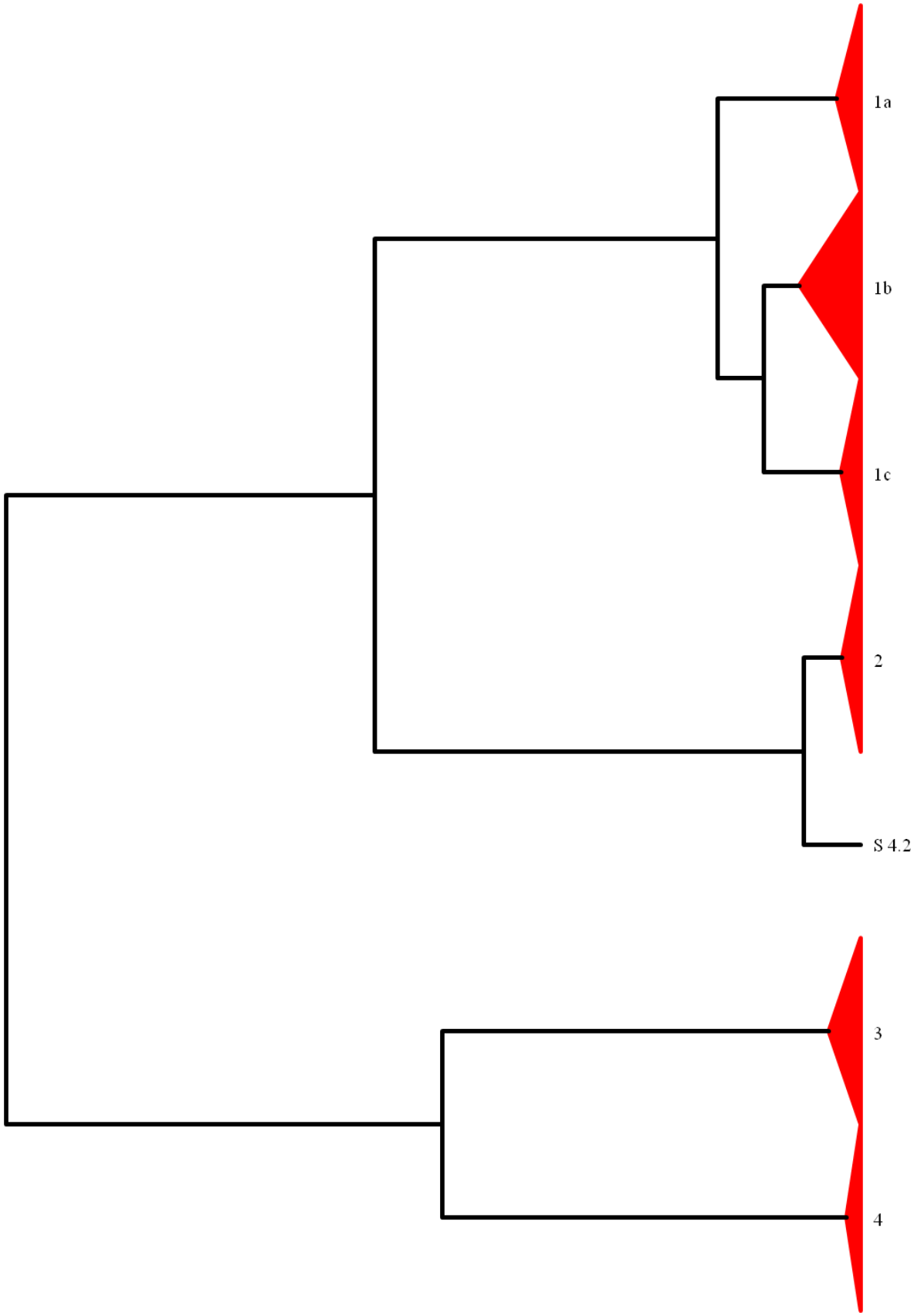

Figure A41: Cluster delimitation of *COI* nucleotide sequences from *Platynothrus peltifer* after the multiple method of the generalized mixed Yule coalescent method (GMYC). Red branches are the different distinct clusters. Tip labels are explained in Table 20.

Table A31: Standard diversity measures of *Achipteria coleoptrata*. Populations with less than two individuals were excluded.

population	sample size n	invarible sites N_{is}	variable sites N_{vs}	parsimony inform. sites N_{pars}	number of singeltons N_s	number of haplotypes N_h	haplotype diversity H_d	variance	nucleotide diversity Π_n
A_5	4	415	97	4	93	4	1	0.031	0.097
A_7	2	487	25	0	25	2	1	0.250	0.049
A_8	3	420	92	0	92	3	1	0.074	0.12
CH_1	5	503	9	2	7	5	1	0.016	0.008
CH_3	2	506	6	0	6	2	1	0.250	0.012
D_1	4	510	2	0	2	3	0.83	0.049	0.002
D_2	2	509	3	0	3	2	1	0.250	0.006
D_3	4	500	12	0	12	3	0.83	0.049	0.012
D_5	4	508	4	0	4	2	0.5	0.07	0.004
D_8	6	509	3	1	2	3	0.73	0.024	0.002
D_9	3	506	6	0	6	2	0.67	0.099	0.008
D_10	5	499	13	11	2	4	0.9	0.026	0.014
D_11	2	512	0	0	0	1	0	0	0
D_12	3	470	42	0	42	3	1	0.074	0.055
D_14	2	511	1	0	1	2	1	0.250	0.002
D_15	3	428	84	0	84	3	1	0.074	0.11
DK_3	4	503	9	1	8	4	1	0.031	0.009
EST_1	4	490	22	6	16	4	1	0.031	0.024
F_1	2	492	20	0	20	2	1	0.250	0.039
F_2	6	477	35	25	10	5	0.93	0.015	0.034
F_4	3	511	1	0	1	2	0.67	0.099	0.001
FIN_1	4	482	30	0	30	2	0.667	0.042	0.039
GB_1	4	471	41	3	38	4	1.00	0.031	0.042
GB_2	3	499	13	0	13	2	0.67	0.099	0.017
GR_1	6	504	8	4	4	3	0.6	0.046	0.007
I_1	8	504	8	7	1	3	0.61	0.027	0.006
I_2	4	504	8	4	4	3	0.83	0.049	0.004
PL_1	4	470	42	1	41	3	0.83	0.049	0.041
PL_2	8	405	107	36	71	6	0.89	0.012	0.074
RUM_1	4	496	16	2	14	3	0.83	0.049	0.016
RUM_2	4	503	9	0	9	2	0.5	0.07	0.009
RUM_5	3	508	4	0	4	3	1	0.074	0.005
RUM_6	2	506	6	0	6	2	1	0.250	0.012
RUS_2	3	509	3	0	3	2	0.67	0.099	0.004
S_2	6	511	1	0	1	2	0.33	0.046	0.001
all	136	293	219	197	22	88	0.98	0	0.114

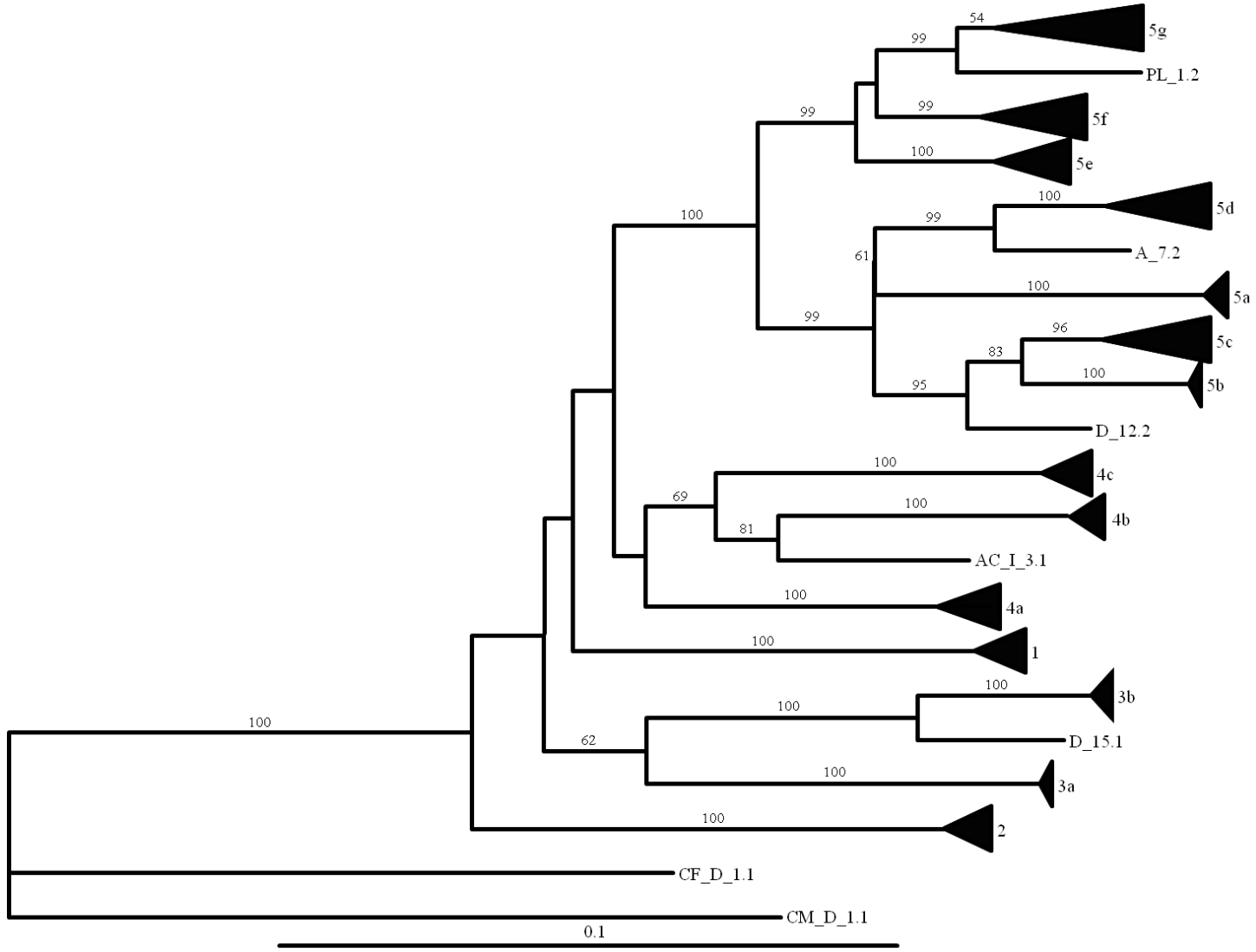

Figure A42: Neighbor-Joining tree of 141 *COI* nucleotide sequences of *Achipteria coleoptrata*. Outgroups are *Carabodes femoralis* (CF_D_1.1) and *C. marginatus* (CM_D_1.1). Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Tip numbers are the different subclades and explain in Table 24.

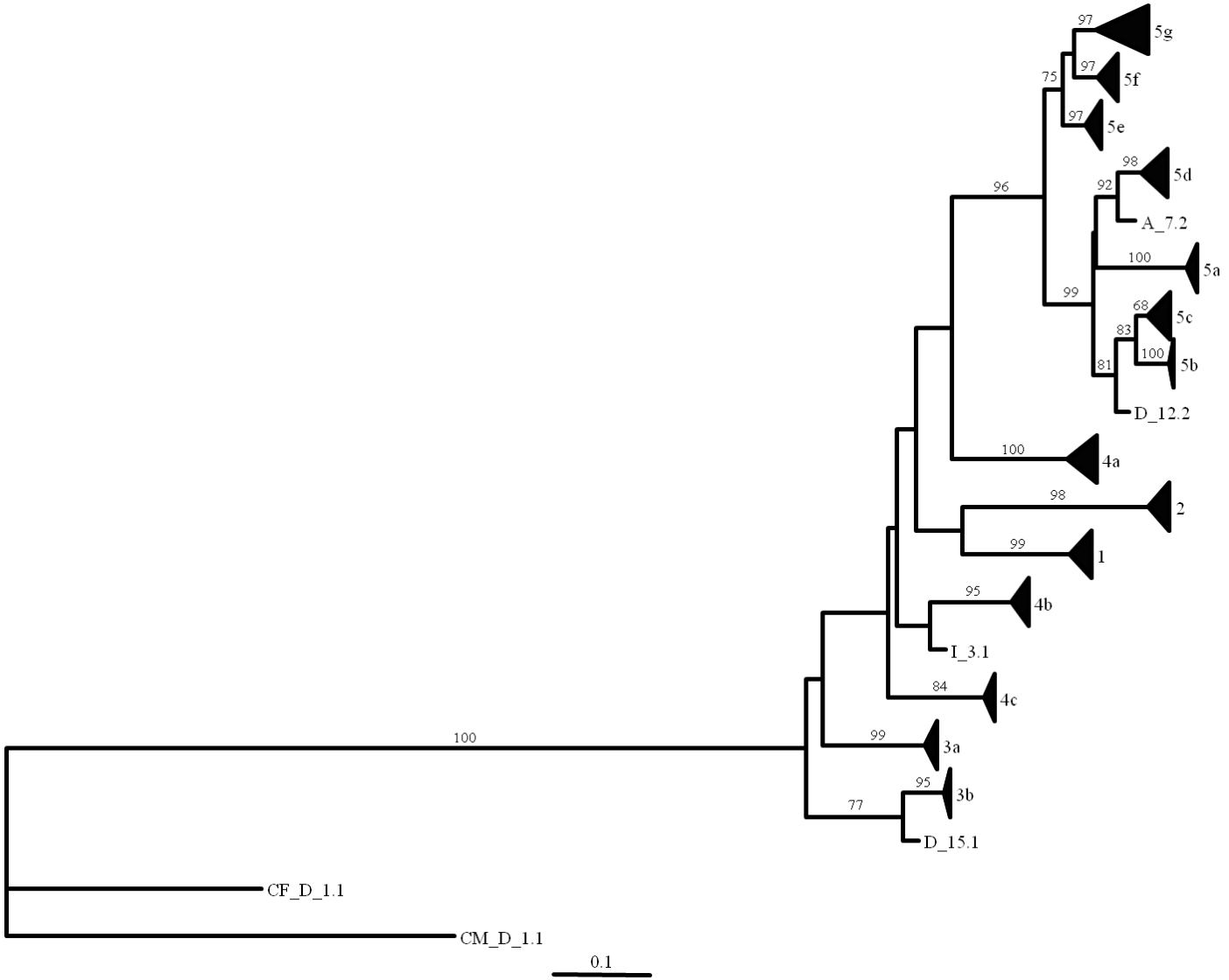

Figure A43: Neighbor-Joining tree of 141 *COI* nucleotide sequences of *Achipteria coleoptrata* with model of sequence evolution TVM+I+G. Outgroups are *Carabodes femoralis* (CF_D_1.1) and *C. marginatus* (CM_D_1.1). Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Tip numbers are the different subclades and explain in Table 24.

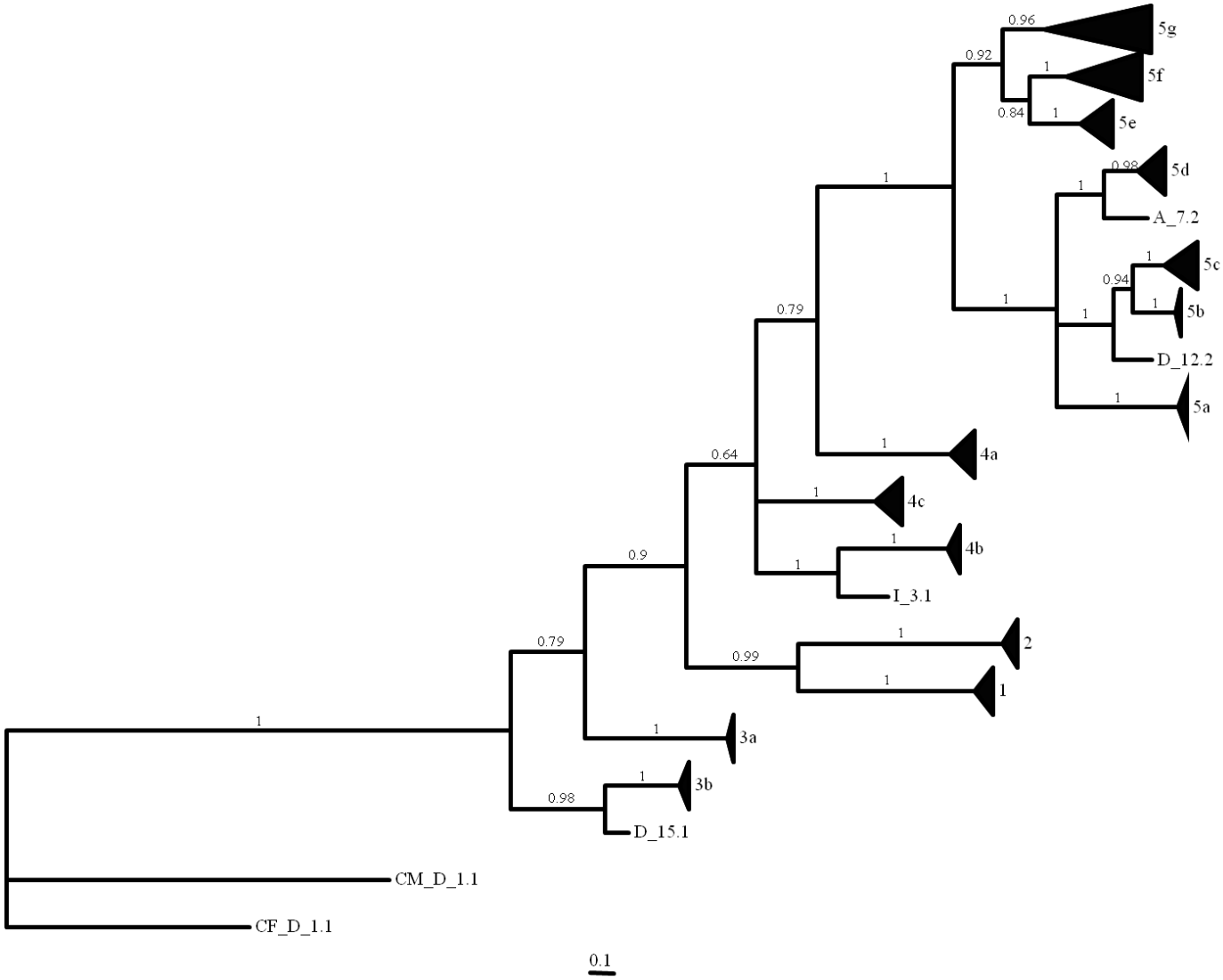

Figure A44: Bayesian tree after $10x10^6$ generations from 141 *COI* nucleotide sequences from *Achipteria coleoptrata* with MrBayes. Split frequencies of 0.012879 and burnin of 25%. Outgroups are *Carabodes femoralis* (CF_D_1.1) and *C. marginatus* (CM_D_1.1). Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 24.

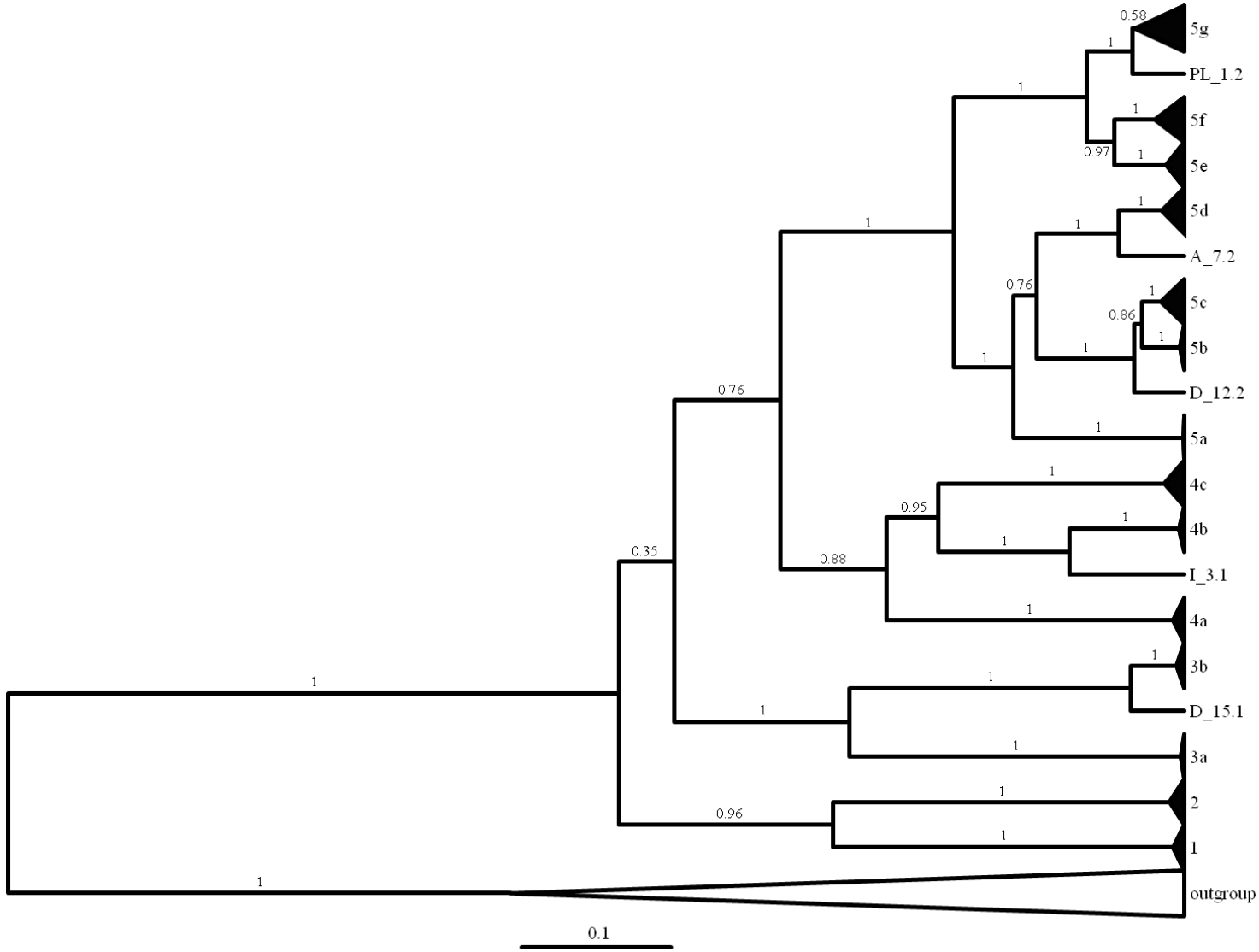

Figure A45: Bayesian phylogeny after $10x10^6$ generations from 141 *COI* nucleotide sequences from *Achipteria coleoptrata* with Beast v1.5.4. Outgroups are *Carabodes femoralis* (CF_D_1.1) and *C. marginatus* (CM_D_1.1).. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 24.

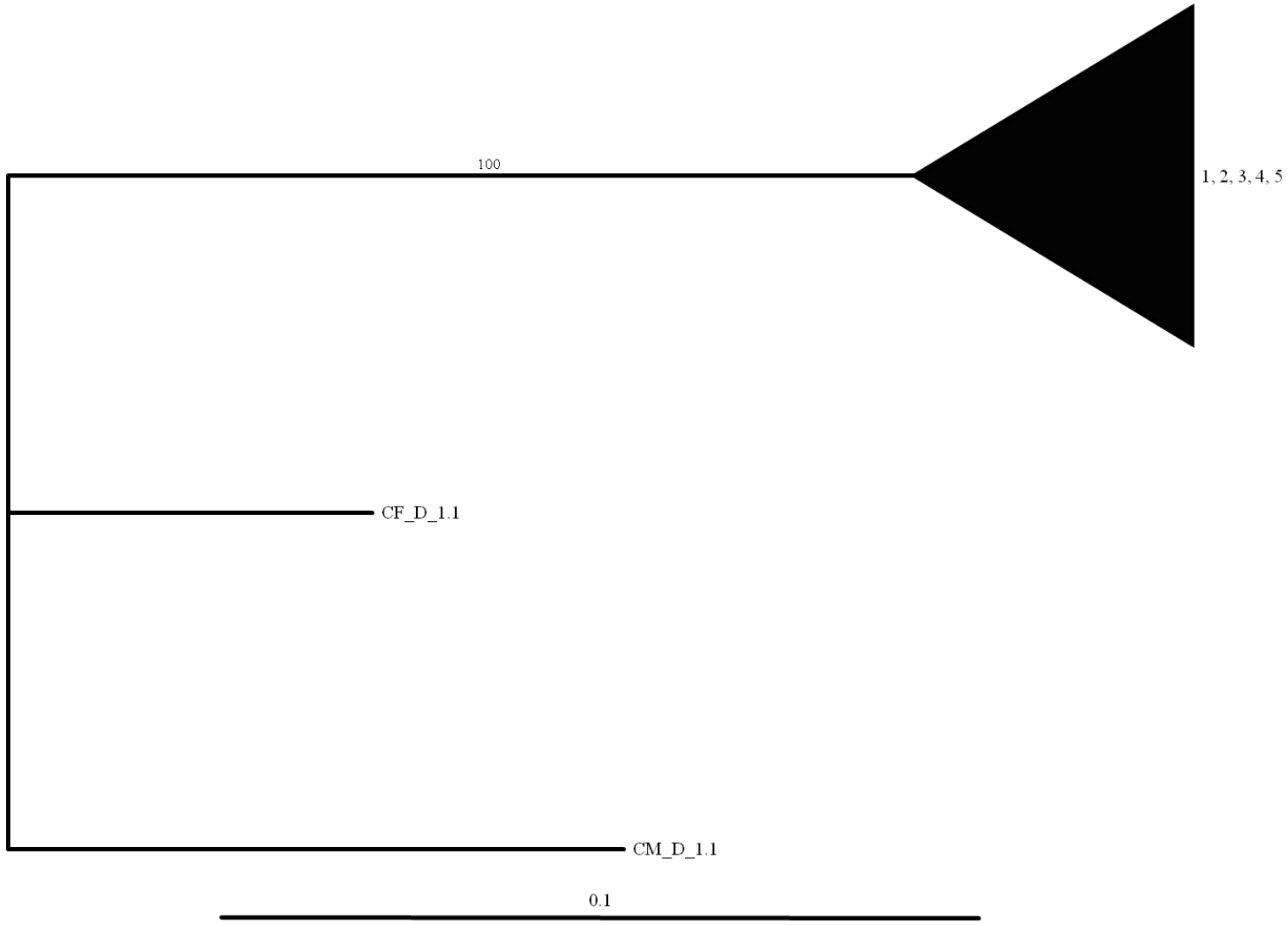

Figure A46: Neighbor-Joining tree of 141 *COI* protein sequences of *Achipteria coleoptrata*. Outgroups are *Carabodes femoralis* (CF_D_1.1) and *C. marginatus* (CM_D_1.1). Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Tip numbers are the different subclades and explain in Table 24.

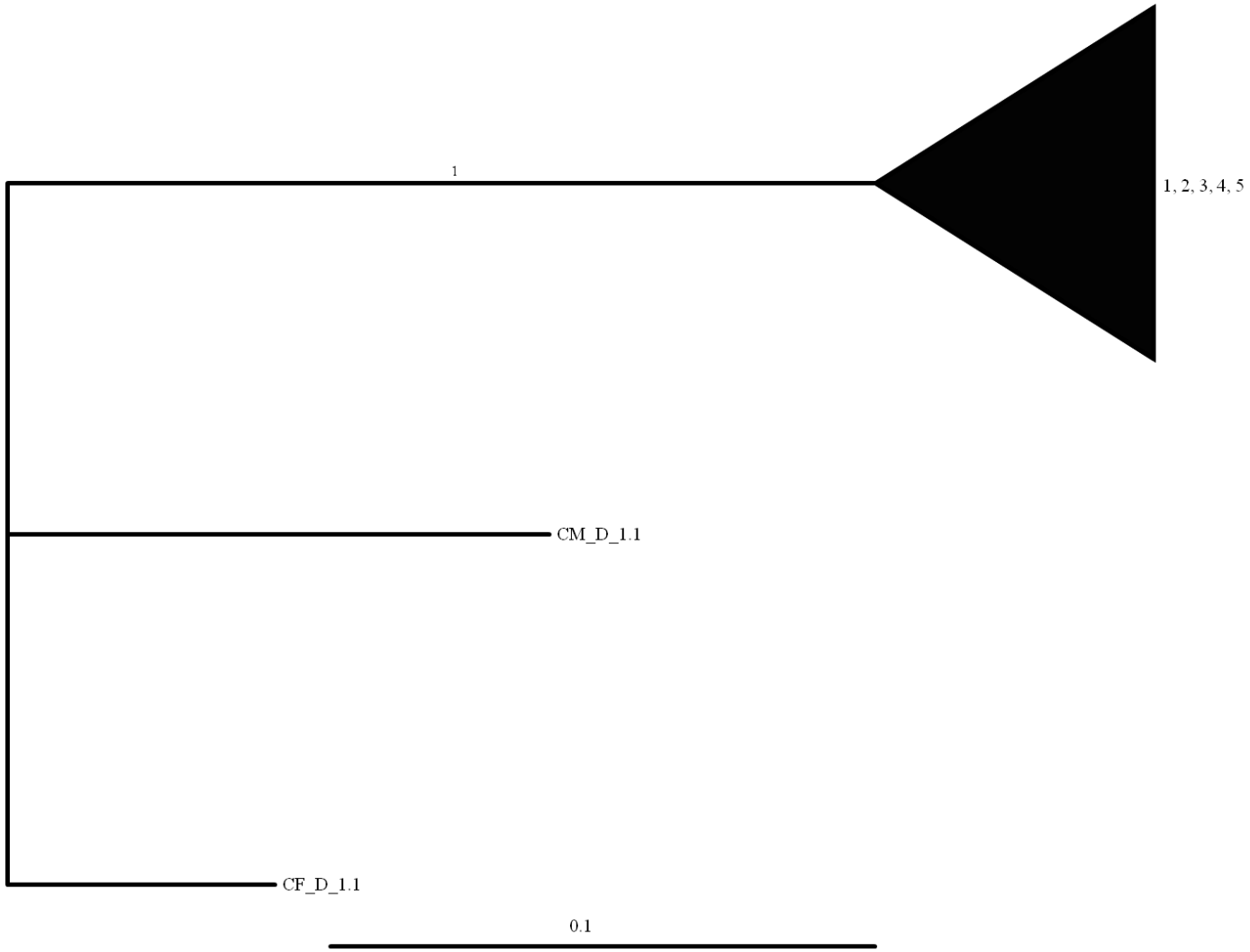

Figure A47: Bayesian tree after 10 x 10^6 generations from 141 *COI* protein sequences of *Achipteria coleoptrata*. Outgroups are *Carabodes femoralis* (CF_D_1.1) and *C. marginatus* (CM_D_1.1). Split frequencies of 0.031752 and burnin of 25%. Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 24.

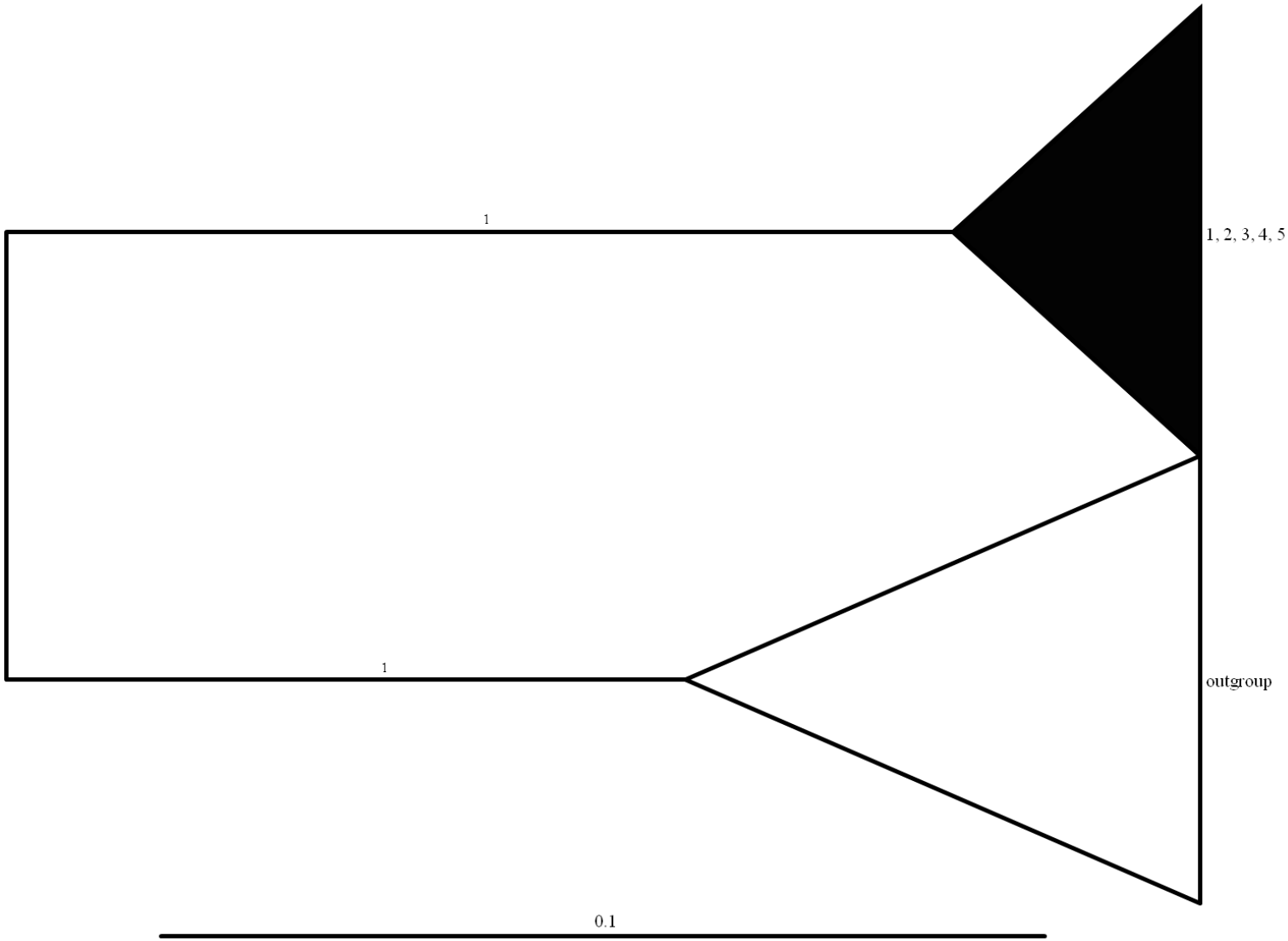

Figure A48: Bayesian phylogeny after $10x10^6$ generations with Beast v1.5.4 from the 141 *COI* protein sequences of *Achipteria coleoptrata*. Outgroups are *Carabodes femoralis* (CF_D_1.1) and *C. marginatus* (CM_D_1.1). Numbers on the branches are posterior probabilities. Tip numbers are the different subclades and explain in Table 24.

Table A32: Mean pairwise percentage differences of uncorrected p-distances of the protein of *Achipteria coleoptrata* from 35 locations. The diagonal is the within population differences (bold) and below the diagonal the among population differences. Bold letters in red are the minimum and maximum differences within and among the populations. Locations with less than two individuals were excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	**A 5**	**0.3**																							
2	**A 7**	0.2	**0**																						
3	**A 8**	0.2	**0**	**0**																					
4	**CH 1**	0.2	**0**	**0**	**0**																				
5	**CH 3**	0.4	0.3	0.3	0.3	**0.6**																			
6	**D 1**	0.2	**0**	**0**	**0**	0.3	**0**																		
7	**D 2**	0.7	0.6	0.6	0.6	0.9	0.6	**1.2**																	
8	**D 3**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**																
9	**D 5**	0.3	0.2	0.2	0.2	0.4	0.2	0.7	0.2	**0.3**															
10	**D 8**	0.3	0.2	0.2	0.2	0.5	0.2	0.8	0.2	0.3	**0.3**														
11	**D 9**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**													
12	**D 10**	0.3	0.1	0.1	0.1	0.4	0.1	0.6	0.1	0.3	0.3	0.1	**0.2**												
13	**D 11**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**											
14	**D 12**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**										
15	**D 14**	0.4	0.3	0.3	0.3	0.6	0.3	0.9	0.3	0.4	0.5	0.3	0.4	0.3	0.3	**0.6**									
16	**D 15**	2.1	2	2	2	1.9	2.0	**2.6**	2.0	2.1	2.2	2	2.1	2	2	1.9	**2.4**								
17	**DK 3**	0.3	0.2	0.2	0.2	0.3	0.2	0.7	0.2	0.3	0.3	0.2	0.3	0.2	0.2	0.4	1.9	**0.3**							
18	**EST 1**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**						
19	**F 1**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**					
20	**F 2**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**	**0**				
21	**F 4**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**	**0**	**0**			
22	**FIN 1**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**	**0**	**0**	**0**		
23	**GB 1**	0.3	0.2	0.2	0.2	0.4	0.2	0.7	0.2	0.3	0.3	0.2	0.3	0.2	0.2	0.3	1.9	0.3	0.2	0.2	0.2	0.2	0.2	**0.3**	
24	**GB 2**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**	**0**	**0**	**0**	0.2	**0**
25	**GR 1**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**	**0**	**0**	**0**	0.2	**0**
26	**I 1**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**	**0**	**0**	**0**	0.2	**0**
27	**I 2**	0.4	0.3	0.3	0.3	0.6	0.6	0.9	0.3	0.4	0.5	0.3	0.4	0.3	0.3	0.6	2.3	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.3
28	**PL 1**	0.6	0.4	0.4	0.4	0.7	0.4	1	0.4	0.6	0.6	0.4	0.6	0.4	0.4	0.6	2.2	0.6	0.4	0.4	0.4	0.4	0.4	0.5	0.4
29	**PL 2**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**	**0**	**0**	**0**	0.2	**0**
30	**RUM 1**	0.7	0.6	0.6	0.6	0.9	0.5	1.2	0.6	0.7	0.8	0.6	0.7	0.6	0.6	0.9	2.6	0.7	0.6	0.6	0.6	0.6	0.6	0.7	0.6
31	**RUM 2**	0.6	0.4	0.4	0.4	0.7	0.4	1.0	0.4	0.6	0.6	0.4	0.6	0.4	0.4	0.7	2.4	0.6	0.4	0.4	0.4	0.4	0.4	0.6	0.4
32	**RUM 5**	0.5	0.4	0.4	0.4	0.7	0.4	1.0	0.4	0.5	0.6	0.4	0.5	0.4	0.4	0.7	2.4	0.5	0.4	0.4	0.4	0.4	0.4	0.5	0.4
33	**RUM 6**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**	**0**	**0**	**0**	0.2	**0**
34	**RUS 2**	0.5	0.4	0.4	0.4	0.7	0.4	1.0	0.4	0.5	0.6	0.4	0.5	0.4	0.4	0.3	1.8	0.5	0.4	0.4	0.4	0.4	0.4	0.3	0.4
35	**S 2**	0.2	**0**	**0**	**0**	0.3	**0**	0.6	**0**	0.2	0.2	**0**	0.1	**0**	**0**	0.3	2	0.2	**0**	**0**	**0**	**0**	**0**	0.2	**0**

Table A32 continue:

Appendix

Population		25	26	27	28	29	30	31	32	33	34	35
25	**GR 1**	**0**										
26	**I 1**	**0**	**0**									
27	**I 2**	0.3	0.3	**0.6**								
28	**PL 1**	0.4	0.4	0.7	**0.7**							
29	**PL 2**	**0**	**0**	0.3	0.4	**0**						
30	**RUM 1**	0.6	0.6	0.9	0.9	0.6	**1**					
31	**RUM 2**	0.4	0.4	0.7	0.9	0.4	0.8	**0.3**				
32	**RUM 5**	0.4	0.4	0.7	0.9	0.4	0.9	0.7	**0.8**			
33	**RUM 6**	**0**	**0**	0.3	0.4	**0**	0.6	0.4	0.4	**0**		
34	**RUS 2**	0.4	0.4	0.7	0.6	0.4	1	0.8	0.8	0.4	**0.4**	
35	**S 2**	**0**	**0**	0.3	0.4	**0**	0.6	0.4	0.4	**0**	0.4	**0**

Table A33: Tajima's D and Fu's FS neutrality tests of *Achipteria coleoptrata COI* nucleotide sequences from 35 locations. Bold red letters show significance (P<0.05).

Neutrality tests	A 5	A 7	A 8	CH 1	CH 3	D 1	D 2	D 3	D 5	D 8	D9	D 10	D 11	D 12	D 14	D 15	DK 3	EST 1	F 1	F 2	F 4	FIN 1
Tajima's D test																						
Samplesize	4	2	3	5	2	4	2	4	4	6	3	5	2	3	2	3	4	4	2	6	3	4
S	97	25	92	9	6	2	3	12	4	3	6	13	0	42	1	84	9	22	20	35	1	30
Pi	49.83	25	61.33	4	6	1	3	6	2	1.2	4	7.4	0	28	1	56.33	4.67	12.33	20	17.53	0.67	20
Tajima's D	-0.61	0	0	-0.53	0	-0.71	0	-0.84	-0.78	-0.45	0	1.35	0	0	0	1536564.26	-0.49	0.29	0	0.91	0	2.30
Tajima's D p-value	0.34	1	0.68	0.41	1	0.27	1	0.1	0.2	0.36	0.78	0.91	1	0.68	1	1	0.46	0.72	1	0.84	0.99	1
Fu's FS test																						
Real no. of alleles	4	2	3	5	2	3	2	3	2	3	2	4	1	3	2	3	4	4	2	5	2	2
Orig. no. of alleles	4	2	3	5	2	3	2	3	2	3	2	4	1	3	2	3	4	4	2	5	2	2
Theta_pi	49.83	25	61.33	4	6	1	3	6	2	1.2	4	7.4	0	28	1	56.33	4.67	12.33	20	17.53	0.67	20
Exp. no. of alleles	3.88	1.96	2.95	3.54	1.86	2.08	1.75	3.27	2.57	2.63	2.47	4.03	0	2.9	1.5	2.95	3.13	3.59	1.95	5.29	1.65	3.73
FS	2.08	3.22	3.01	-1.72	1.79	-0.89	1.1	1.79	2.2	0.12	2.64	1.12	0	2.21	0	2.92	-0.62	0.58	3	1.75	0.2	7.48
FS p-value	0.53	0.6	0.57	0.06	0.51	0.09	0.43	0.76	0.83	0.45	0.83	0.63	N.A.	0.55	0.25	0.57	0.19	0.38	0.61	0.73	0.38	1

Neutrality tests	GB 1	GB 2	GR 1	I 1	I 2	PL 1	PL 2	RUM 1	RUM 2	RUM 5	RUM 6	RUS 2	S 2	mean	s. d.
Tajima's D test															
Samplesize	4	3	6	8	4	4	8	4	4	3	2	3	6	3.89	1.59
S	41	13	8	8	4	42	107	16	9	4	6	3	1	22.23	29.32
Pi	21.33	8.67	3.73	3.25	2	21.17	37.64	8.33	4.5	2.67	6	2	0.33	12.94	16.26
Tajima's D	-0.48	0	0.39	0.26	-0.78	-0.79	-0.48	-0.46	-0.83	0	0	0	-0.93	43901.73	259726.78
Tajima's D p-value	0.46	0.71	0.66	0.62	0.2	0.17	0.33	0.47	0.12	0.83	1	0.88	0.26	0.64	0.32
Fu's FS test															
Real no. of alleles	4	2	3	3	3	3	6	3	2	3	2	2	2	2.89	1.08
Orig. no. of alleles	4	2	3	3	3	3	6	3	2	3	2	2	2	2.89	1.08
Theta_pi	21.33	8.67	3.73	3.25	2	21.17	37.64	8.33	4.5	2.67	6	2	0.33	12.94	16.26
Exp. no. of alleles	3.75	2.71	3.9	4.41	2.57	3.74	7.34	3.43	3.11	2.30	1.86	2.17	1.63	2.93	1.28
FS	1.18	3.92	2.28	2.75	0.13	3.93	3.89	2.32	3.78	-0.34	1.79	1.61	0.00	1.75	1.79
FS p-value	0.45	0.92	0.87	0.91	0.33	0.92	0.94	0.81	0.94	0.19	0.51	0.7	0.27	N.A.	N.A.

Table A34: Tajima's D and Fu's FS neutrality tests of *Achipteria coleoptrata COI* protein sequences from 35 locations. Bold red letters show significance (P<0.05).

Neutrality tests	A 5	A 7	A 8	CH 1	CH 3	D 1	D 2	D 3	D 5	D 8	D 9	D 10	D 11	D 12	D 14	D 15	DK 3	EST 1	F 1	F 2	F 4	FIN 1
Tajima's D test																						
Samplesize	4	2	3	5	2	4	2	4	4	6	3	5	2	3	2	3	4	4	2	6	3	4
S	1	0	0	0	1	0	2	0	1	1	0	1	0	0	1	6	1	0	0	0	0	0
Pi	0.5	0	0	0	1	0	2	0	0.5	0.53	0	0.4	0	0	1	4	0.5	0	0	0	0	0
Tajima's D	-0.61	0	0	0	0	0	0	0	-0.61	0.85	0	-0.82	0	0	0	0	-0.61	0	0	0	0	0
Tajima's D p-value	0.38	1	1	1	1	1	1	1	0.38	0.88	1	0.3	1	1	1	0.78	0.38	1	1	1	1	1
Fu's FS test																						
Real no. of alleles	3	1	2	2	2	2	2	1	2	2	1	2	1	2	2	3	3	1	1	3	1	1
Orig. no. of alleles	3	1	2	2	2	2	2	1	2	2	1	2	1	2	2	3	3	1	1	3	1	1
Theta_pi	0.5	0	0	0	1	0	2	0	0.5	0.53	0	0.4	0	0	1	4	0.5	0	0	0	0	0
Exp. no. of alleles	1.68	0	2	2	1.5	2	1.67	0	1.68	1.92	0	1.66	0	2	1.5	2.47	1.68	0	0	3	0	0
FS	0.17	0	0	0	0	0	0.69	0	0.17	0.63	0	0.09	0	0	0	0.13	0.17	0	0	0	0	0
FS p-value	0.35	N.A.	N.A.	N.A.	0.24	N.A.	0.37	N.A.	0.34	0.47	N.A.	0.3	N.A.	N.A.	0.25	0.27	0.34	N.A.	N.A.	N.A.	N.A.	N.A.

Neutrality tests	GB 1	GB 2	GR 1	I 1	I 2	PL 1	PL 2	RUM 1	RUM 2	RUM 5	RUM 6	RUS 2	S 2	mean	s. d.
Tajima's D test															
Samplesize	4	3	6	8	4	4	8	4	4	3	2	3	6	3.89	1.59
S	41	13	8	8	4	42	107	16	9	4	6	3	1	22.23	29.32
Pi	21.33	8.67	3.73	3.25	2	21.17	37.64	8.33	4.5	2.67	6	2	0.33	12.94	16.26
Tajima's D	-0.48	0	0.39	0.26	-0.78	-0.79	-0.48	-0.46	-0.83	0	0	0	-0.93	43901.73	259726.78
Tajima's D p-value	0.46	0.71	0.66	0.62	0.2	0.17	0.33	0.47	0.12	0.83	1	0.88	0.26	0.64	0.32
Fu's FS test															
Real no. of alleles	4	2	3	3	3	3	6	3	2	3	2	2	2	2.89	1.08
Orig. no. of alleles	4	2	3	3	3	3	6	3	2	3	2	2	2	2.89	1.08
Theta_pi	21.33	8.67	3.73	3.25	2	21.17	37.64	8.33	4.5	2.67	6	2	0.33	12.94	16.26
Exp. no. of alleles	3.75	2.71	3.9	4.41	2.57	3.74	7.34	3.43	3.11	2.30	1.86	2.17	1.63	2.93	1.28
FS	1.18	3.92	2.28	2.75	0.13	3.93	3.89	2.32	3.78	-0.34	1.79	1.61	0.00	1.75	1.79
FS p-value	0.45	0.92	0.87	0.91	0.33	0.92	0.94	0.81	0.94	0.19	0.51	0.7	0.27	N.A.	N.A.

Table A35: Results of the McDonald-Kreitman test for *Achipteria coleoptrata*. The differences between 58 populations are significant (*0.01<P<0.05), between other 41 populations high significant (**0.001<P<0.01) and between 37 populations extremely high significant (***P<0.001). Number of fixed and polymorph synonymous and non-synonymous mutations are shown. Locations with less than two individuals were excluded.

Population		A 5			A 7			A 8			CH 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
A 7	fixed	0	0	- ns									
Roggendorf	poly	107	4										
A 8	fixed	0	0	- ns	3	0	1 ns						
Imst	poly	98	4		102	3							
CH 1	fixed	0	0	- ns	1	0	1 ns	0	0	- ns			
Basel	poly	100	4		32	1		95	3				
CH 3	fixed	0	0	- ns	1	0	1 ns	3	0	1 ns	0	0	- ns
Rohrschach	poly	98	5		28	1		92	4		10	2	
D 1	fixed	50	0	0.1709 ns	76	3	1 ns	49	0	0.299 ns	84	2	0.0622 ns
Kranichstein	poly	96	5		25	1		90	4		9	2	
D 2	fixed	26	1	0.6899 ns	46	1	0.1435 ns	27	1	0.6916 ns	58	1	0.0023**
Goettingen	poly	95	7		25	3		89	6		8	4	
D 3	fixed	23	1	1 ns	43	1	1 ns	24	1	1 ns	54	1	0.4649 ns
Lake Constance	poly	103	4		34	0		96	3		19	1	
D 5	fixed	51	0	0.1007 ns	76	3	0.6091 ns	50	0	0.1655 ns	85	2	0.0151*
Moerfelden	poly	97	6		27	2		91	5		10	3	
D 8	fixed	24	1	1 ns	44	1	1 ns	25	1	1 ns	56	1	0.076 ns
Cuxhaven	poly	97	5		27	1		91	4		10	2	
D 9	fixed	25	1	1 ns	45	1	1 ns	26	1	1 ns	57	1	0.371 ns
Bonn	poly	97	4		29	0		91	3		14	1	
D 10	fixed	22	1	1 ns	43	1	1 ns	23	1	1 ns	54	1	0.1947 ns
Cologne	poly	104	5		33	1		98	4		20	2	
D 11	fixed	28	0	- ns	43	0	- ns	31	0	0.5712 ns	56	0	0.1385 ns
Wittmoor	poly	95	4		25	0		89	3		8	1	
D 12	fixed	0	0	- ns	1	0	1 ns	1	0	1 ns	1	0	1 ns
Sonthofen	poly	118	5		58	1		114	4		48	2	
D 14	fixed	26	1	1 ns	33	1	1 ns	29	1	1 ns	41	1	0.091 ns
Steineberg	poly	95	5		25	1		89	4		8	2	
D 15	fixed	6	0	1 ns	20	1	0.69 ns	6	0	1 ns	27	0	0.0683 ns
Langenwang	poly	140	14		96	10		136	13		82	11	
DK 3	fixed	24	1	1 ns	46	1	0.5626 ns	25	1	1 ns	56	1	0.0408*
Arhus	poly	99	6		30	2		92	5		15	3	
EST 1	fixed	22	0	0.6157 ns	37	0	- ns	24	0	1 ns	50	0	0.375 ns
Tallin	poly	113	4		46	0		107	3		29	1	
F 1	fixed	20	1	1 ns	42	1	1 ns	21	1	1 ns	54	1	1 ns
Mont Blanc	poly	115	4		42	0		109	3		28	1	
F 2	fixed	18	1	1 ns	40	1	1 ns	19	1	1 ns	46	1	0.3559 ns
Loire	poly	118	6		49	2		112	5		42	3	
F 4	fixed	28	1	1 ns	46	1	1 ns	29	1	1 ns	58	1	0.2707 ns
Haute Loire	poly	95	4		25	0		89	3		9	1	
FIN 1	fixed	17	0	1 ns	1	0	1 ns	18	0	1 ns	42	0	0.4615 ns
Lahti	poly	116	4		50	0		110	3		35	1	
GB 1	fixed	18	0	0.596 ns	34	0	0.2924 ns	19	0	0.5932 ns	43	0	0.0584 ns
Ascot	poly	118	8		55	4		113	7		44	5	
GB 2	fixed	21	1	1 ns	41	1	1 ns	22	1	1 ns	51	1	1 ns
Braemar	poly	102	4		35	0		95	3		21	1	
GR 1	fixed	33	0	0.3402 ns	64	0	0.3402 ns	33	0	0.5717 ns	70	0	0.04*
Thessaloniki	poly	102	5		32	1		96	4		16	2	
I 1	fixed	37	0	0.5725 ns	72	1	1 ns	38	0	0.5611 ns	81	0	0.1735 ns
Grosseto	poly	103	4		32	0		97	3		16	1	
I 2	fixed	38	0	0.1895 ns	70	2	0.141 ns	37	0	0.1856 ns	80	1	0.0011**
Parma	poly	96	7		26	3		90	6		9	4	
PL 1	fixed	16	0	0.5978 ns	32	0	0.1639 ns	16	0	0.5976 ns	43	0	0.0296*
Krakow	poly	123	9		58	5		119	8		45	6	
PL 2	fixed	7	0	1 ns	19	0	1 ns	7	0	1 ns	25	0	0.6047 ns
Warsaw	poly	173	7		125	4		168	3		118	4	

Table A35 continue:

Population		A 5			A 7			A 8			CH 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM 1	fixed	24	0	0.2144 ns	32	0	0.0614 ns	27	0	0.2073 ns	41	0	0.0016**
Sibiu 1	poly	102	9		35	5		98	8		18	6	
RUM 2	fixed	25	0	0.3562 ns	33	0	0.4923 ns	28	0	0.3558 ns	42	0	0.0209*
Sibiu 2	poly	100	6		31	2		96	5		14	3	
RUM 5	fixed	42	1	0.447 ns	67	2	0.5794 ns	42	1	0.6654 ns	76	2	0.0197*
Busteni	poly	96	6		27	2		90	5		10	3	
RUM 6	fixed	41	1	1 ns	65	2	0.563 ns	41	1	1 ns	76	2	0.3942 ns
Sinaia	poly	100	4		31	0		95	3		13	1	
RUS 2	fixed	40	1	0.6732 ns	75	3	0.6059 ns	39	1	0.6694 ns	84	2	0.0125*
Novosibirsk	poly	96	6		26	2		90	5		9	3	
S 2	fixed	26	1	1 ns	46	1	1 ns	27	1	1 ns	58	1	0.053 ns
Stroemstad	poly	95	5		25	1		89	4		8	2	

Population		CH 3			D 1			D 2			D 3		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 1	fixed	86	3	0.0528 ns									
Kranichstein	poly	6	2										
D 2	fixed	58	1	0.0007***	81	4	0.0001***						
Goettingen	poly	5	4		1	4							
D 3	fixed	54	1	0.4190 ns	78	4	1 ns	1	0	1 ns			
Lake Constance	poly	16	1		12	1		12	3				
D 5	fixed	87	3	0.0126*	0	0	- ns	82	4	0.0001***	78	4	0.2526 ns
Moerfelden	poly	7	3		3	3		2	5		14	2	
D 8	fixed	56	1	0.0467*	81	4	0.0341*	0	0	- ns	1	0	1 ns
Cuxhaven	poly	7	2		3	2		2	4		13	1	
D 9	fixed	57	1	0.3155 ns	80	4	0.3722 ns	0	0	- ns	0	0	- ns
Bonn	poly	11	2		7	1		6	3		18	0	
D 10	fixed	54	1	0.16 ns	77	4	0.2354 ns	0	0	- ns	0	0	- ns
Cologne	poly	17	2		13	2		12	3		23	1	
D 11	fixed	55	0	0.0984 ns	80	3	0.0884 ns	30	1	0.0007***	26	1	1 ns
Wittmoor	poly	5	1		1	1		0	3		12	0	
D 12	fixed	1	0	1 ns	67	3	1 ns	36	0	0.125 ns	36	0	1 ns
Sonthofen	poly	45	2		41	2		41	4		48	1	
D 14	fixed	41	1	0.0498*	90	4	0.0094**	60	2	0.0000***	58	2	0.4498 ns
Steineberg	poly	5	2		1	2		0	4		12	1	
D 15	fixed	28	1	0.2896 ns	54	0	0.0068**	39	2	0.1423 ns	38	2	0.3493 ns
Langenwang	poly	79	10		76	11		75	13		84	10	
DK 3	fixed	57	1	0.0946 ns	77	4	0.0347*	0	0	- ns	0	0	- ns
Arhus	poly	12	2		8	3		7	5		19	2	
EST 1	fixed	49	0	0.3636 ns	78	3	1 ns	20	1	0.6174 ns	16	1	0.34 ns
Tallin	poly	27	1		24	1		23	3		33	0	
F 1	fixed	54	1	1 ns	77	4	1 ns	5	0	1 ns	4	0	- ns
Mont Blanc	poly	25	1		21	1		20	3		31	0	
F 2	fixed	46	1	0.3399 ns	71	5	1 ns	0	0	- ns	0	0	- ns
Loire	poly	39	3		35	3		34	5		44	2	
F 4	fixed	58	1	0.2023 ns	78	4	0.1682 ns	3	0	0.1429 ns	3	0	- ns
Haute Loire	poly	6	1		2	1		1	3		13	0	
FIN 1	fixed	41	0	0.4605 ns	68	3	1 ns	15	1	1 ns	12	1	0.2453 ns
Lahti	poly	34	1		31	1		30	3		40	0	
GB 1	fixed	42	0	0.0575 ns	69	3	0.2558 ns	0	0	- ns	0	0	- ns
Ascot	poly	42	5		39	5		38	7		46	4	
GB 2	fixed	52	1	0.4609 ns	76	4	1 ns	0	0	- ns	0	0	- ns
Braemar	poly	18	1		14	1		13	3		22	0	
GR 1	fixed	72	0	0.0281*	76	3	0.1111 ns	68	1	0.0014**	62	1	0.4398 ns
Thessaloniki	poly	13	2		9	2		8	4		20	1	
I 1	fixed	83	1	0.2666 ns	82	2	0.2892 ns	73	2	0.0139*	69	2	1 ns
Grosseto	poly	13	1		9	1		8	3		20	0	

Table A35 continue:

Population		CH 3			D 1			D 2			D 3		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
I 2	fixed	80	2	0.001**	79	1	0.0000***	74	3	0.0000***	68	3	0.073 ns
Parma	poly	6	4		2	4		1	6		13	3	
PL 1	fixed	43	0	0.0276*	72	3	0.0747 ns	7	0	0.3506 ns	6	0	1 ns
Krakow	poly	42	6		38	6		37	8		45	5	
PL 2	fixed	26	0	0.5864 ns	1	0	1 ns	8	1	0.4487 ns	6	1	0.2452 ns
Warsaw	poly	116	5		113	5		113	7		119	4	
RUM 1	fixed	41	0	0.0009***	84	4	0.0015**	50	0	0.0000***	48	0	0.0053**
Sibiu 1	poly	15	6		12	6		11	8		23	5	
RUM 2	fixed	42	0	0.0131*	88	3	0.0159*	51	0	0.0001***	49	0	0.087 ns
Sibiu 2	poly	11	3		8	3		7	5		19	2	
RUM 5	fixed	76	2	0.0086**	73	1	0.0001***	75	3	0.0000***	72	3	0.2107 ns
Busteni	poly	7	3		3	3		2	5		14	2	
RUM 6	fixed	75	2	0.3559 ns	70	1	0.1934 ns	72	3	0.0148*	69	3	1 ns
Sinaia	poly	11	1		7	1		6	3		18	0	
RUS 2	fixed	84	3	0.0101*	93	2	0.0001***	81	4	0.0000***	79	4	0.2277 ns
Novosibirsk	poly	6	3		2	3		1	5		13	2	
S 2	fixed	58	1	0.0278*	81	3	0.0078**	0	0	- ns	1	0	1 ns
Stroemstad	poly	5	2		1	2		0	4		12	1	

Population		D 5			D 8			D 9			D 10		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 8	fixed	82	4	0.0082 ns									
Cuxhaven	poly	4	3		.								
D 9	fixed	81	4	0.1194 ns	0	0	- ns						
Bonn	poly	8	2		8	1							
D 10	fixed	78	4	0.0955 ns	0	0	- ns	0	0	- ns			
Cologne	poly	14	3		14	2		15	1				
D 11	fixed	85	3	0.0137*	29	1	0.1761 ns	27	1	1 ns	25	1	1 ns
Wittmoor	poly	2	2		2	1		6	0		12	1	
D 12	fixed	67	3	0.6802 ns	34	0	0.5034 ns	36	0	1 ns	33	0	0.5215 ns
Sonthofen	poly	43	3		43	1		46	1		51	2	
D 14	fixed	90	4	0.0021**	58	2	0.0171*	59	2	0.2819 ns	57	2	0.1637 ns
Steineberg	poly	2	3		2	2		6	1		12	2	
D 15	fixed	55	0	0.0035**	38	2	0.2257 ns	38	2	0.3415 ns	27	2	0.3474 ns
Langenwang	poly	7	12		76	11		80	10		86	11	
DK 3	fixed	78	4	0.0113*	0	0	- ns	0	0	- ns	0	0	- ns
Arhus	poly	9	4		9	3		12	2		17	3	
EST 1	fixed	79	3	0.5958 ns	19	1	1 ns	18	1	0.413 ns	15	1	1 ns
Tallin	poly	25	2		25	1		27	0		33	1	
F 1	fixed	78	4	0.6161 ns	5	0	1 ns	3	0	- ns	1	0	1 ns
Mont Blanc	poly	22	2		21	1		25	0		28	1	
F 2	fixed	72	5	0.7164 ns	0	0	- ns	0	0	- ns	0	0	- ns
Loire	poly	36	4		35	3		37	2		36	3	
F 4	fixed	79	4	0.0356*	3	0	1 ns	3	0	- ns	2	0	1 ns
Haute Loire	poly	3	2		3	1		6	0		12	1	
FIN 1	fixed	69	3	1 ns	14	1	1 ns	14	1	0.3125 ns	12	1	0.4412 ns
Lahti	poly	32	2		32	1		33	0		38	1	
GB 1	fixed	70	3	0.1508 ns	0	0	- ns	0	0	- ns	0	0	- ns
Ascot	poly	40	6		39	5		38	4		44	5	
GB 2	fixed	77	4	0.2779 ns	0	0	- ns	0	0	- ns	0	0	- ns
Braemar	poly	15	2		14	1		18	0		22	1	
GR 1	fixed	77	3	0.03392*	67	1	0.0573 ns	65	1	0.338 ns	62	1	0.1629 ns
Thessaloniki	poly	10	3		10	2		14	1		20	2	
I 1	fixed	83	2	0.0735 ns	72	2	0.3437 ns	70	2	1 ns	68	2	1 ns
Grosseto	poly	10	2		10	1		14	0		20	1	
I 2	fixed	80	1	0.0000***	73	3	0.0006***	71	3	0.0206*	69	3	0.023*
Parma	poly	3	5		3	4		14	1		13	4	

Table A35 continue:

Population		D 5			D 8			D 9			D 10		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
PL 1	fixed	73	3	0.0403*	7	0	0.5779 ns	5	0	1 ns	4	0	1 ns
Krakow	poly	39	7		38	6		41	5		42	6	
PL 2	fixed	1	0	1 ns	7	1	0.3265 ns	8	1	0.3097 ns	6	1	0.2894 ns
Warsaw	poly	115	6		115	5		115	4		117	5	
RUM 1	fixed	84	4	0.0006***	48	0	0.0003***	50	0	0.0019**	47	0	0.0022**
Sibiu 1	poly	13	7		13	6		17	5		23	6	
RUM 2	fixed	88	3	0.0043**	49	0	0.0061**	51	0	0.049*	48	0	0.0281*
Sibiu 2	poly	9	4		9	3		13	2		19	3	
RUM 5	fixed	74	1	0.0002***	75	3	0.0063**	73	3	0.1011 ns	67	3	0.086 ns
Busteni	poly	4	4		4	3		8	2		14	3	
RUM 6	fixed	71	1	0.0379*	72	3	0.3701 ns	70	3	1 ns	64	3	1 ns
Sinaia	poly	8	2		8	1		12	0		18	1	
RUS 2	fixed	94	0	0.0000***	81	4	0.0052**	79	4	0.1042 ns	78	4	0.0836 ns
Novosibirsk	poly	3	4		3	3		7	2		13	3	
S 2	fixed	82	3	0.0016**	0	0	- ns	0	0	- ns	0	0	- ns
Stroemstad	poly	2	3		2	2		6	1		12	2	

Population		D 11			D 12			D 14			D 15		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 12	fixed	37	0	1 ns									
Sonthofen	poly	41	1										
D 14	fixed	59	1	0.0328*	15	1	1 ns						
Steineberg	poly	0	1		41	2							
D 15	fixed	34	1	0.1726 ns	18	1	0.6975 ns	38	2	0.335 ns			
Langenwang	poly	75	10		104	11		75	10				
DK 3	fixed	29	1	0.1274 ns	35	0	0.2674 ns	58	2	0.0186*	38	2	0.3422 ns
Arhus	poly	7	2		48	3		7	3		80	11	
EST 1	fixed	0	0	- ns	31	0	1 ns	51	1	1 ns	29	1	0.4548 ns
Tallin	poly	23	0		60	1		23	1		94	10	
F 1	fixed	26	1	1 ns	33	0	1 ns	55	2	1 ns	38	2	0.5096 ns
Mont Blanc	poly	20	0		58	1		20	1		92	10	
F 2	fixed	21	1	1 ns	29	0	0.5527 ns	49	2	0.646 ns	32	2	0.5249 ns
Loire	poly	34	2		66	3		34	3		103	12	
F 4	fixed	28	1	1 ns	35	0	1 ns	59	2	0.0937 ns	39	2	0.3348 ns
Haute Loire	poly	1	0		42	1		1	1		76	10	
FIN 1	fixed	6	0	- ns	25	0	1 ns	47	1	1 ns	22	1	0.6898 ns
Lahti	poly	30	0		66	1		30	1		103	10	
GB 1	fixed	2	0	1 ns	24	0	0.5704 ns	45	1	0.1881 ns	23	1	0.4687 ns
Ascot	poly	38	4		74	4		38	4		111	13	
GB 2	fixed	24	1	1 ns	33	0	1 ns	57	2	0.4773 ns	34	2	0.509 ns
Braemar	poly	13	0		51	1		13	1		84	10	
GR 1	fixed	66	0	0.12 ns	57	0	0.216 ns	79	1	0.0317*	43	1	0.1028 ns
Thessaloniki	poly	8	1		48	2		8	2		82	11	
I 1	fixed	68	1	1 ns	70	1	1 ns	87	2	0.2533 ns	46	0	0.0175*
Grosseto	poly	8	0		49	1		8	1		83	10	
I 2	fixed	70	2	0.0006***	66	2	0.2189 ns	81	3	0.0001***	48	0	0.0042**
Parma	poly	1	3		42	4		1	4		76	13	
PL 1	fixed	7	0	0.5891 ns	26	0	0.3252 ns	45	1	0.0992 ns	25	1	0.3108 ns
Krakow	poly	37	5		71	5		37	5		107	14	
PL 2	fixed	3	0	1 ns	12	0	1 ns	27	1	1 ns	7	0	1 ns
Warsaw	poly	113	4		139	5		113	5		170	12	
RUM 1	fixed	52	0	0.0004***	15	0	0.3462 ns	20	1	0.0314*	33	1	0.0692 ns
Sibiu 1	poly	11	5		49	5		11	6		83	15	
RUM 2	fixed	53	0	0.019*	15	0	1 ns	20	1	0.0868 ns	35	1	0.1088 ns
Sibiu 2	poly	7	2		46	2		7	3		80	12	
RUM 5	fixed	69	2	0.0125*	61	2	0.6482 ns	81	3	0.0017**	51	1	0.0313*
Busteni	poly	2	2		43	3		2	3		76	12	

Table A35 continue:

Population		D 11			D 12			D 14			D 15		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM 6	fixed	67	2	1 ns	60	2	1 ns	80	3	0.2808 ns	49	1	0.0968 ns
Sinaia	poly	6	0		46	1		6	1		81	10	
RUS 2	fixed	81	3	0.0078**	67	3	0.6774 ns	94	4	0.0087**	22	0	0.1159 ns
Novosibirsk	poly	1	2		42	3		1	2		76	11	
S 2	fixed	30	1	0.0625 ns	36	0	0.4976 ns	50	2	0.003**	39	2	0.3343 ns
Stroemstad	poly	0	1		41	2		0	2		75	10	

Population		DK 3			EST 1			F 1			F 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
EST 1	fixed	19	1	1 ns									
Tallin	poly	28	2										
F 1	fixed	2	0	1 ns	19	1	0.3509 ns						
Mont Blanc	poly	26	2		37	0							
F 2	fixed	0	0	- ns	15	1	1 ns	0	0	- ns			
Loire	poly	37	4		48	2		39	2				
F 4	fixed	3	0	1 ns	18	1	0.4419 ns	4	0	- ns	1	0	1 ns
Haute Loire	poly	7	2		24	0		21	0		34	2	
FIN 1	fixed	14	1	1 ns	0	0	- ns	15	1	0.254 ns	9	1	0.3843 ns
Lahti	poly	35	2		42	0		47	0		56	2	
GB 1	fixed	0	0	- ns	0	0	- ns	1	0	1 ns	0	0	- ns
Ascot	poly	42	6		48	4		54	4		60	6	
GB 2	fixed	0	0	- ns	15	1	0.3333 ns	3	0	- ns	0	0	- ns
Braemar	poly	15	2		32	0		31	0		39	2	
GR 1	fixed	63	1	0.0316*	58	0	0.3483 ns	63	1	1 ns	57	1	0.31588 ns
Thessaloniki	poly	15	3		30	1		28	1		42	3	
I 1	fixed	69	2	0.1667 ns	61	1	1 ns	69	2	1 ns	64	3	1 ns
Grosseto	poly	15	2		29	0		26	0		40	2	
I 2	fixed	69	3	0.0017**	61	2	0.157 ns	67	3	0.3324 ns	63	4	0.2897 ns
Parma	poly	5	8		24	3		21	3		35	5	
PL 1	fixed	6	0	0.5889 ns	0	0	- ns	5	0	1 ns	3	0	1 ns
Krakow	poly	43	7		50	5		52	5		60	7	
PL 2	fixed	7	1	0.3683 ns	0	0	- ns	8	1	0.2899 ns	6	1	0.3036 ns
Warsaw	poly	115	6		117	4		125	4		129	6	
RUM 1	fixed	48	0	0.0003***	45	0	0.0187*	47	0	0.013*	41	0	0.0309*
Sibiu 1	poly	18	7		34	5		31	5		44	6	
RUM 2	fixed	49	0	0.004**	46	0	0.1652 ns	48	0	0.1388 ns	42	0	0.1166 ns
Sibiu 2	poly	14	4		30	2		27	2		40	4	
RUM 5	fixed	73	3	0.0079**	64	2	0.5768 ns	70	3	0.5947 ns	65	3	0.4184 ns
Busteni	poly	9	4		25	2		22	2		35	4	
RUM 6	fixed	70	3	0.1998 ns	62	2	1 ns	67	3	0.5605 ns	61	4	1 ns
Sinaia	poly	13	2		26	0		26	0		39	2	
RUS 2	fixed	79	4	0.0081**	76	3	0.5954 ns	75	4	0.615 ns	74	4	0.438 ns
Novosibirsk	poly	8	4		24	2		21	2		35	4	
S 2	fixed	0	0	- ns	20	1	1 ns	5	0	1 ns	0	0	- ns
Stroemstad	poly	7	3		23	1		20	1		34	3	
FIN 1	fixed	14	1	0.3333 ns									
Lahti	poly	30	0										
GB 1	fixed	1	0	1 ns	1	0	1 ns						
Ascot	poly	38	4		51	4							
GB 2	fixed	3	0	- ns	9	1	0.1961 ns	0	0	- ns			
Braemar	poly	13	0		41	0		43	4				
GR 1	fixed	68	1	0.2386 ns	52	0	0.4286 ns	52	0	0.0254*	61	1	0.4576 ns
Thessaloniki	poly	9	1		38	1		45	5		21	1	
I 1	fixed	72	2	1 ns	58	1	1 ns	58	1	0.1712 ns	68	2	1 ns
Grosseto	poly	9	0		37	0		44	4		21	0	
I 2	fixed	75	3	0.0021**	61	2	0.3354 ns	58	2	0.0385*	65	3	0.0913 ns
Parma	poly	2	3		30	3		39	7		14	3	

Table A35 continue:

Population		F 4			FIN 1			GB 1			GB 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
PL 1	fixed	5	0	1 ns	1	0	1 ns	0	0	- ns	6	0	1 ns
Krakow	poly	38	5		56	5		56	4		44	5	
PL 2	fixed	7	1	0.2856 ns	0	0	- ns	0	0	- ns	4	1	0.1833 ns
Warsaw	poly	113	4		113	4		126	8		119	4	
RUM 1	fixed	51	1	0.0006***	41	0	0.0574 ns	37	0	0.0205*	46	0	0.0069**
Sibiu 1	poly	12	5		41	5		49	8		24	5	
RUM 2	fixed	52	0	0.0238*	42	0	0.2287 ns	38	0	0.0669 ns	47	0	0.0985 ns
Sibiu 2	poly	8	2		37	2		45	5		20	2	
RUM 5	fixed	74	3	0.0279*	56	2	0.6245 ns	59	2	0.0724 ns	70	3	0.2372 ns
Busteni	poly	3	2		32	2		40	6		15	2	
RUM 6	fixed	71	3	1 ns	54	2	0.5214 ns	57	2	0.4007 ns	68	3	1 ns
Sinaia	poly	7	0		35	0		42	4		18	0	
RUS 2	fixed	81	4	0.0216*	72	3	0.6402 ns	69	3	0.2558 ns	77	4	0.2567 ns
Novosibirsk	poly	2	2		31	2		39	5		14	2	
S 2	fixed	3	0	0.4 ns	15	1	1 ns	0	0	- ns	0	0	- ns
Stroemstad	poly	1	1		30	1		38	5		13	1	

Population		GR 1			I 1			I 2			PL 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
I 1	fixed	59	1	0.3951 ns									
Grosseto	poly	16	1										
I 2	fixed	64	2	0.0058**	54	1	0.0164*						
Parma	poly	9	4		9	3							
PL 1	fixed	55	0	0.0099**	53	1	0.097 ns	59	2	0.0182*			
Krakow	poly	44	6		43	5		38	8				
PL 2	fixed	29	0	0.5842 ns	32	0	0.5807 ns	34	0	0.2104 ns	59	1	0.3951 ns
Warsaw	poly	120	5		118	4		113	7		16	1	
RUM 1	fixed	76	0	0.0001***	85	2	0.005**	82	3	0.0001***	64	2	0.0058**
Sibiu 1	poly	19	6		19	5		12	8		9	4	
RUM 2	fixed	78	0	0.0057**	88	1	0.0663 ns	85	2	0.0003***	55	0	0.0099**
Sibiu 2	poly	15	3		15	2		8	5		44	6	
RUM 5	fixed	80	2	0.0174*	70	3	0.1439 ns	77	2	0.0000***	29	0	0.5842 ns
Busteni	poly	10	3		10	2		3	5		120	5	
RUM 6	fixed	78	2	0.4064 ns	69	3	1 ns	75	2	0.00997**	76	0	0.0001***
Sinaia	poly	14	1		14	0		7	3		19	6	
RUS 2	fixed	76	3	0.0285*	79	2	0.0687 ns	88	1	0.0000***	78	0	0.0057**
Novosibirsk	poly	9	3		9	2		2	5		15	3	
S 2	fixed	68	1	0.0408*	73	2	0.2913 ns	74	3	0.0001***	80	2	0.0174*
Stroemstad	poly	8	2		8	1		1	4		10	3	

Population		PL 2			RUM 1			RUM 2			RUM 5		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM 1	fixed	23	0	0.3567 ns									
Sibiu 1	poly	121	9										
RUM 2	fixed	24	0	0.5899 ns	0	0	- ns						
Sibiu 2	poly	119	6		11	5							
RUM 5	fixed	28	4	1 ns	82	2	0.0001***	83	2	0.0025**			
Busteni	poly	114	6		12	7		8	4				
RUM 6	fixed	25	0	0.6026 ns	78	3	0.0105*	81	2	0.1098 ns	1	0	1 ns
Sinaia	poly	116	4		17	5		13	2		8	2	
RUS 2	fixed	51	1	0.4439 ns	86	3	0.0002***	89	3	0.0031**	78	1	0.0001***
Novosibirsk	poly	114	6		12	7		8	4		3	4	
S 2	fixed	8	1	0.314 ns	50	0	0.0001***	51	0	0.0033**	75	2	0.0011**
Stroemstad	poly	113	4		11	6		7	3		2	3	

Table A35 continue:

Population		RUM 6			RUS 2		
		syn.	nons.	sign.	syn.	nons.	sign.
RUS 2	fixed	79	1	0.0261*			
Novosibirsk	poly	7	2				
S 2	fixed	72	2	0.2402 ns	81	1	0.0007***
Stroemstad	poly	6	1		1	3	

Table A36: Neutrality indices of *Achipteria coleoptrata* computed in the McDonald-Kreitman test with DnaSP v5 for 35 locations. Locations with less than two individuals were excluded.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	**A 5**																																		
2	**A 7**	-																																	
3	**A 8**	-	-																																
4	**CH 1**	-	-	-																															
5	**CH 3**	-	-	-	-																														
6	**D 1**	-	1	-	9.3	9.6																													
7	**D 2**	1.9	5.5	1.8	29	46.4	81																												
8	**D 3**	0.9	0	0.8	2.8	3.4	1.6	-																											
9	**D 5**	-	1.9	-	12.8	12.4	-	51.3	2.8																										
10	**D 8**	1.2	1.6	1.1	11.2	16	13.5	-	-	15.4																									
11	**D 9**	1.0	0	0.9	4.1	5.2	2.9	-	-	5.1	-																								
12	**D 10**	1.1	1.3	0.9	5.4	6.4	3.0	-	-	4.2	-	-																							
13	**D 11**	-	-	-	-	-	28	-	0	28.3	14.5	0	2.1																						
14	**D 12**	-	-	-	-	-	1.1	-	-	1.6	-	-	-	-																						
15	**D 14**	1.4	1.3	1.3	10.3	16.4	45	-	2.4	33.8	29	4.9	1.2	-	0.0																					
16	**D 15**	-	2.1	-	-	3.5	-	3.4	2.3	-	2.8	2.4	4.8	-	0.7	2.5																			
17	**DK 3**	1.5	3.1	1.4	11.2	9.5	7.2	-	-	8.7	-	-	2.4	4.5	1.9	12.4	2.6																		
18	**EST 1**	-	-	-	-	-	1.1	2.6	0	2.1	0.76	0	-	8.3	-	2.2	3.1	1.4																	
19	**F 1**	0.7	0	0.6	1.9	2.2	0.9	-	-	1.8	-	-	0.5	-	-	1.4	2.1	-	0																
20	**F 2**	0.9	1.6	0.8	3.3	3.5	1.2	-	-	1.6	-	-	-	0	-	2.2	1.9	-	0.6	-															
21	**F 4**	1.2	0	1.0	6.4	9.7	9.8	-	-	13.2	-	-	-	1.2	-	29.5	2.6	-	0	-	-														
22	**FIN 1**	-	-	-	-	-	0.7	1.5	0	1.4	0.4	0	-	0	-	1.6	2.1	0.8	-	0	0.3	0													
23	**GB 1**	-	-	-	-	-	2.9	-	-	3.5	-	-	0.3	-	-	4.7	2.7	-	-	-	-	-	-												
24	**GB 2**	0.8	0	0.7	2.4	2.9	1.4	-	-	2.6	-	-	-	-	-	2.2	2	-	0	-	-	-	0	-											
25	**GR 1**	-	-	-	-	-	5.63	34	3.1	7.7	13.4	4.6	-	0	-	19.8	5.8	12.6	-	2.3	4.1	7.6	-	-	2.9										
26	**I 1**	-	0	-	-	6.4	4.6	13.7	0	8.3	3.6	0	6.2	-	-	5.4	-	4.6	0	0	1.1	0	0	5.3	0	3.7								
27	**I 2**	-	4.0	-	35.6	26.7	158	148	5.2	133.3	32.4	10.1	1.7	0	3.1	5.4	-	4.6	0	0	1.1	0	3.1	5.2	4.6	14.2	18							
28	**PL 1**	-	-	-	-	-	3.8	-	-	4.4	-	-	7.1	105	-	108	-	14.4	3.8	3.2	2.3	37.5	-	-	-	-	6.2	6.2						
29	**PL 2**	-	-	-	-	-	-	0.5	0.2	-	0.3	0.3	-	-	-	6.1	3.3	-	-	-	-	-	-	-	0.1	-	-	-	-					
30	**RUM 1**	-	-	-	-	-	10.5	-	-	11.3	-	-	0.3	-	-	1.2	-	0.4	-	0.3	0.3	0.2	-	-	-	-	11.2	18.2	-	-				
31	**RUM 2**	-	-	-	-	-	11	-	-	13.0	-	-	-	-	-	10.9	6	-	-	-	-	-	-	-	-	-	11.7	26.6	-	-	-			
32	**RUM 5**	2.6	2.5	2.3	11.4	16.3	73	62.5	3.4	74	18.8	6.1	-	-		8.6	5.3	-	-	-	-	-	1.8	4.4	3.1	12	4.7	64.2	5.2	1.5	23.9	18.4		
33	**RUM 6**	1.6	0	1.3	2.9	3.4	10	12	0	17.8	3	0	4.8	34.5	2.1	40.5	8.1	10.8	2.6	2.1	2.5	16.4	0	2.7	0	2.8	0	16.1	3.3	-	7.6	6.2	-	
34	**RUS 2**	2.5	1.9	2.2	14	14	-	101.3	3.0	-	20.3	5.6	1.2	0	0.7	4.4	6	3.6	0	0	0.8	0	1.5	2.9	2.8	8.4	8.8	220	3.7	2.7	16.7	14.8	104	22.6
35	**S 2**	1.4	1.8	1.2	14.5	23.2	54	-	-	41	-	-	4.5	54	1.6	47	-	9.9	2.1	1.8	2.1	20.3	0.5	-	-	17	4.6	98.7	-	0.3	-	-	56.3	6	81

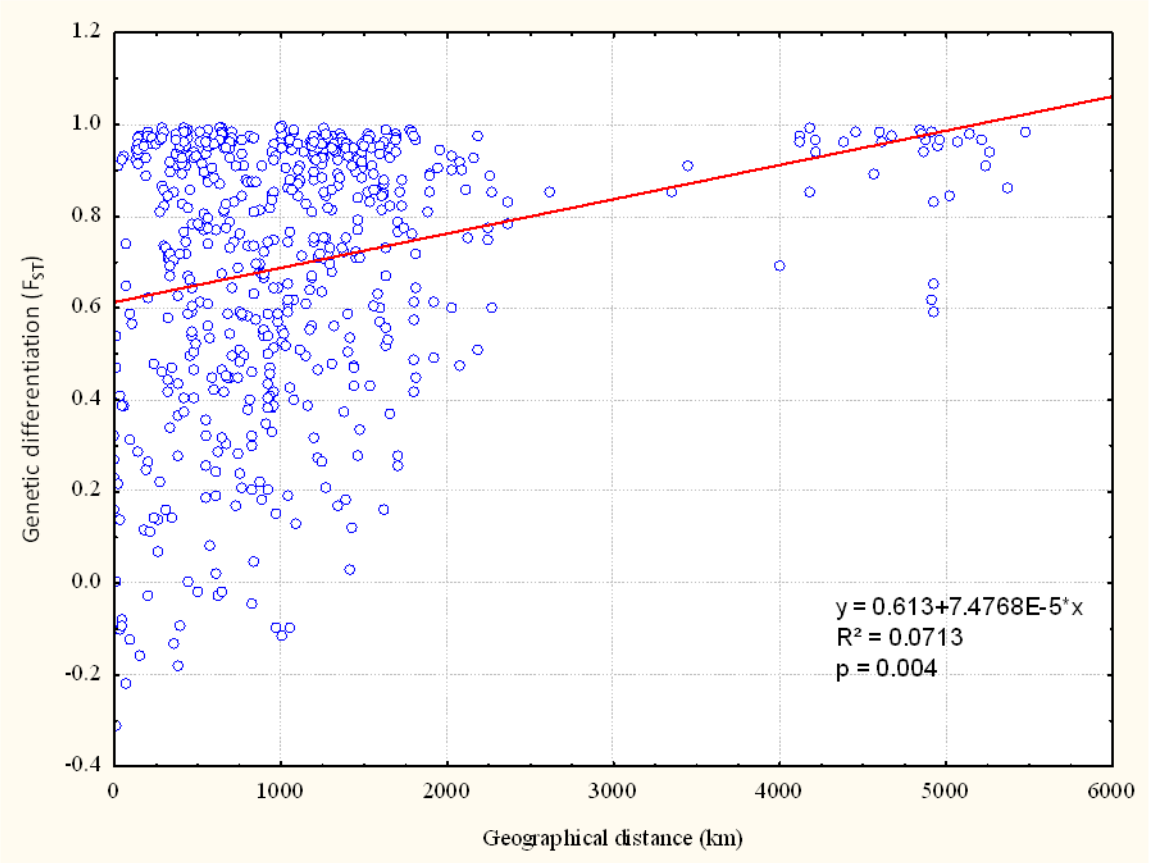

Figure A49: Linear regression of geographical distances (kilometers) vs. genetic differentiations (F_{ST}) based on *COI* of *Achipteria coleoptrata*. Regression is significant (p=0.004**; (*0.01<P<0.05; **0.001<P<0.01; ***P<0.001)) using 1000 randomizations.

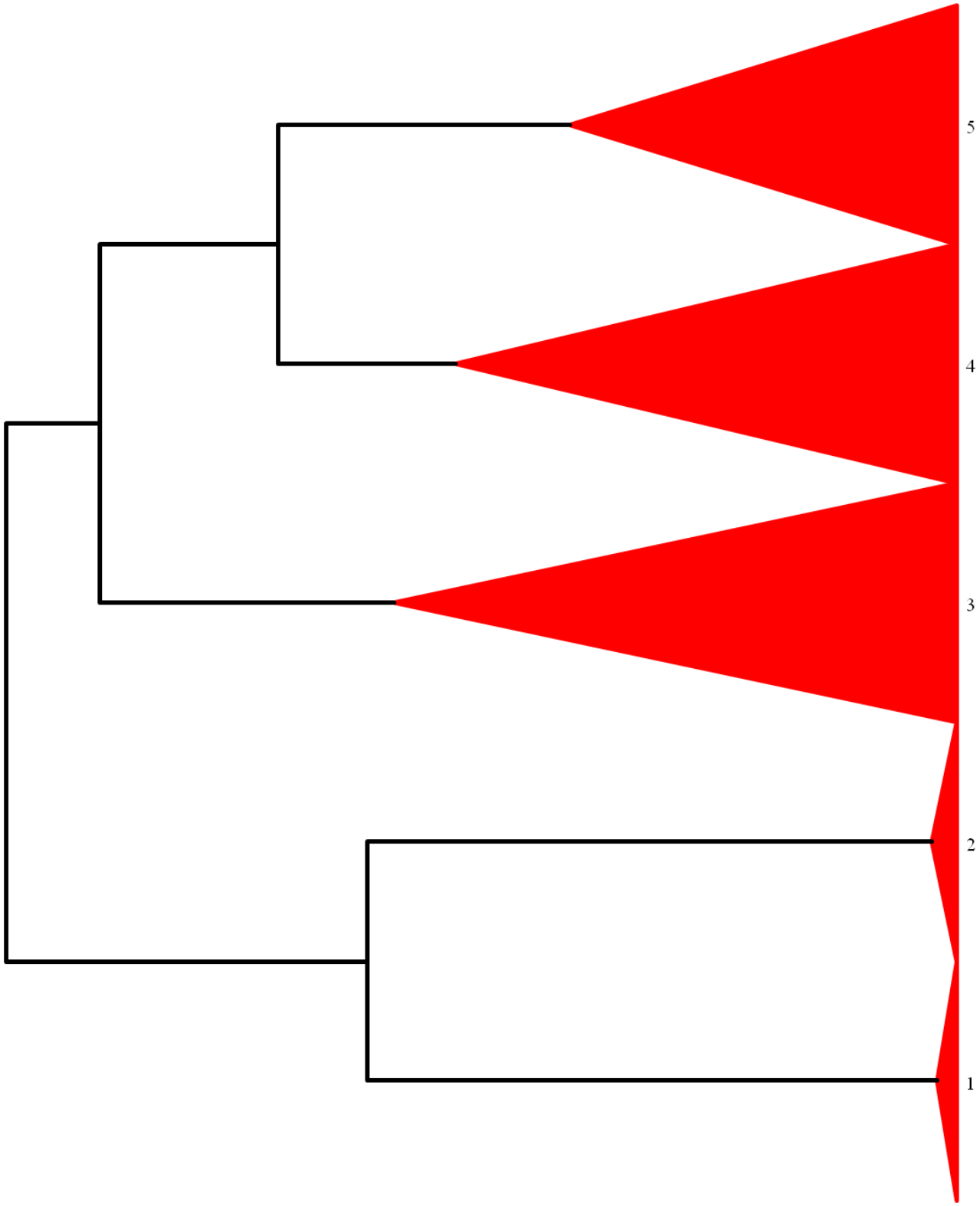

Figure A50: Cluster delimitation of *COI* nucleotide sequences from *Achipteria coleoptrata* after the single method of the generalized mixed Yule coalescent method (GMYC). Red branches are the different distinct clusters. Tip labels are explained in Table 24.

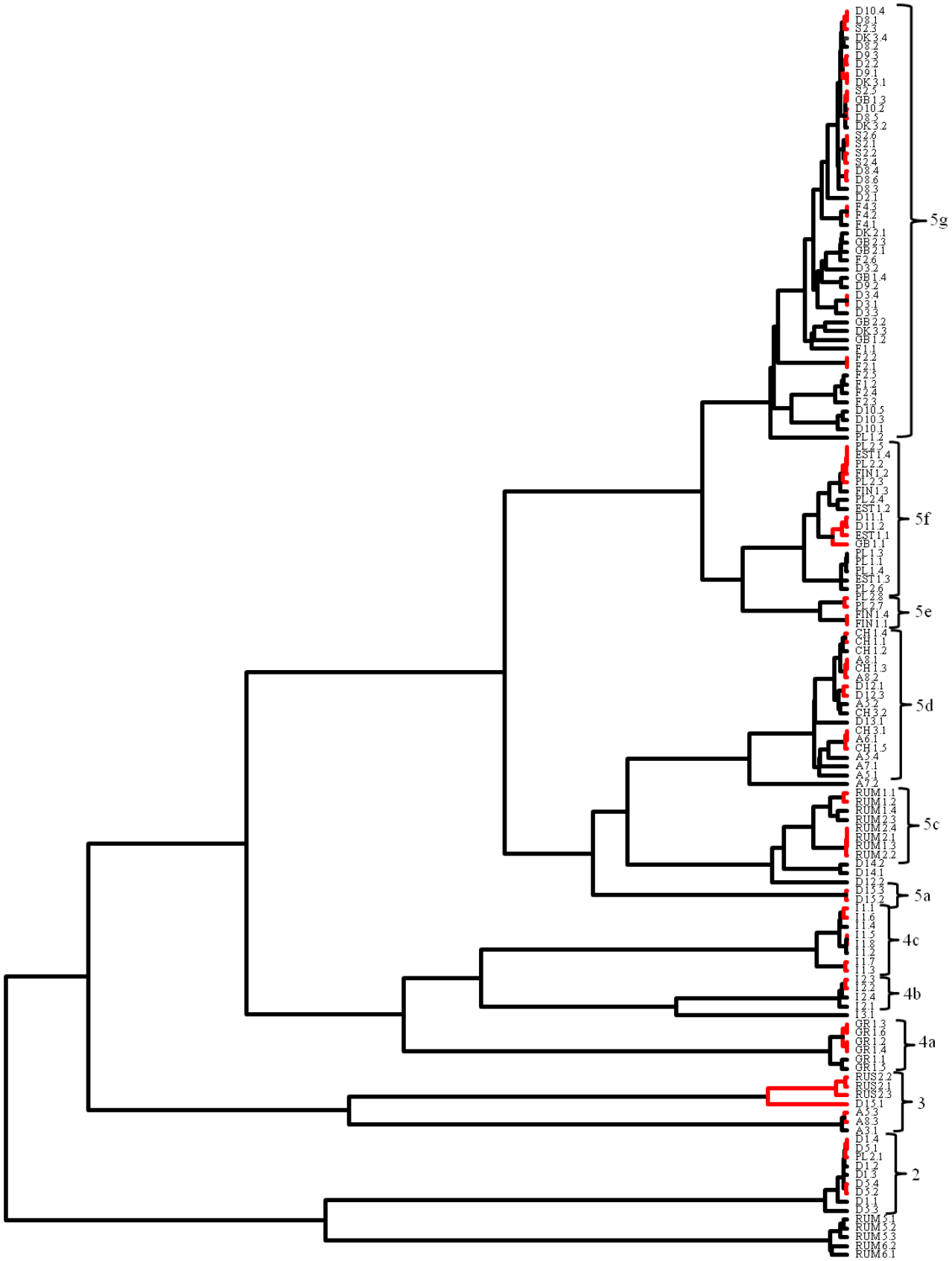

Figure A51: Cluster delimitation of *COI* nucleotide sequences from *Achipteria coleoptrata* after the multiple method of the generalized mixed Yule coalescent method (GMYC). Red branches are the different distinct clusters. Tip labels are explained in Table 24.

Table A37: Standard diversity measures of *Steganacarus magnus*. Populations with less than two individuals were excluded.

population	sample size n	invarible sites N_{is}	variable sites N_{vs}	parsimony inform. sites N_{pars}	number of singeltons N_s	number of haplotypes N_h	haplotype diversity H_d	variance	nucleotide diversity Π_n
A_1	2	530	0	0	0	1	0	0	0
CZ_1	5	358	172	35	137	4	0.9	0.026	0.161
D_1	14	503	27	12	15	8	0.77	0.014	0.012
D_2	9	524	6	1	5	5	0.69	0.022	0.003
D_3	3	522	8	0	8	3	1	0.074	0.01
D_4	9	336	194	128	66	5	0.83	0.01	0.163
D_5	5	336	194	51	143	3	0.7	0.048	0.183
D_7	4	397	133	96	37	3	0.83	0.049	0.159
D_8	3	528	2	0	2	2	0.67	0.099	0.003
D_9	4	518	12	2	10	3	0.833	0.049	0.012
DK_1	3	391	139	0	139	3	1	0.074	0.179
DK_2	5	530	0	0	0	1	0	0	0
DK_3	3	529	1	0	1	2	0.67	0.099	0.001
F_1	6	339	191	50	151	3	0.6	0.046	0.154
F_2	4	528	2	0	2	3	0.83	0.049	0.002
F_3	5	459	71	0	71	4	0.9	0.026	0.054
F_4	2	530	0	0	0	1	0	0	0
FIN_1	3	527	3	0	3	2	0.67	0.099	0.004
GB_1	2	528	2	0	2	2	1	0.25	0.004
GB_2	3	525	5	0	5	2	0.67	0.099	0.006
I_1	10	520	10	8	2	4	0.71	0.014	0.009
I_2	4	517	13	2	11	4	1	0.031	0.013
N_1	3	387	143	0	143	2	0.67	0.099	0.18
NL_1	3	521	9	0	9	3	1	0.074	0.011
PL_1	6	387	143	136	7	6	1	0.009	0.143
PL_2	3	515	15	0	15	2	0.67	0.099	0.019
RUM_1	3	527	3	0	3	2	0.67	0.099	0.004
RUM_2	4	517	13	3	10	4	1	0.031	0.013
RUM_3	4	512	18	0	18	2	0.5	0.073	0.017
RUM_4	3	523	7	0	7	3	1	0.074	0.009
RUM_5	4	523	7	1	6	4	1	0.031	0.007
RUM_6	3	437	93	0	93	3	1	0.074	0.118
RUS_1	4	402	128	4	124	4	1	0.031	0.125
RUS_2	4	530	0	0	0	1	0	0	0
S_2	3	337	193	0	193	3	1	0.074	0.257
all	155	216	314	276	38	93	0.98	0	0.21

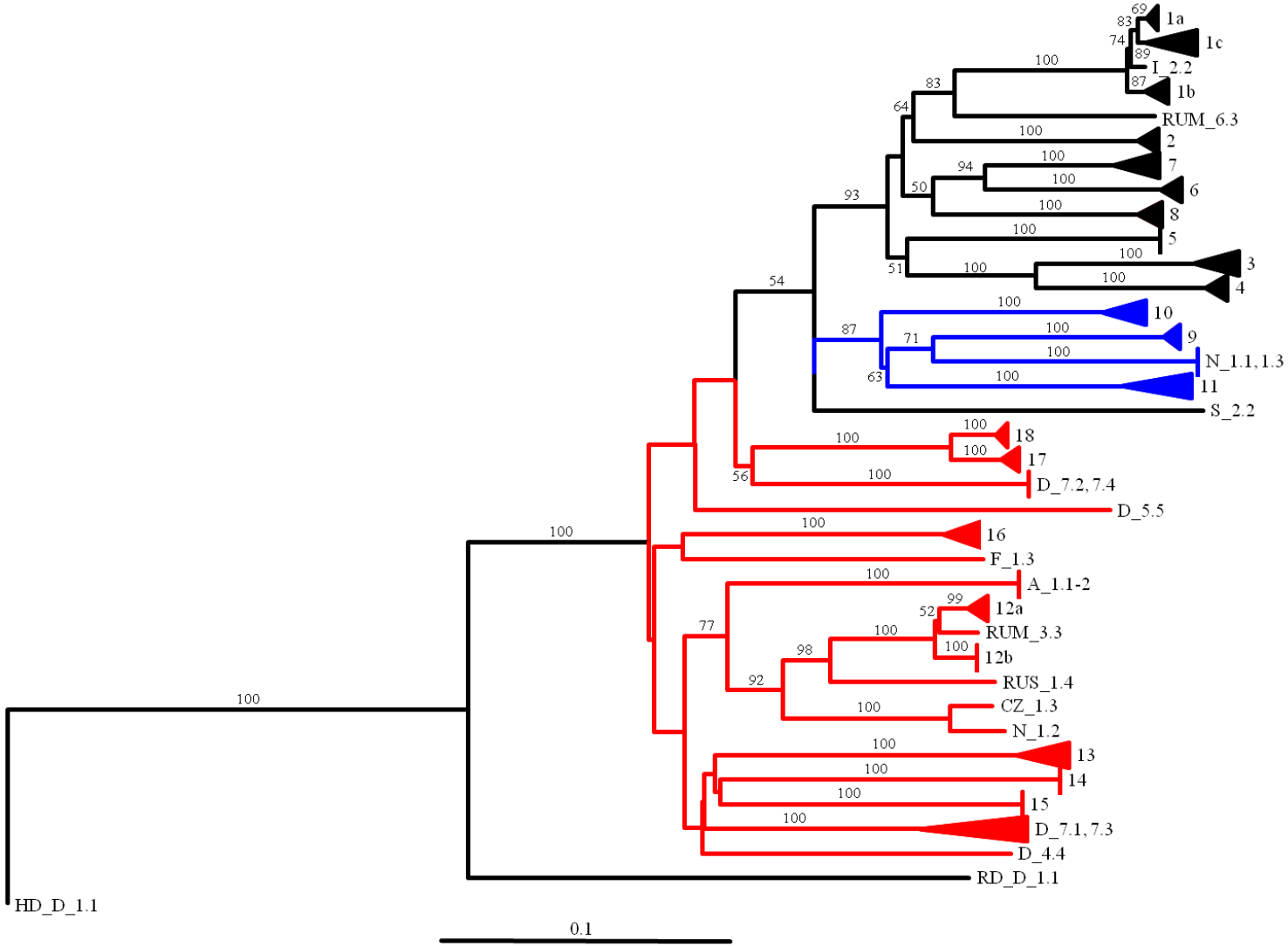

Figure A52: Neighbor-Joining tree of 157 *COI* nucleotide sequences of *Steganacarus magnus*. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Coloured branches show the three different main clades (black, blue, red). Tip numbers are the different subclades and explain in Table 28.

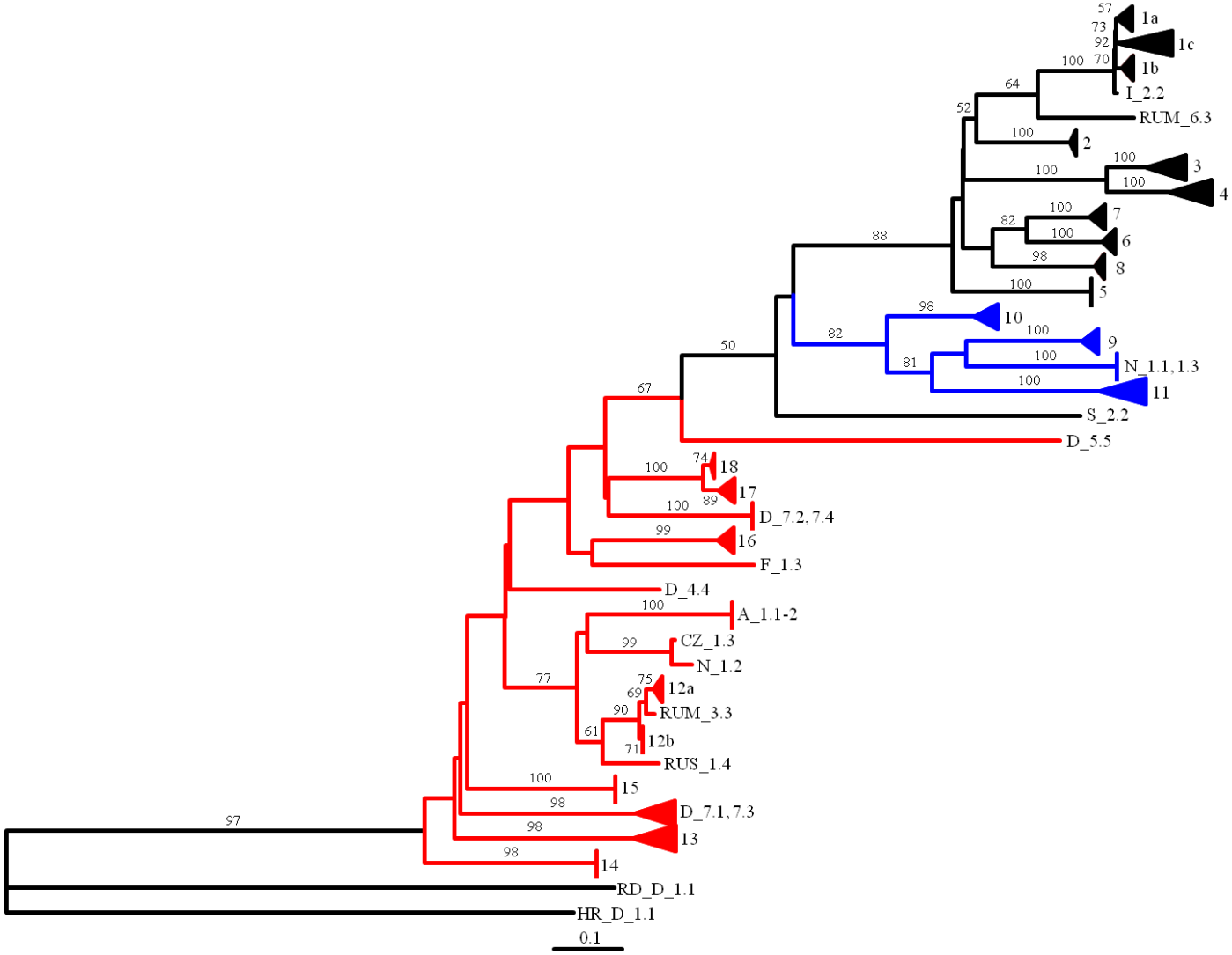

Figure A53: Neighbor-Joining tree of 157 *COI* nucleotide sequences of *Steganacarus magnus* with model of sequence evolution GTR+I+G. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Coloured branches show the three different main clades (black, blue, red). Tip numbers are the different subclades and explain in Table 28.

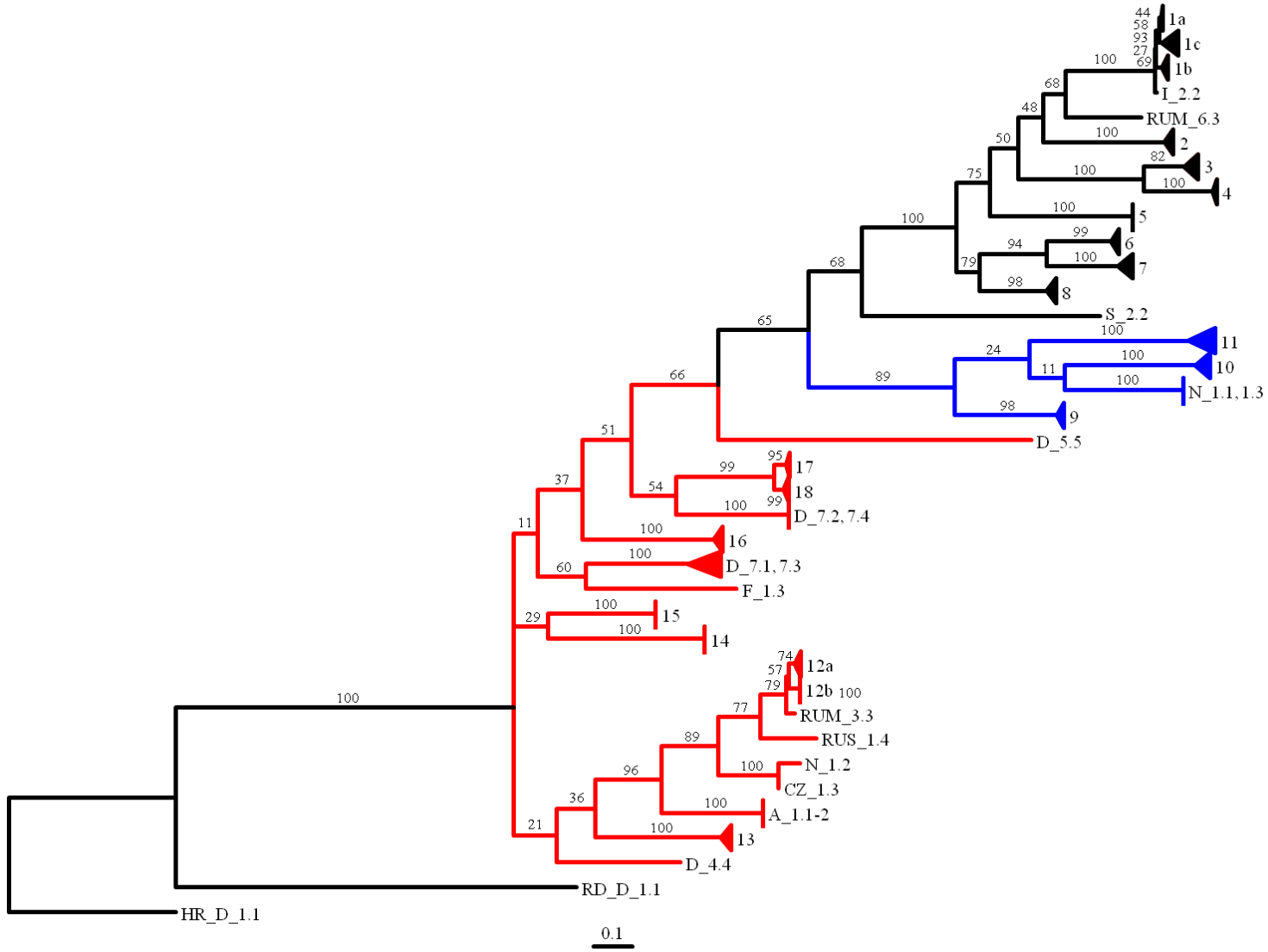

Figure A54: Maximum likelihood tree of 157 *COI* nucleotide sequences of *Steganacarus magnus* with model of sequence evolution GTR+I+G. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1) Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Coloured branches show the three different main clades (black, blue, red). Tip numbers are the different subclades and explain in Table 28.

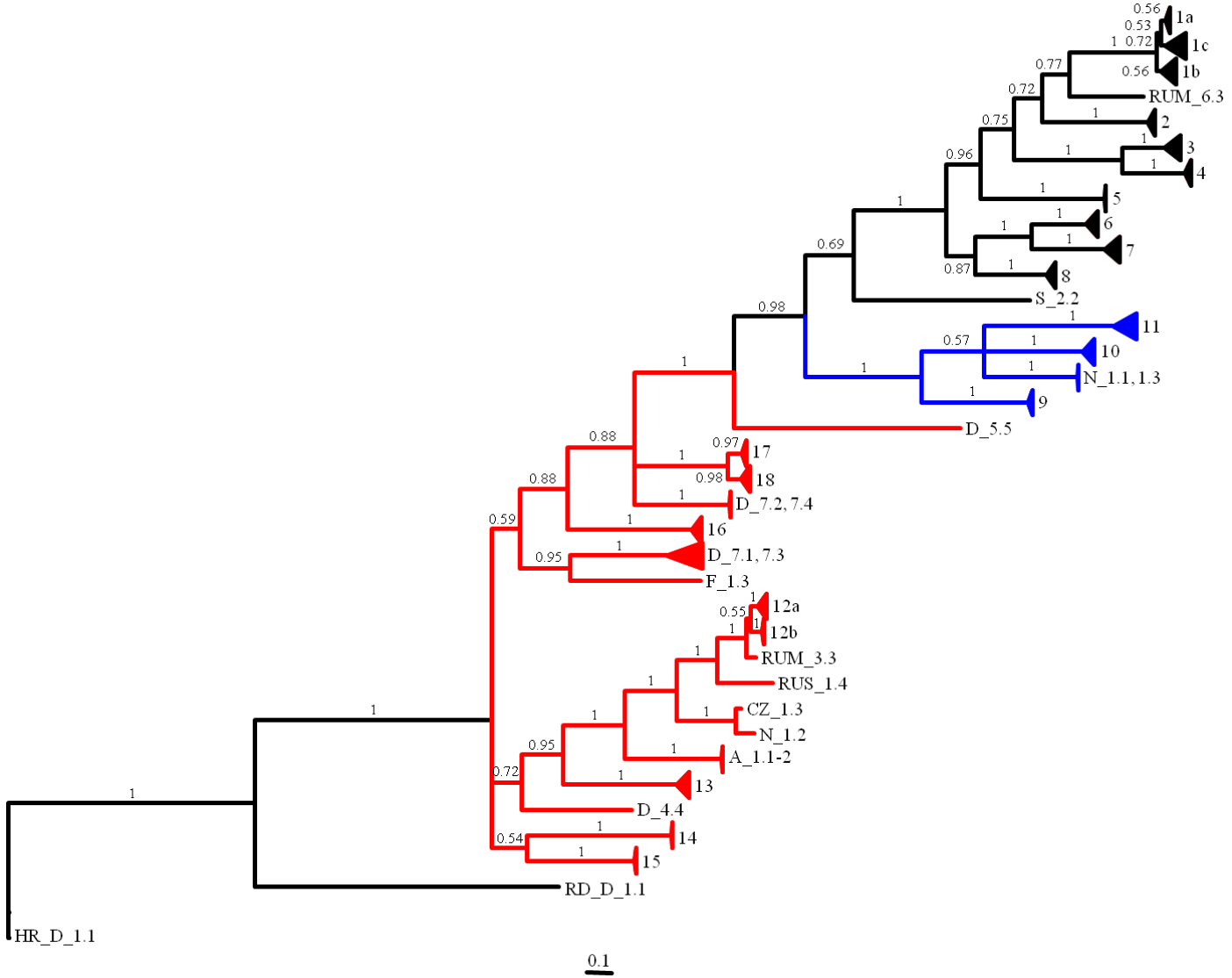

Figure A55: Bayesian tree from the nucleotide of *Steganacarus magnus* after $10x10^6$ generations. Split frequencies of 0.024252 and burnin of 25%. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are posterior probabilities. Coloured branches show the three different main clades (black, blue, red). Tip numbers are the different subclades and explain in Table 28.

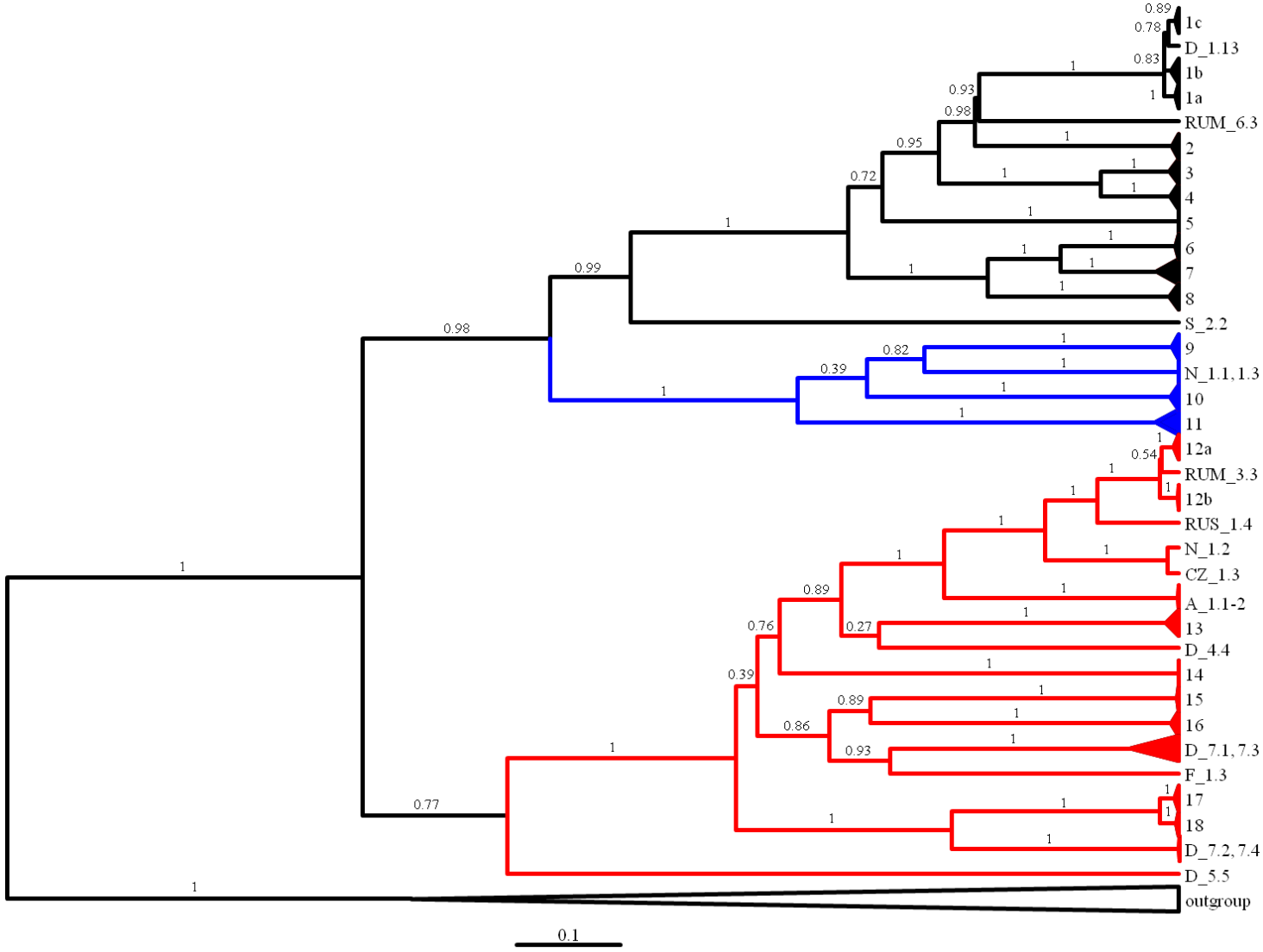

Figure A56: Bayesian phylogeny approach from the nucleotide of *Steganacarus magnus* after $10x10^6$ generations with Beast v1.5.4. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are posterior probabilities. Coloured branches show the three different main clades (black, blue, red). Tip numbers are the different subclades and explain in Table 28.

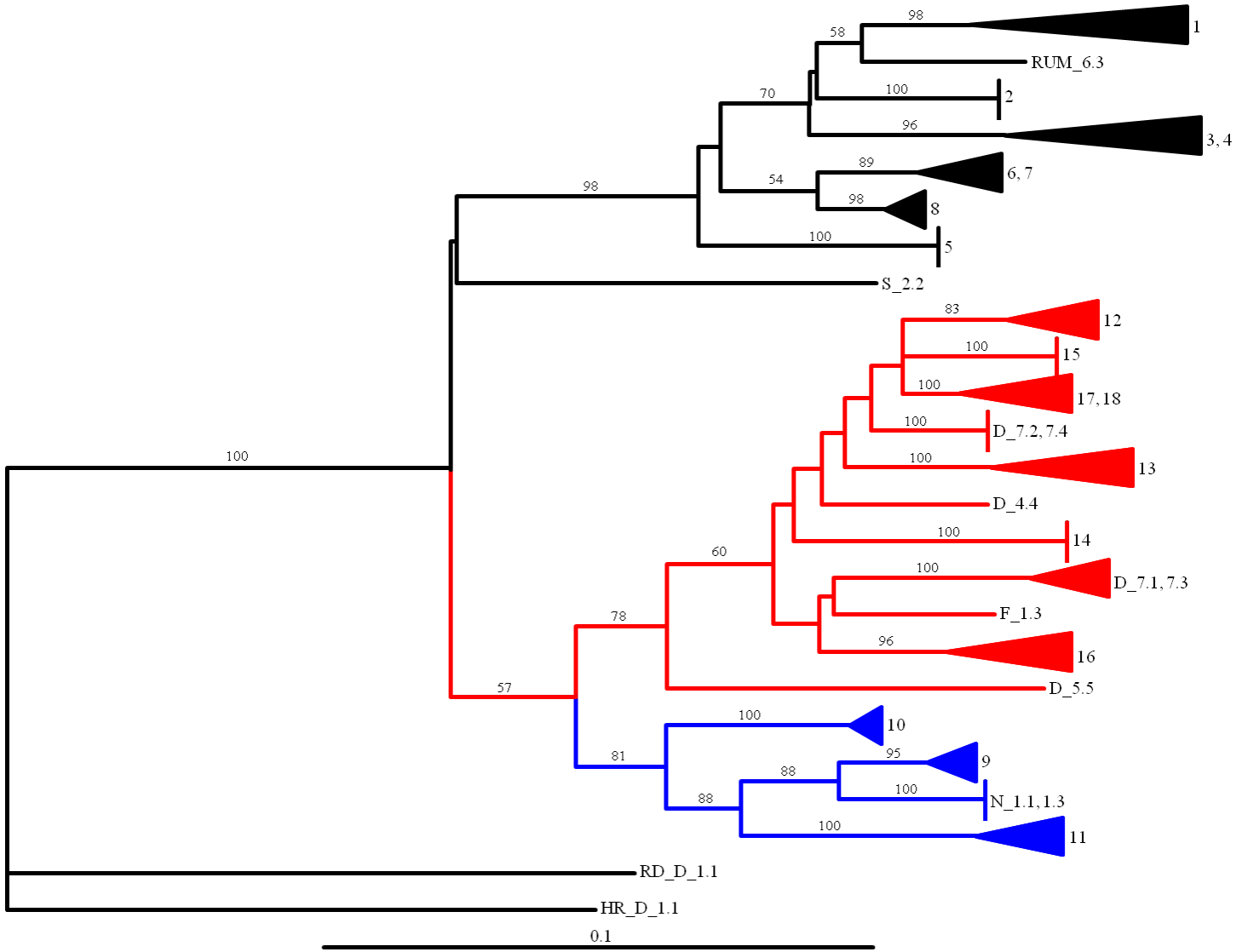

Figure A57: Neighbor-Joining tree of 157 *COI* protein sequences of *Steganacarus magnus*. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are bootstrap values of 100,000 pseudo-replicates. Coloured branches show the three different main clades (black, blue, red). Tip numbers are the different subclades and explain in Table 28.

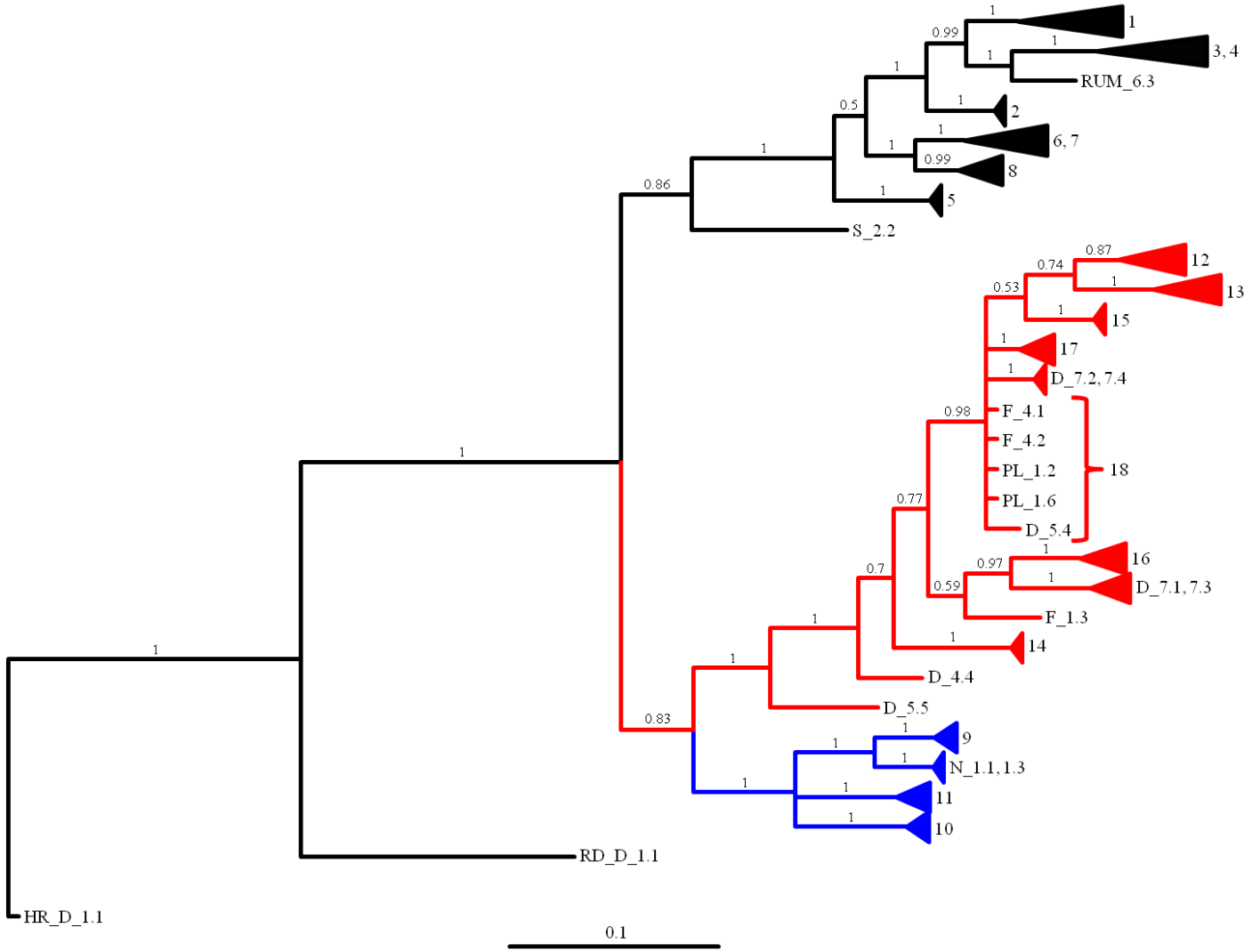

Figure A58: Bayesian tree from the protein of *Steganacarus magnus* after $10x10^6$ generations with the model equalin, split frequencies of 0.006509 and burnin of 25%. are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers show the posterior probabilities. Coloured branches show the three different main clades (black, blue, red). Tip numbers are the different subclades and explain in Table 28.

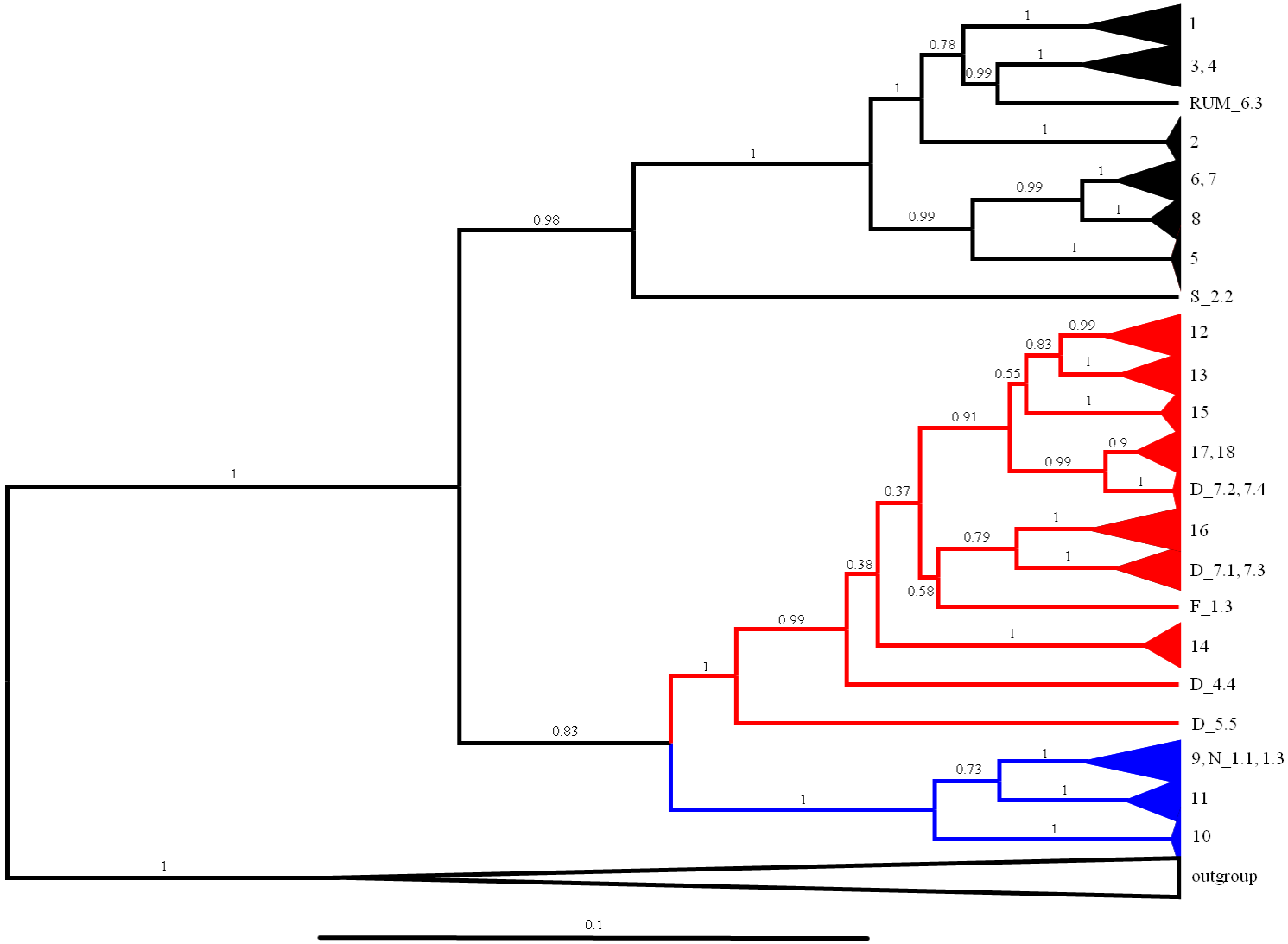

Figure A59: Bayesian tree from the protein of *Steganacarus magnus* after $10x10^6$ generations. Split frequencies of 0.024252 and burnin of 25%. Outgroups are *Hypochthonius rufulus* (HD_D_1) and *Rhysotritia duplicata* (RD_D_1.1). Numbers on the branches are posterior probabilities. Coloured branches show the three different main clades (black, blue, red). Tip numbers are the different subclades and explain in Table 28.

Appendix

Table A38: Mean pairwise percentage differences of uncorrected p-distances of the protein of *Steganacarus magnus* from 35 locations. The diagonal is the within population differences (bold) and below the diagonal the among population differencess. Bold letters in red are the minimum and maximum differences within and among the populations. Bold letters in pink is the maximum divergences, if Russia is excluded. Locations with less than two individuals were excluded from the analysis.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	A 1	0																																		
2	CZ 1	1.8	**1.1**																																	
3	D 1	2.9	1.7	**0.2**																																
4	D 2	2.8	1.6	0.1	0																															
5	D 3	4	2.7	1.3	1.1	0																														
6	D 4	1.6	1.2	2.3	2.2	2.7	**1.2**																													
7	D 5	2.5	1.7	1.5	1.4	2.7	2	2																												
8	D 7	2.1	1.6	2.8	2.7	3.8	1.5	2.1	**1**																											
9	D 8	3	2.7	1.5	1.3	1.3	3.1	2.6	3.5	**0.4**																										
10	D 9	2.8	1.6	0.1	0	1.1	2.2	1.4	2.7	1.3	0																									
11	DK 1	2.7	1.4	1.5	1.3	1.7	1.5	1.9	2.3	2.7	1.3	**1.5**																								
12	DK 2	0.6	1.6	2.9	2.8	4	1.4	2.3	1.3	3	2.8	2.7	0																							
13	DK 3	2.8	1.6	0.1	0	1.1	2.2	1.4	2.7	1.3	0	1.3	2.8	0																						
14	F 1	1.5	1.9	2.6	2.5	4	1.7	2.3	2.1	3.8	2.5	2.5	1	2.5	**1.4**																					
15	F 2	2.8	1.6	0.1	0	1.1	2.2	1.4	2.7	1.3	0	1.3	2.8	0	2.5	0																				
16	F 3	3.1	2.7	1.5	1	1.4	3.1	2.6	3.5	0.4	1.4	2.7	3.1	1.4	3.8	1.4	**0.5**																			
17	F 4	0.6	1.6	2.9	2.8	4	1.4	2.3	1.3	3	2.8	2.7	0	2.8	1	2.8	3.1	0																		
18	FIN 1	1.1	1.5	2.4	2.3	3.4	1.3	1.9	1	2.5	2.3	2.1	0.6	2.3	1.5	2.3	2.5	0.6	0																	
19	GB 1	2.8	2.5	1.3	1.1	1.1	2.9	2.4	3.3	0.2	1.1	2.5	2.8	1.1	3.6	1.1	0.2	2.8	2.3	0																
20	GB 2	3	2.7	1.5	1.3	1.3	3.1	2.6	3.5	0.3	1.3	2.7	3	1.3	3.8	1.3	0.4	3	2.5	0.2	**0.4**															
21	I 1	2.8	1.6	0.1	0	1.1	2.2	1.4	2.7	1.3	0	1.3	2.8	0	2.5	0	1.4	2.8	2.3	1.1	1.3	0														
22	I 2	2.8	1.6	0.1	0	1.1	2.2	1.4	2.7	1.3	0	1.3	2.8	0	2.5	0	1.4	2.8	2.3	1.1	1.3	0	0													
23	N 1	1.5	1.3	2.2	2.1	3.2	1.5	2.1	1.8	3	2.1	1.9	1.7	2.1	1.9	2.8	3.1	1.7	1.5	2.8	3	2.1	2.1	**1.5**												
24	NL 1	3	2.5	1.3	1.1	1.3	3	2.5	3.5	0.4	1.1	2.5	3	1.1	3.5	1.1	0.4	3	2.5	0.2	0.4	1.1	1.1	2.9	**0.8**											
25	PL 1	1	1.2	2.4	2.3	3.4	1.3	1.9	1.4	2.8	2.3	2.1	0.6	2.3	1.2	2.3	2.9	0.6	0.8	2.7	2.8	2.3	2.3	1.5	2.8	**0.9**										
26	PL 2	3.6	2.2	0.7	0.6	0.8	2.1	2.2	3.2	2.1	0.6	1.1	3.6	0.6	3.3	0.6	2.1	3.6	3	1.9	2.1	0.6	0.6	2.7	2	3	**0.4**									
27	RUM 1	2.8	1.6	0.1	0	1.1	2.1	1.5	2.4	1.3	0	1.3	2.8	0	2.8	0	1.4	2.8	2.3	1.1	1.3	0	0	2.1	1.3	2.3	0.8	0								
28	RUM 2	2.8	1.6	0.1	0	1.1	2.1	1.5	2.4	1.3	0	1.3	2.8	0	2.8	0	1.4	2.8	2.3	1.1	1.3	0	0	2.1	1.3	2.3	0.8	0	0							
29	RUM 3	0.4	2.0	2.9	2.8	4	1.6	2.5	1.9	3	2.8	2.7	0.6	2.8	1.9	2.8	3.1	0.6	1.1	2.8	3	2.8	2.8	1.9	3	1.1	3.6	2.8	2.8	**0.3**						
30	RUM 4	0	1.8	2.9	2.8	4	1.6	2.5	1.9	3	2.8	2.7	0.6	2.8	1.9	2.8	3.1	0.6	1.1	2.8	3	2.8	2.8	1.5	3	1	3.6	2.8	2.8	0.4	0					
31	RUM 5	3	1.7	0.2	0.1	1.3	2.2	1.5	2.6	1.5	0.1	1.5	3	0.1	2.9	0.1	1.5	3	2.4	1.3	1.5	0.1	0.1	2.2	1.5	2.4	0.9	0.1	0.1	3	3	**0.3**				
32	RUM 6	2.7	1.6	0.5	0.4	1.7	2.1	1.6	2.5	1.5	0.4	1.5	2.7	0.4	2.5	0.4	1.6	2.7	2.1	1.3	1.5	0.4	0.4	2	1.4	2.2	1.2	0.6	0.6	2.7	2.7	0.7	**1.1**			
33	RUS 1	1.9	2.4	2.4	2.3	3.8	2.1	2.6	2.8	4.2	2.3	2.3	2.4	2.3	2.4	2.3	**4.2**	2.4	2.6	4	4.2	2.3	2.3	2.1	3.9	2.2	3.2	2.7	2.7	2.7	2.3	2.8	2.5	**1.7**		
34	RUS 2	3.4	2.1	2.4	2.3	4	2.8	2.4	1.6	3.6	2.3	2.8	2.8	2.3	2.8	2.3	3.6	2.8	2.3	3.4	3.6	2.3	2.3	2.7	3.6	2.3	3	2.3	2.3	3.4	3.4	2.4	2.5	3.6	**0**	
35	S 2	1.3	0.8	1.8	1.7	2.8	1.1	1.6	1.5	2.7	1.7	2.7	1.1	1.7	1.5	1.7	2.7	1.1	1	2.5	2.7	1.7	1.7	1.2	2.6	1	2.4	1.7	1.7	1.6	1.3	1.9	1.7	1.9	2.3	**1.1**

Table A39: Tajima's D and Fu's FS neutrality tests of *Steganacarus magnus COI* nucleotide sequences from 35 locations. Bold red letters show significance (P<0.05).

Neutrality tests	A 1	CZ 1	D 1	D 2	D 3	D 4	D 5	D 7	D 8	D 9	DK 1	DK 2	DK 3	F 1	F 2	F 3	F 4	FIN 1	GB 1	GB 2	I 1	I 2
Tajima's D test																						
Samplesize	2	5	14	9	3	9	5	4	3	4	3	5	3	6	4	5	2	3	2	3	10	4
S	0	173	27	7	8	195	195	134	2	12	139	0	1	191	2	71	0	3	2	5	10	14
Pi	0	85.5	6.44	1.72	5.33	86.78	97.7	84.67	1.33	6.33	95	0	0.67	81.67	1	28.4	0	2	2	3.33	4.67	7.33
Tajima's D	0	0.23	-1.03	-1.48	0	1.09	0.33	1.66	0	-0.33	6428365.59	0	0	-0.15	-0.71	-1.26	0	0	0	0	1.42	-0.4
Tajima's D p-value	0.76	0.62	0.15	0.07	0.75	0.9	0.64	0.93	0.94	0.53	1	1	0.99	0.46	0.28	**0**	1	0.88	1	0.8	0.94	0.5
Fu's FS test																						
Real no. of alleles	1	4	8	5	3	5	3	3	2	3	3	1	2	3	3	4	1	2	2	2	4	4
Orig. no. of alleles	1	4	8	5	3	5	3	3	2	3	3	1	2	3	3	4	1	2	2	2	4	4
Theta_pi	0	85.5	6.44	1.72	5.33	86.78	97.7	84.67	1.33	6.33	95	0	0.67	81.67	1	28.4	0	2	2	3.33	4.67	7.33
Exp. no. of alleles	0	4.89	7.79	3.61	2.57	8.61	4.9	3.93	1.97	3.3	2.97	0	1.65	5.82	2.08	4.68	0	2.17	1.67	2.39	5.7	3.38
FS	0	5.44	0.27	-1.19	0.46	11.58	9.93	6.54	1.06	1.88	3.45	0	0.2	12.17	-0.89	3.39	0	1.61	0.69	2.36	2.77	-0.04
FS p-value	N.A.	0.96	0.55	0.13	0.4	1	1	0.98	0.6	0.76	0.58	N.A.	0.38	1	0.09	0.89	N.A.	0.7	0.38	0.8	0.91	0.28

Neutrality tests	N 1	NL 1	PL 1	PL 2	RUM 1	RUM 2	RUM 3	RUM 4	RUM 5	RUM 6	RUS 1	RUS 2	S 2	mean	s. d.
Tajima's D test															
Samplesize	3	3	6	3	3	4	4	3	4	3	4	4	3	4.43	2.52
S	143	9	144	15	3	13	18	7	7	93	129	0	194	56.17	72.90
Pi	95.33	6	76.47	10	2	7	9	4.67	3.67	62.33	66.5	0	137	30.91	41.00
Tajima's D	0	0	1.38	0	0	-0.13	-0.85	0	-0.39	1384076.56	-0.58	0	15062332.57	653564.96	2739489.35
Tajima's D p-value	0.2	0.73	0.96	0.7	0.88	0.61	0.08	0.76	0.49	1	0.38	1	1.00	0.69	0.32
Fu's FS test															
Real no. of alleles	2	3	6	2	2	4	2	3	4	3	4	1	3	3.06	1.47
Orig. no. of alleles	2	3	6	2	2	4	2	3	4	3	4	1	3	3.06	1.47
Theta_pi	95.33	6	76.47	10	2	7	9	4.67	3.67	62.33	66.5	0	137	30.91	41.00
Exp. no. of alleles	2.97	2.61	5.81	2.74	2.17	3.35	3.47	2.52	2.98	2.95	3.91	0	2.98	3.22	1.97
FS	8.45	0.59	1.55	4.17	1.61	-0.09	5.39	0.31	-0.95	3.02	2.38	0	3.82	2.63	3.48
FS p-value	0.99	0.4	0.48	0.93	0.71	0.27	0.98	0.37	0.11	0.57	0.53	N.A.	0.59	N.A.	N.A.

Table A40: Tajima's D and Fu's FS neutrality tests of *Steganacarus magnus COI* protein sequences from 35 locations. Bold red letters show significance (P<0.05).

Neutrality tests	A 1	CZ 1	D 1	D 2	D 3	D 4	D 5	D 7	D 8	D 9	DK 1	DK 2	DK 3	F 1	F 2	F 3	F 4	FIN 1	GB 1	GB 2	I 1	I 2	N 1
Tajima's D test																							
Samplesize	2	5	14	9	3	9	5	4	3	4	3	5	3	6	4	5	2	3	2	3	10	4	3
S	0	5	3	0	0	4	7	3	1	0	4	0	0	5	0	2	0	0	0	1	0	0	4
Pi	0	1.9	0.43	0	0	2.11	3.5	1.83	0.67	0	2.67	0	0	2.53	0	0.8	0	0	0	0.67	0	0	2.67
Tajima's D	0	-1.4	-1.67	0	0	1.77	0.29	1.09	0	0	0	0	0	0.88	0	-0.97	0	0	0	0	0	0	0
Tajima's D p-value	1	0	**0.03**	1	1	0.98	0.66	0.85	0.99	1	0.84	1	1	0.81	1	0.2	1	1	1	0.99	1	1	0.84
Fu's FS test																							
Real no. of alleles	1	4	3	1	1	5	3	3	2	1	3	1	1	3	1	4	1	2	2	2	2	1	2
Orig. no. of alleles	1	4	3	1	1	5	3	3	2	1	3	1	1	3	1	4	1	2	2	2	2	1	2
Theta_pi	0	1.9	0.43	0	0	2.11	3.5	1.83	0.67	0	2.67	0	0	2.53	0	0.8	0	0	0	0.67	0	0	2.67
Exp. no. of alleles	0	2.85	2.14	0	0	3.95	3.42	2.5	1.65	0	2.3	0	0	3.46	0	2.11	0	2	0	1.65	2	0	2.3
FS	0	0.56	-0.76	0	0	1.92	1.62	0.01	0.2	0	-0.34	0	0	1.47	0	1.04	0	0	0	0.2	0	0	2.02
FS p-value	N.A.	0.54	0.08	N.A.	N.A.	0.85	0.76	0.3	0.39	N.A.	0.19	N.A.	N.A.	0.77	N.A.	0.62	N.A.	N.A.	N.A.	0.39	N.A.	N.A.	0.76

Neutrality tests	NL 1	PL 1	PL 2	RUM 1	RUM 2	RUM 3	RUM 4	RUM 5	RUM 6	RUS 1	RUS 2	S 2	mean	s. d.
Tajima's D test														
Samplesize	3	6	3	3	4	4	3	4	3	4	4	3	4.43	2.52
S	1	3	1	0	0	1	0	1	3	6	0	3	1.66	2.04
Pi	0.67	1.6	0.67	0	0	0.5	0	0.5	2	3	0	2	0.88	1.09
Tajima's D	0	1.12	0	0	0	-0.61	0	-0.61	0	-0.81	0	0	-0.03	0.63
Tajima's D p-value	0.99	0.88	0.98	1	1	0.38	1	0.38	0.88	0.15	1	0.88	0.82	0.31
Fu's FS test														
Real no. of alleles	2	5	2	1	2	2	1	2	2	4	1	3	2.17	1.18
Orig. no. of alleles	2	5	2	1	2	2	1	2	2	4	1	3	2.17	1.18
Theta_pi	0.67	1.6	0.67	0	0	0.5	0	0.5	2	3	0	2	0.88	1.09
Exp. no. of alleles	1.65	2.94	1.65	0	2	1.68	0	1.68	2.17	2.85	0	2.17	1.46	1.25
FS	0.2	2.51	0.2	0	0	0.17	0	0.17	1.61	0.73	0	1.61	0.43	0.77
FS p-value	0.37	0.87	0.39	N.A.	N.A.	0.34	N.A.	0.35	0.69	0.57	N.A.	0.7	N.A.	N.A.

Table A41: Results of the McDonald-Kreitman test for *Steganacarus magnus*. The differences between 64 populations are significant (*0.01<P<0.05), between other 64 populations high significant (**0.001<P<0.01) and between 41 populations extremely high significant (***P<0.001). Number of fixed and polymorph synonymous and non-synonymous mutations are shown. Locations with less than two individuals were excluded.

Population		A 1			CZ 1			D 1			D 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
CZ 1	fixed	50	3	0.0303*									
Decin	poly	151	32										
D 1	fixed	98	45	0.2658 ns	32	18	0.0263*						
Kranichstein	poly	15	12		162	42							
D 2	fixed	99	46	0.1782 ns	33	18	0.0107*	0	0	- ns			
Goettingen	poly	6	0		155	32		18	11				
D 3	fixed	109	48	0.1063 ns	40	19	0.0157*	70	11	0.0375*	70	12	0.2038 ns
Lake Constance	poly	8	0		158	32		24	11		14	0	
D 4	fixed	42	5	0.4955 ns	6	0	0.599 ns	27	18	0.0028**	28	18	0.0007***
Meckl. Seenpl.	poly	167	30		252	43		177	40		169	30	
D 5	fixed	55	6	0.0186*	9	1	0.693 ns	21	7	1 ns	20	8	0.6394 ns
Moerfelden	poly	153	49		237	59		165	58		157	49	
D 7	fixed	64	10	0.8399 ns	24	2	0.3923 ns	55	30	0.0081**	54	30	0.0004***
Uelzen	poly	115	20		224	42		125	30		119	20	
D 8	fixed	100	48	1 ns	43	23	0.0092**	87	13	0.0022**	87	15	1 ns
Cuxhaven	poly	1	1		151	33		17	12		7	1	
D 9	fixed	99	46	0.1098 ns	33	18	0.011*	0	0	- ns	0	0	- ns
Bonn	poly	11	1		158	33		18	11		13	1	
DK 1	fixed	72	24	0.2458 ns	9	2	1 ns	0	0	- ns	0	0	- ns
Copenhagen	poly	94	46		202	61		108	56		99	45	
DK 2	fixed	101	15	- ns	47	3	0.0457*	79	39	0.5033 ns	80	39	0.1756 ns
Hjorring	poly	0	0		151	32		16	11		6	0	
DK 3	fixed	100	46	0.3197 ns	35	18	0.0216*	0	0	- ns	0	0	- ns
Arhus	poly	0	1		151	33		16	12		6	1	
F 1	fixed	51	4	0.0878 ns	0	0	- ns	26	17	0.0103*	30	17	0.0047**
Mont Blanc	poly	167	34		237	39		175	45		169	34	
F 2	fixed	101	46	0.5703 ns	35	18	0.0125*	0	0	- ns	0	0	- ns
Loire	poly	2	0		153	32		18	11		8	0	
F 3	fixed	80	46	0.0018**	29	20	0.001**	68	10	0.1147 ns	67	12	1 ns
Saint Isidore	poly	60	11		187	41		73	22		65	11	
F 4	fixed	101	12	- ns	45	2	0.021*	76	40	0.6561 ns	76	40	0.1009 ns
Haute Loire	poly	0	0		151	32		16	11		6	0	
FIN 1	fixed	106	18	0.004**	60	9	0.352 ns	89	37	0.0843 ns	92	37	1 ns
Lahti	poly	0	3		151	35		16	14		6	3	
GB 1	fixed	101	49	1 ns	46	24	0.0071**	92	14	0.0023**	93	16	1 ns
Ascot	poly	1	1		151	33		17	12		6	1	
GB 2	fixed	101	48	0.0447*	46	23	0.0287*	93	13	0.0001***	93	15	0.0741 ns
Braemar	poly	1	4		152	36		17	15		7	4	
I 1	fixed	100	46	1 ns	33	18	0.0127*	0	0	- ns	0	0	- ns
Grosseto	poly	7	3		157	35		16	13		11	3	
I 2	fixed	99	45	0.3467 ns	33	17	0.018*	0	0	- ns	0	0	- ns
Parma	poly	11	2		160	34		18	12		13	2	
N 1	fixed	69	5	0.0041**	2	0	1 ns	56	24	0.6517 ns	55	14	0.1488 ns
Narvik	poly	112	31		187	37		121	45		116	32	
NL 1	fixed	98	48	0.7192 ns	43	23	0.0094**	88	13	0.0052**	88	15	1 ns
Wageningen	poly	7	2		155	34		23	13		13	2	
PL 1	fixed	66	8	0.0197*	1	0	1 ns	41	22	0.3333 ns	40	22	0.0899 ns
Krakow	poly	111	36		200	40		122	47		117	36	
PL 2	fixed	106	44	0.559 ns	32	20	0.0026**	62	18	0.1947 ns	59	20	0.3876 ns
Warsaw	poly	12	3		156	34		27	14		18	3	
RUM 1	fixed	104	44	0.5564 ns	40	18	0.039*	72	14	0.0366*	74	16	0.3476 ns
Sibiu 1	poly	3	0		154	32		19	11		9	0	
RUM 2	fixed	101	43	0.5108 ns	40	18	0.04*	70	13	0.0338*	72	15	0.7207 ns
Sibiu 2	poly	10	2		156	33		26	13		16	2	
RUM 3	fixed	88	5	0.119 ns	24	1	0.0911 ns	87	43	0.8555 ns	91	43	0.0548 ns
Bagau	poly	15	3		158	33		31	14		21	3	

Table A41 continue:

Population		A 1			CZ 1			D 1			D 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM 4	fixed	92	5	1 ns	42	2	0.0358*	92	44	1 ns	96	44	0.0117*
Cluj Napoca	poly	7	0		170	34		23	11		13	0	
RUM 5	fixed	108	43	1 ns	42	19	0.0464*	70	18	0.0624 ns	71	20	1 ns
Busteni	poly	5	2		154	34		21	13		10	2	
RUM 6	fixed	85	37	0.1172 ns	17	13	0.0034**	37	5	0.1229 ns	39	6	0.4792 ns
Sinaia	poly	75	19		201	45		89	29		78	19	
RUS 1	fixed	60	3	0.119 ns	20	2	0.5461 ns	66	34	0.0068*	67	35	0.0001***
Altai Mountains	poly	116	16		221	43		126	28		120	16	
RUS 2	fixed	112	17	- ns	63	8	0.2544 ns	110	44	0.2565 ns	110	44	0.1890 ns
Novosibirsk	poly	0	0		151	32		16	11		6	0	
S 2	fixed	55	6	0.02788*	0	0	- ns	29	15	0.2598 ns	30	16	0.0873 ns
Stroemstad	poly	157	46		229	54		168	55		162	46	

Population		D 3			D 4			D 5			D 7		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 4	fixed	29	15	0.0048**									
Meckl. Seenpl.	poly	172	30										
D 5	fixed	27	7	0.8279 ns	0	0	- ns						
Moerfelden	poly	147	49		240	57							
D 7	fixed	48	34	0.0000***	13	1	0.4884 ns	17	1	0.1395 ns			
Uelzen	poly	122	20		226	42		223	58				
D 8	fixed	90	17	1 ns	42	23	0.0012**	40	9	0.4528 ns	55	32	0.0004***
Cuxhaven	poly	9	1		167	31		153	50		116	21	
D 9	fixed	69	11	0.4505 ns	27	18	0.0006***	21	7	1 ns	55	30	0.0005***
Bonn	poly	19	1		172	31		160	49		120	21	
DK 1	fixed	42	4	0.0018**	0	0	- ns	5	1	0.6895 ns	32	12	0.8502 ns
Copenhagen	poly	100	46		193	52		190	69		179	59	
DK 2	fixed	79	41	0.0541 ns	0	0	- ns	8	1	0.4647 ns	53	4	0.1583 ns
Hjorring	poly	8	0		167	30		153	49		115	20	
DK 3	fixed	73	12	1 ns	28	18	0.0008***	21	8	0.8188 ns	57	30	0.0017**
Arhus	poly	8	1		167	31		153	50		115	21	
F 1	fixed	31	20	0.0009***	6	0	0.5979 ns	6	1	1 ns	16	0	0.1408 ns
Mont Blanc	poly	173	34		247	43		247	60		227	42	
F 2	fixed	71	12	0.3498 ns	29	18	0.0008***	24	8	1 ns	56	30	0.0008***
Loire	poly	10	0		168	30		155	49		117	20	
F 3	fixed	67	13	0.8254 ns	24	19	0.0001***	24	6	0.82 ns	45	30	0.0002***
Saint Isidore	poly	67	11		207	40		190	59		156	31	
F 4	fixed	77	42	0.0517 ns	6	1	1 ns	0	1	0.2463 ns	55	3	0.0586 ns
Haute Loire	poly	8	0		167	30		153	49		115	20	
FIN 1	fixed	92	39	1 ns	40	7	0.8315 ns	43	4	0.0111*	55	5	0.1294 ns
Lahti	poly	8	3		167	33		153	52		115	23	
GB 1	fixed	92	18	1 ns	45	24	0.0016**	43	9	0.2784 ns	58	33	0.0004***
Ascot	poly	9	1		167	31		153	50		116	21	
GB 2	fixed	93	17	0.2337 ns	45	23	0.0055**	43	8	0.1462 ns	58	32	0.0014**
Braemar	poly	9	4		168	34		154	53		116	23	
I 1	fixed	70	11	0.7153 ns	27	18	0.0008**	22	7	1 ns	55	30	0.0019**
Grosseto	poly	15	3		171	33		158	51		119	23	
I 2	fixed	69	11	0.7317 ns	27	18	0.0006***	21	7	1 ns	55	30	0.0005***
Parma	poly	19	2		173	31		160	49		121	21	
N 1	fixed	60	24	0.341 ns	11	0	0.3716 ns	15	0	0.0469*	34	5	0.5013 ns
Narvik	poly	116	33		232	39		221	63		201	45	
NL 1	fixed	88	17	0.7403 ns	42	23	0.0013**	40	8	0.3404 ns	55	32	0.0004***
Wageningen	poly	15	2		171	32		158	50		119	22	
PL 1	fixed	41	24	0.048*	0	0	- ns	0	0	- ns	30	1	0.0151*
Krakow	poly	117	36		213	42		202	63		194	49	

Table A41 continue:

Population		D 3			D 4			D 5			D 7		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
PL 2	fixed	75	17	0.7601 ns	32	13	0.0068**	29	6	0.5146 ns	43	29	0.0001***
Warsaw	poly	20	3		172	32		159	49		122	22	
RUM 1	fixed	80	16	0.3532 ns	37	14	0.0614 ns	37	4	0.0403*	59	28	0.0024**
Sibiu 1	poly	10	0		169	30		154	49		118	20	
RUM 2	fixed	78	15	0.7326 ns	37	14	0.0627 ns	36	4	0.059 ns	57	28	0.0014**
Sibiu 2	poly	17	2		172	31		158	50		124	21	
RUM 3	fixed	81	46	0.0123*	30	3	0.4387 ns	40	5	0.0726 ns	52	8	0.8308 ns
Bagau	poly	23	3		174	30		162	50		126	23	
RUM 4	fixed	83	46	0.0028**	29	3	0.586 ns	43	5	0.0493*	55	10	0.8342 ns
Cluj Napoca	poly	15	0		172	30		156	49		120	20	
RUM 5	fixed	79	18	0.7365 ns	35	16	0.0165*	32	5	0.1448 ns	55	29	0.0016**
Busteni	poly	13	2		169	32		154	51		119	22	
RUM 6	fixed	44	7	0.4977 ns	18	12	0.0052**	11	2	0.5373 ns	36	25	0.0004***
Sinaia	poly	80	19		206	41		192	61		165	36	
RUS 1	fixed	53	37	0.0000***	17	1	0.4845 ns	22	1	0.0576 ns	31	4	0.6231 ns
Altai Mountains	poly	123	16		235	40		228	61		195	35	
RUS 2	fixed	105	48	0.1065 ns	46	8	1 ns	57	7	0.0226*	61	9	0.8336 ns
Novosibirsk	poly	8	0		167	30		153	49		115	20	
S 2	fixed	29	14	0.1682 ns	0	0	- ns	2	0	1 ns	20	1	0.1411 ns
Stroemstad	poly	163	46		246	52		225	67		232	55	

Population		D 8			D 9			DK 1			DK 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
D 9	fixed	87	14	1 ns									
Bonn	poly	12	2										
DK 1	fixed	54	9	0.0062**	0	0	- ns						
Copenhagen	poly	95	47		104	46							
DK 2	fixed	101	45	1 ns	78	39	0.103 ns	46	17	0.4187 ns			
Hjorring	poly	1	1		11	1		94	46				
DK 3	fixed	91	15	0.0628 ns	0	0	- ns	0	0	- ns	82	39	0.3279 ns
Arhus	poly	1	2		11	2		95	46		0	1	
F 1	fixed	46	25	0.0026**	28	17	0.0038**	12	2	0.5379 ns	34	1	0.0362*
Mont Blanc	poly	167	35		171	35		212	63		167	34	
F 2	fixed	89	15	0.4786 ns	0	0	- ns	4	0	0.3107 ns	83	39	0.5653 ns
Loire	poly	3	1		13	1		96	45		2	0	
F 3	fixed	2	0	1 ns	69	11	0.8252 ns	40	6	0.0265*	82	42	0.0071**
Saint Isidore	poly	60	12		67	12		139	56		60	11	
F 4	fixed	99	46	1 ns	75	40	0.1015 ns	44	17	0.513 ns	17	3	- ns
Haute Loire	poly	1	1		11	1		94	46		0	0	
FIN 1	fixed	106	39	0.024*	90	37	1 ns	63	21	0.183 ns	94	16	0.0041**
Lahti	poly	1	4		11	4		95	48		0	3	
GB 1	fixed	6	1	0.4909 ns	92	15	1 ns	60	10	0.0031**	103	46	1 ns
Ascot	poly	2	2		12	2		94	47		1	1	
GB 2	fixed	6	0	0.021*	93	14	0.1377 ns	60	9	0.00098***	104	45	0.0357*
Braemar	poly	2	5		12	5		95	50		1	4	
I 1	fixed	90	14	0.0904 ns	0	0	- ns	2	0	0.5619 ns	76	39	1 ns
Grosseto	poly	8	4		11	3		100	48		7	3	
I 2	fixed	87	14	0.6944 ns	0	0	- ns	0	0	- ns	79	38	0.3422 ns
Parma	poly	12	3		13	2		103	46		11	2	
N 1	fixed	59	31	0.049*	56	24	0.2047 ns	26	3	0.0444*	74	5	0.0023**
Narvik	poly	112	32		119	34		177	69		111	32	
NL 1	fixed	0	0	- ns	88	14	1 ns	55	9	0.0066**	101	45	0.7238 ns
Wageningen	poly	8	3		18	3		100	48		7	2	
PL 1	fixed	60	30	0.1827 ns	41	22	0.1299 ns	14	3	0.413 ns	0	0	- ns
Krakow	poly	111	37		117	37		173	69		111	36	

Table A41 continue:

Population		D 8			D 9			DK 1			DK 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
PL 2	fixed	83	20	0.745 ns	61	19	0.4264 ns	34	9	0.1887 ns	87	35	0.5589 ns
Warsaw	poly	13	4		22	4		103	48		12	3	
RUM 1	fixed	83	15	1 ns	71	15	0.4544 ns	44	7	0.0105*	89	37	0.5569 ns
Sibiu 1	poly	4	1		14	1		96	46		3	0	
RUM 2	fixed	81	15	0.6978 ns	69	14	0.7578 ns	43	7	0.0166*	87	36	0.5086 ns
Sibiu 2	poly	11	3		21	3		101	47		10	2	
RUM 3	fixed	88	47	0.2143 ns	88	43	0. 0447*	56	23	0.7647 ns	87	11	0.693 ns
Bagau	poly	16	4		25	4		104	48		15	3	
RUM 4	fixed	94	48	0.2725 ns	93	44	0. 0143*	58	23	0.6531 ns	89	13	0.5955 ns
Cluj Napoca	poly	8	1		18	1		99	46		7	0	
RUM 5	fixed	96	17	0.1649 ns	69	19	0.758 ns	43	10	0.0764 ns	95	36	1 ns
Busteni	poly	6	3		16	3		98	47		5	2	
RUM 6	fixed	59	7	0.0921 ns	37	5	0.3389 ns	18	3	0.2082 ns	64	29	0.137 ns
Sinaia	poly	76	20		83	20		146	56		74	20	
RUS 1	fixed	64	43	0.0000***	66	35	0.0001***	44	14	1 ns	65	9	1 ns
Altai Mountains	poly	117	17		121	17		182	60		116	16	
RUS 2	fixed	110	44	0.495 ns	108	44	0.1824 ns	78	22	0.0812 ns	112	22	- ns
Novosibirsk	poly	1	1		11	1		94	46		0	0	
S 2	fixed	48	18	0.5094 ns	30	15	0.1233 ns	9	2	0.7356 ns	0	0	- ns
Stroemstad	poly	157	47		164	46		206	71		157	46	

Population		DK 3			F 1			F 2			F 3		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
F 1	fixed	31	17	0.0086**									
Mont Blanc	poly	167	34										
F 2	fixed	4	0	0.4286 ns	32	17	0.0092**						
Loire	poly	2	1		168	34							
F 3	fixed	71	12	0.8245 ns	31	20	0.0023**	69	12	1 ns			
Saint Isidore	poly	60	12		200	44		62	11				
F 4	fixed	78	40	0.3445 ns	34	1	0.0362*	79	40	0.5537 ns	79	44	0.0028**
Haute Loire	poly	0	1		167	34		2	0		60	11	
FIN 1	fixed	95	37	0.0074**	36	2	0.054 ns	94	37	0.1514 ns	82	37	0.067 ns
Lahti	poly	0	4		167	37		2	3		60	14	
GB 1	fixed	96	16	0.0634 ns	47	26	0.0018**	94	16	0.4808 ns	0	0	- ns
Ascot	poly	1	2		168	35		3	1		61	12	
GB 2	fixed	97	15	0.0005***	47	25	0.0084**	95	15	0.0065**	0	0	- ns
Braemar	poly	1	5		167	38		2	4		61	15	
I 1	fixed	2	0	0.5385 ns	28	17	0.0048**	0	0	- ns	71	11	0.5171 ns
Grosseto	poly	7	4		171	37		9	3		65	14	
I 2	fixed	0	0	- ns	28	16	0.0073**	0	0	- ns	69	11	0.8252 ns
Parma	poly	11	3		172	36		13	2		67	13	
N 1	fixed	58	25	0.2693 ns	9	1	0.705 ns	58	25	0.2043 ns	42	28	0.0022**
Narvik	poly	111	33		234	47		113	32		156	40	
NL 1	fixed	92	15	0.357 ns	45	25	0.0022**	90	15	1 ns	0	0	- ns
Wageningen	poly	7	3		171	35		9	2		63	12	
PL 1	fixed	42	22	0.1831 ns	16	1	0.3225 ns	43	22	0.1806 ns	40	25	0.0149*
Krakow	poly	111	37		219	46		113	36		159	46	
PL 2	fixed	62	20	1 ns	31	19	0.0018**	61	20	0.7548 ns	61	17	0.5512 ns
Warsaw	poly	12	4		172	35		14	3		66	14	
RUM 1	fixed	76	16	1 ns	34	18	0.0067**	77	16	0.5879 ns	61	12	0.824 ns
Sibiu 1	poly	3	1		169	34		5	0		63	11	
RUM 2	fixed	74	15	0.6962 ns	33	18	0.0061**	75	15	1 ns	59	12	1 ns
Sibiu 2	poly	10	3		173	35		12	2		70	13	
RUM 3	fixed	92	43	0.4312 ns	39	2	0.0563 ns	93	43	0.1893 ns	66	45	0.0002***
Bagau	poly	15	4		175	34		16	3		74	14	

Table A41 continue:

Population		DK 3			F 1			F 2			F 3		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM 4	fixed	97	44	0.436 ns	42	2	0.0358*	97	44	0.0588 ns	71	46	0.0003***
Cluj Napoca	poly	7	1		170	34		9	0		64	11	
RUM 5	fixed	72	20	0.3804 ns	28	19	0.0014**	73	20	1 ns	70	14	1 ns
Busteni	poly	5	3		170	36		7	2		65	12	
RUM 6	fixed	39	6	0.3544 ns	18	13	0.0046**	42	6	0.3527 ns	39	6	0.3908 ns
Sinaia	poly	75	20		208	47		76	19		122	29	
RUS 1	fixed	69	35	0.0001***	24	0	0.0323*	67	36	0.0000***	47	40	0.0000***
Altai Mountains	poly	116	17		230	44		118	16		164	27	
RUS 2	fixed	113	45	0.2893 ns	46	8	0.8375 ns	112	44	1 ns	94	42	0.0313*
Novosibirsk	poly	0	1		167	34		2	0		59	12	
S 2	fixed	32	16	0.1938 ns	7	0	0.3586 ns	33	16	0.142 ns	32	15	0.1941 ns
Stroemstad	poly	157	47		248	56		159	46		191	56	

Population		F 4			FIN 1			GB 1			GB 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
FIN 1	fixed	94	15	0.0036**									
Lahti	poly	0	3										
GB 1	fixed	100	47	1 ns	104	40	0.0268*						
Ascot	poly	1	1		1	4							
GB 2	fixed	101	46	0.0405*	104	39	0.0011**	0	1	1 ns			
Braemar	poly	1	4		1	7		2	5				
I 1	fixed	76	40	1 ns	91	37	0.216 ns	95	15	0.0921 ns	96	14	0.0038**
Grosseto	poly	0	3		0	3		8	4		8	7	
I 2	fixed	75	39	0.2207 ns	89	37	1 ns	92	15	0.696 ns	93	14	0.0748 ns
Parma	poly	11	2		11	5		12	3		12	6	
N 1	fixed	71	4	0.0016**	81	11	0.0279*	61	32	0.0509 ns	61	31	0.1028 ns
Narvik	poly	112	31		111	35		113	32		113	35	
NL 1	fixed	98	46	0.7206 ns	102	39	0.3276 ns	0	0	- ns	0	0	- ns
Wageningen	poly	7	2		7	5		8	3		8	6	
PL 1	fixed	0	0	- ns	61	9	0.0349*	62	31	0.1858 ns	63	30	0.3821 ns
Krakow	poly	111	36		111	39		112	37		112	40	
PL 2	fixed	84	38	0.5522 ns	94	38	0.7834 ns	83	21	0.7509 ns	82	20	0.1461 ns
Warsaw	poly	12	3		12	6		13	4		13	7	
RUM 1	fixed	90	40	0.5536 ns	96	37	0.3544 ns	85	16	1 ns	84	16	0.0374*
Sibiu 1	poly	3	0		3	3		4	1		4	4	
RUM 2	fixed	88	39	0.3535 ns	94	36	0.7627 ns	83	16	0.7022 ns	82	16	0.0919 ns
Sibiu 2	poly	10	2		10	5		11	3		11	6	
RUM 3	fixed	88	9	0.3976 ns	97	17	0.2014 ns	89	48	0.2132 ns	89	47	0.8141 ns
Bagau	poly	15	3		15	6		16	4		16	7	
RUM 4	fixed	91	10	0.6225 ns	96	18	0.371 ns	95	49	0.2726 ns	95	48	1 ns
Cluj Napoca	poly	7	0		7	3		8	1		8	4	
RUM 5	fixed	90	39	1 ns	93	38	0.283 ns	97	18	0.1359 ns	96	17	0.009**
Busteni	poly	5	2		5	5		5	3		6	6	
RUM 6	fixed	59	32	0.0492*	71	31	0.3395 ns	60	9	0.2172 ns	60	8	0.0691 ns
Sinaia	poly	74	20		74	23		75	20		75	23	
RUS 1	fixed	60	6	0.6351 ns	68	11	1 ns	66	44	0.0000***	67	43	0.0000***
Altai Mountains	poly	116	16		116	19		117	17		117	20	
RUS 2	fixed	113	19	- ns	117	21	0.0044**	113	45	0.4936 ns	113	44	0.0274*
Novosibirsk	poly	0	0		0	3		1	1		1	4	
S 2	fixed	8	1	0.6872 ns	49	7	0.0694 ns	51	19	0.5185 ns	51	18	0.7482 ns
Stroemstad	poly	157	46		157	49		157	47		158	50	

Table A41 continue:

Population		I 1			I 2			N 1			NL 1		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
I 2	fixed	0	0	- ns									
Parma	poly	11	4										
N 1	fixed	56	24	0.3439 ns	56	23	0.3372 ns						
Narvik	poly	117	36		119	35							
NL 1	fixed	91	14	0.1688 ns	88	14	0.7383 ns	58	31	0.0349*			
Wageningen	poly	14	5		18	4		117	33				
PL 1	fixed	41	22	0.1835 ns	41	21	0.1773 ns	26	0	0.0067**	59	30	0.1382 ns
Krakow	poly	115	39		118	38		196	47		118	38	
PL 2	fixed	62	19	1 ns	61	18	0.7895 ns	52	25	0.1088 ns	81	20	1 ns
Warsaw	poly	18	6		22	5		121	34		18	4	
RUM 1	fixed	72	15	0.6987 ns	70	14	1 ns	66	26	0.2811 ns	81	15	1 ns
Sibiu 1	poly	10	3		14	2		114	32		10	2	
RUM 2	fixed	70	14	0.5377 ns	68	13	1 ns	64	26	0.2187 ns	79	15	0.7483 ns
Sibiu 2	poly	17	5		21	4		119	33		17	4	
RUM 3	fixed	89	43	0.2703 ns	87	42	0.0817 ns	45	1	0.0008***	85	47	0.1147 ns
Bagau	poly	22	6		26	5		123	34		22	5	
RUM 4	fixed	94	44	0.2761 ns	92	43	0.0624 ns	47	1	0.0012**	91	48	0.0929 ns
Cluj Napoca	poly	14	3		18	2		117	31		14	2	
RUM 5	fixed	72	19	0.5254 ns	70	18	1 ns	59	25	0.2097 ns	93	17	0.47 ns
Busteni	poly	12	5		16	4		116	33		12	4	
RUM 6	fixed	39	5	0.1713 ns	37	5	0.3404 ns	33	18	0.0687 ns	57	7	0.138 ns
Sinaia	poly	81	22		84	20		173	48		80	20	
RUS 1	fixed	66	35	0.0003***	65	34	0.0001***	32	1	0.0239*	63	43	0.0000***
Altai Mountains	poly	119	19		121	18		199	44		122	17	
RUS 2	fixed	110	44	1 ns	109	44	0.3589 ns	84	13	0.1265 ns	109	44	1 ns
Novosibirsk	poly	7	3		11	2		112	31		7	2	
S 2	fixed	30	15	0.1813 ns	30	15	0.1225 ns	15	0	0.0495*	47	18	0.5061 ns
Stroemstad	poly	162	48		165	46		228	58		163	48	

Population		PL 1			PL 2			RUM 1			RUM 2		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
PL 2	fixed	44	19	0.3978 ns									
Warsaw	poly	122	39										
RUM 1	fixed	48	21	0.4075 ns	67	9	0.6942 ns						
Sibiu 1	poly	113	36		15	3							
RUM 2	fixed	47	21	0.321 ns	64	8	0.505 ns	0	0	- ns			
Sibiu 2	poly	117	37		22	5		10	2				
RUM 3	fixed	48	8	0.1823 ns	90	43	0.1401 ns	99	42	0.1929 ns	96	23	0.2503 ns
Bagau	poly	119	37		26	6		18	3		23	5	
RUM 4	fixed	51	9	0.193 ns	90	44	0.0848 ns	100	43	0.0627 ns	97	42	0.1007 ns
Cluj Napoca	poly	114	36		19	3		10	0		17	2	
RUM 5	fixed	53	20	0.7451 ns	55	3	0.0326*	72	8	0.5946 ns	71	7	0.2177 ns
Busteni	poly	115	38		17	5		8	2		15	4	
RUM 6	fixed	25	14	0.1624 ns	35	3	0.1273 ns	41	4	0.1423 ns	41	3	0.051 ns
Sinaia	poly	165	52		84	20		77	19		82	21	
RUS 1	fixed	35	4	0.19 ns	73	35	0.0003***	72	36	0.0001***	72	35	0.0003***
Altai Mountains	poly	201	48		124	18		118	16		122	18	
RUS 2	fixed	76	12	0.047*	107	43	0.5621 ns	104	43	0.5577 ns	103	43	0.5112 ns
Novosibirsk	poly	111	36		12	3		3	0		10	2	
S 2	fixed	0	0	- ns	30	12	0.4246 ns	95	36	0.5636 ns	94	35	0.5185 ns
Stroemstad	poly	165	52		164	47		3	0		10	2	

Table A41 continue:

Population		RUM 3			RUM 4			RUM 5			RUM 6		
		syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.	syn.	nons.	sign.
RUM 4	fixed	5	0	1 ns									
Cluj Napoca	poly	20	3										
RUM 5	fixed	106	42	0.4764 ns	107	46	0.3541 ns						
Busteni	poly	19	5		12	2							
RUM 6	fixed	75	35	0.0935 ns	76	36	0.0609 ns	0	0	- ns			
Sinaia	poly	84	23		79	20		77	21				
RUS 1	fixed	29	0	0.0461*	35	1	0.1283 ns	79	36	0.0006***	51	30	0.0004***
Altai Mountains	poly	125	19		120	16		121	18		171	34	
RUS 2	fixed	99	17	1 ns	99	18	0.5921 ns	114	42	1 ns	91	35	0.3451 ns
Novosibirsk	poly	15	3		7	0		5	2		74	20	
S 2	fixed	39	7	0.3261 ns	40	8	0.4397 ns	36	10	1 ns	13	7	0.2649 ns
Stroemstad	poly	165	47		160	46		159	48		202	56	

Population		RUS 1			RUS 2		
		syn.	nons.	sign.	syn.	nons.	sign.
RUS 2	fixed	59	14	0.2156 ns			
Novosibirsk	poly	116	16				
S 2	fixed	26	2	0.1287 ns	54	7	0.0679 ns
Stroemstad	poly	234	58		157	46	

Table A42: Neutrality indices of *Steganacarus magnus* computed in the McDonald-Kreitman test with DnaSP v5. Locations with less than two individuals were excluded.

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	A 1																																		
2	CZ 1	3.5																																	
3	D 1	1.7	0.5																																
4	D 2	0	0.4	-																															
5	D 3	0	0.4	2.9	0																														
6	D 4	1.5	-	0.3	0.3	0.3																													
7	D 5	2.9	2.2	1.1	0.8	1.2	-																												
8	D 7	1.1	2.3	0.4	0.3	0.2	2.4	4.4																											
9	D 8	2.1	0.4	4.7	0.8	0.6	0.3	1.5	0.3																										
10	D 9	0.2	0.4	-	-	0.3	0.3	0.9	0.3	1																									
11	DK 1	1.5	1.4	-	-	4.8	-	1.8	0.9	3	-																								
12	DK 2	-	3.3	1.4	0	0	-	2.6	2.3	2.2	0.2	1.3																							
13	DK 3	-	0.4	-	-	0.8	0.3	0.9	0.3	12.1	-	-	-																						
14	F 1	2.6	-	0.4	0.4	0.3	-	1.5	-	0.4	0.3	1.8	6.9	0.4																					
15	F 2	0	0.4	-	-	0	0.3	0.9	0.3	2	-	-	0	-	0.4																				
16	F 3	0.3	0.3	2	0.9	0.8	0.2	1.2	0.3	-	1.1	2.7	0.4	1.2	0.3	1																			
17	F 4	-	4.8	1.3	0	0	1.1	0	3.2	2.2	0.2	1.3	-	-	6.9	0	0.3																		
18	FIN 1	-	1.5	2.1	1.2	0.9	1.1	3.7	2.2	10.9	0.9	1.5	-	-	4	3.8	0.5	-																	
19	GB 1	-	0.4	4.6	1	0.6	0.3	1.6	0.3	6	1	3	2.2	12	0.4	2	-	2.1	10.4																
20	GB 2	8.4	0.5	6.3	3.5	2.4	0.4	1.9	0.4	-	2.8	3.5	9.2	32.3	0.4	12.7	-	8.8	18.7	0															
21	I 1	0.9	0.4	-	-	1.3	0.3	1	0.4	3.2	-	-	0.9	-	0.4	-	1.4	0.8	2.1	3.2	6														
22	I 2	0.4	0.4	-	-	0.7	0.3	0.9	0.3	1.6	-	-	0.4	-	0.4	-	1.2	0.4	1.1	1.5	3.3	-													
23	N 1	3.8	-	0.9	0.6	0.7	-	-	1.5	0.5	0.7	3.4	4.3	0.7	1.8	0.7	0.4	4.9	2.3	0.5	0.6	0.7	0.7											
24	NL 1	0.6	0.4	3.8	0.9	0.7	0.3	1.6	0.3	-	1	2.9	0.6	2.6	0.4	1.3	-	0.6	1.9	-	-	2.3	1.4	0.5										
25	PL 1	2.7	-	0.7	0.6	0.5	-	-	7.6	0.7	0.6	1.9	-	0.6	3.4	0.7	0.5	-	2.4	0.7	0.8	0.6	0.6	-	0.6									
26	PL 2	0.6	0.3	1.8	0.5	0.7	0.4	1.5	0.3	1.3	0.6	1.8	0.6	1	0.3	0	0.8	0.6	1.2	1.2	2.2	1.1	0.8	0.6	0.9	0.7								
27	RUM 1	0	0.5	3	0	0	0.5	2.9	0.4	1.4	0.3	3	0	1.6	0.4	0.6	0.9	0	2.6	1.3	5.3	1.4	0.7	0.7	1.1	0.7	1.5							
28	RUM 2	0.5	0.5	2.7	0.6	0.6	0.5	2.8	0.3	1.5	0.7	2.9	0.5	1.5	0.4	0.8	0.9	0.5	1.3	1.4	2.8	1.5	1	0.7	1.2	0.7	1.8	-						
29	RUM 3	3.5	5	0.9	0.3	0.2	1.7	2.5	1.2	0.5	0.3	1.1	1.6	0.6	3.8	0.4	0.3	2	2.3	0.5	0.8	0.6	0.4	12.4	0.4	1.9	0.5	0.4	0.5					
30	RUM 4	0	4.2	1	0	0	1.7	2.7	0.9	0.2	0.1	1.2	0	0.3	4.2	0	0.3	0	2.3	0.2	1	0.5	0.2	12.5	0.3	1.8	0.3	0	0.3	-				
31	RUM 5	1	0.5	2.4	0.7	0.7	0.4	2.1	0.4	2.8	0.7	2.1	1.1	2.2	0.3	1	0.9	0.9	2.4	3.2	5.6	1.6	1	0.7	1.8	0.9	5.4	2.3	2.7	0.7	0.4			
32	RUM 6	0.6	0.3	2.4	1.6	1.5	0.3	1.7	0.3	2.2	1.8	2.3	0.6	1.7	0.3	1.8	1.5	0.5	0.7	1.8	2.3	2.1	1.8	0.5	2	0.6	2.8	2.5	3.5	0.6	0.5	-		
33	RUS 1	2.8	1.9	0.4	0.3	0.2	2.9	5.9	1.4	0.2	0.3	1	1	0.3	-	0.3	0.2	1.4	1	0.2	0.3	0.3	0.3	7.1	0.2	2.1	0.3	0.3	0.3	-	4.7	0.3	0.3		
34	RUS 2	-	1.7	1.7	0	0	1	2.6	1.2	2.5	0.2	1.7	-	-	1.2	0	0.5	-	-	2.5	10.3	1.1	0.5	1.8	0.7	2.1	0.6	0	0.5	1.2	0	1.1	0.7	0.6	
35	S 2	2.7	-	0.6	0.5	0.6	-	-	4.7	0.8	0.6	1.6	-	0.6	-	0.6	0.6	2.3	2.2	0.8	0.9	0.6	0.6	-	0.8	-	0.7	0	0.5	1.6	1.4	1.1	0.5	3.2	2.3

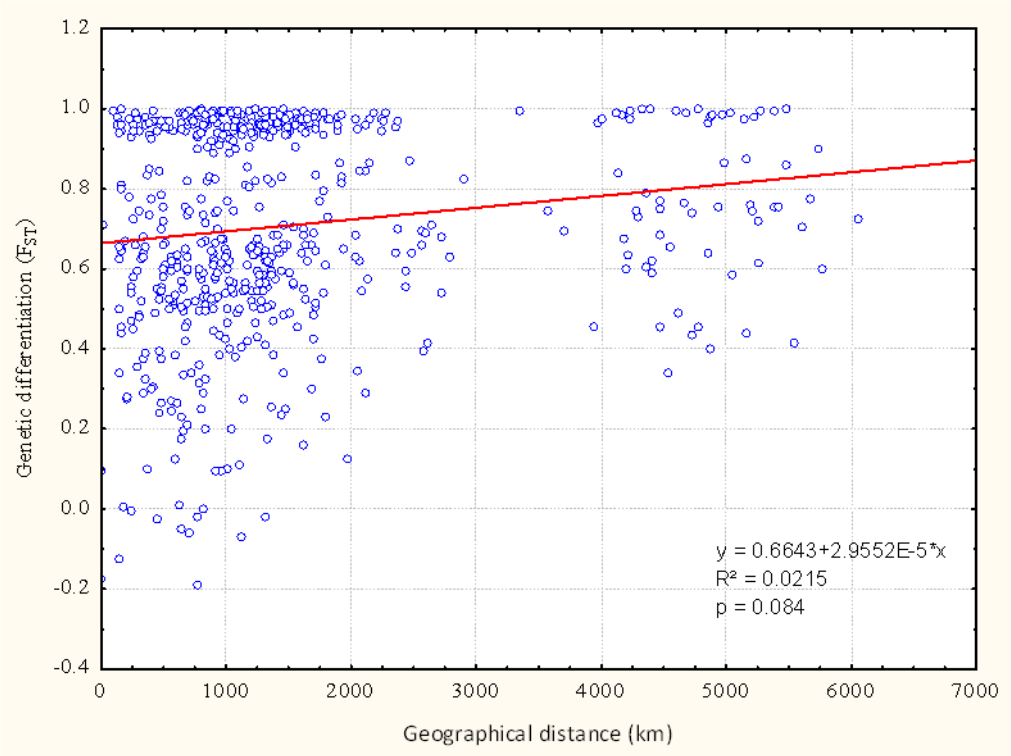

Figure A60: Linear regression of geographical distances (kilometers) vs. genetic differentiations (F_{ST}) based on *COI* of *Steganacarus magnus*. Regression is not significant (p=0.084; (*0.01<P<0.05; **0.001<P<0.01; ***P<0.001)) using 1000 randomizations.

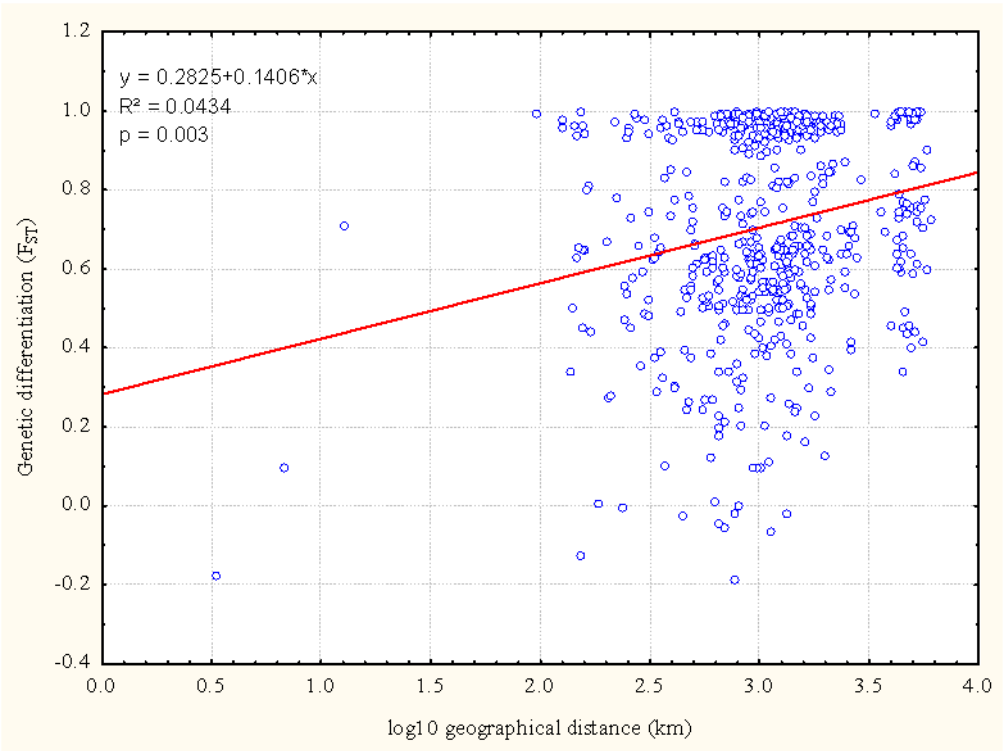

Figure A61: Linear regression of log10 geographical distances (kilometers) vs. genetic differentiations (F_{ST}) based on *COI* of *Steganacarus magnus*. Regression is significant (p=0.003**) using 1000 randomizations.

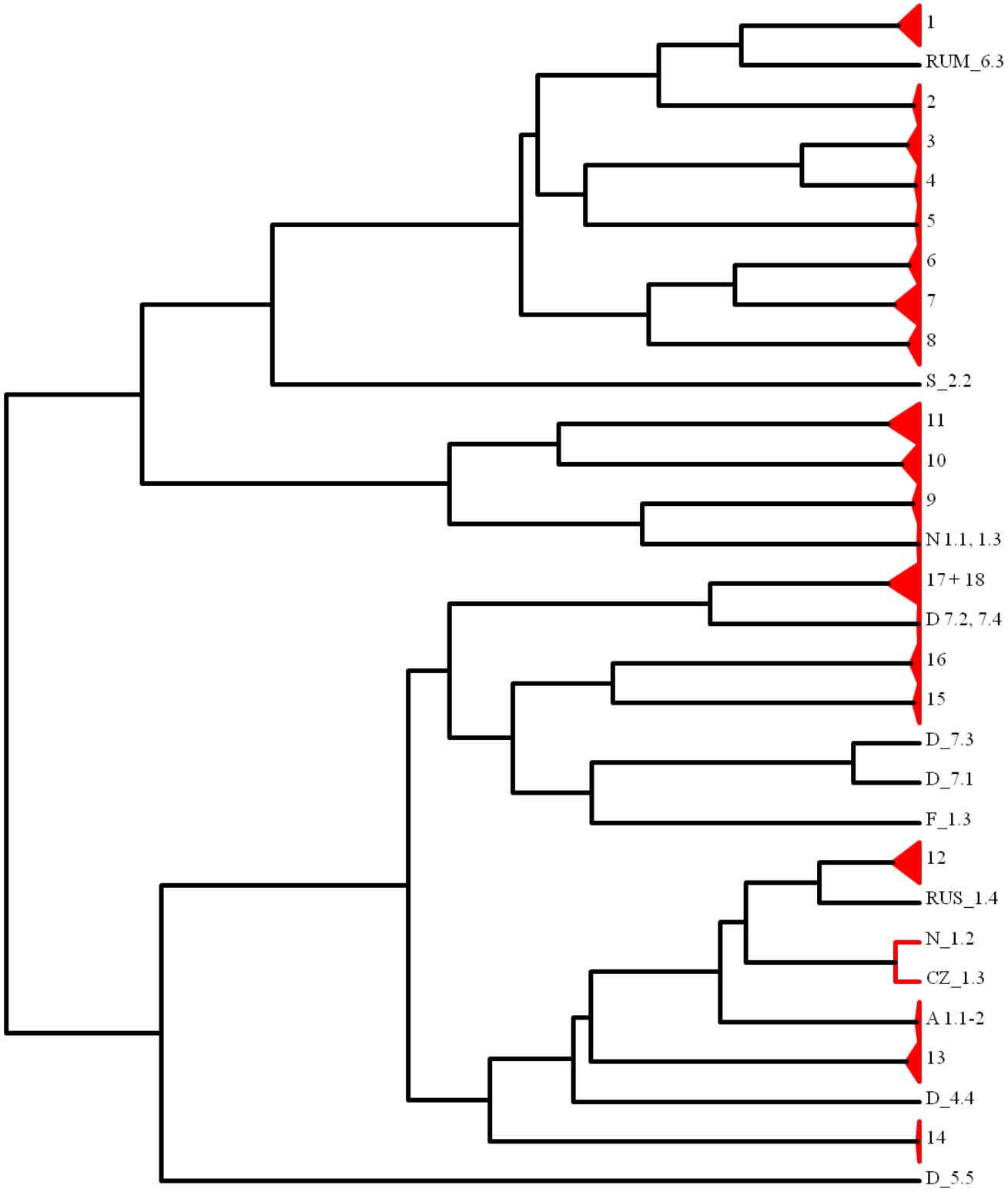

Figure A62: Cluster delimitation of *COI* nucleotide sequences from *Steganacarus magnus* after the single method of the generalized mixed Yule coalescent method (GMYC). Red branches are the different distinct clusters. Tip labels are explained in Table 28.

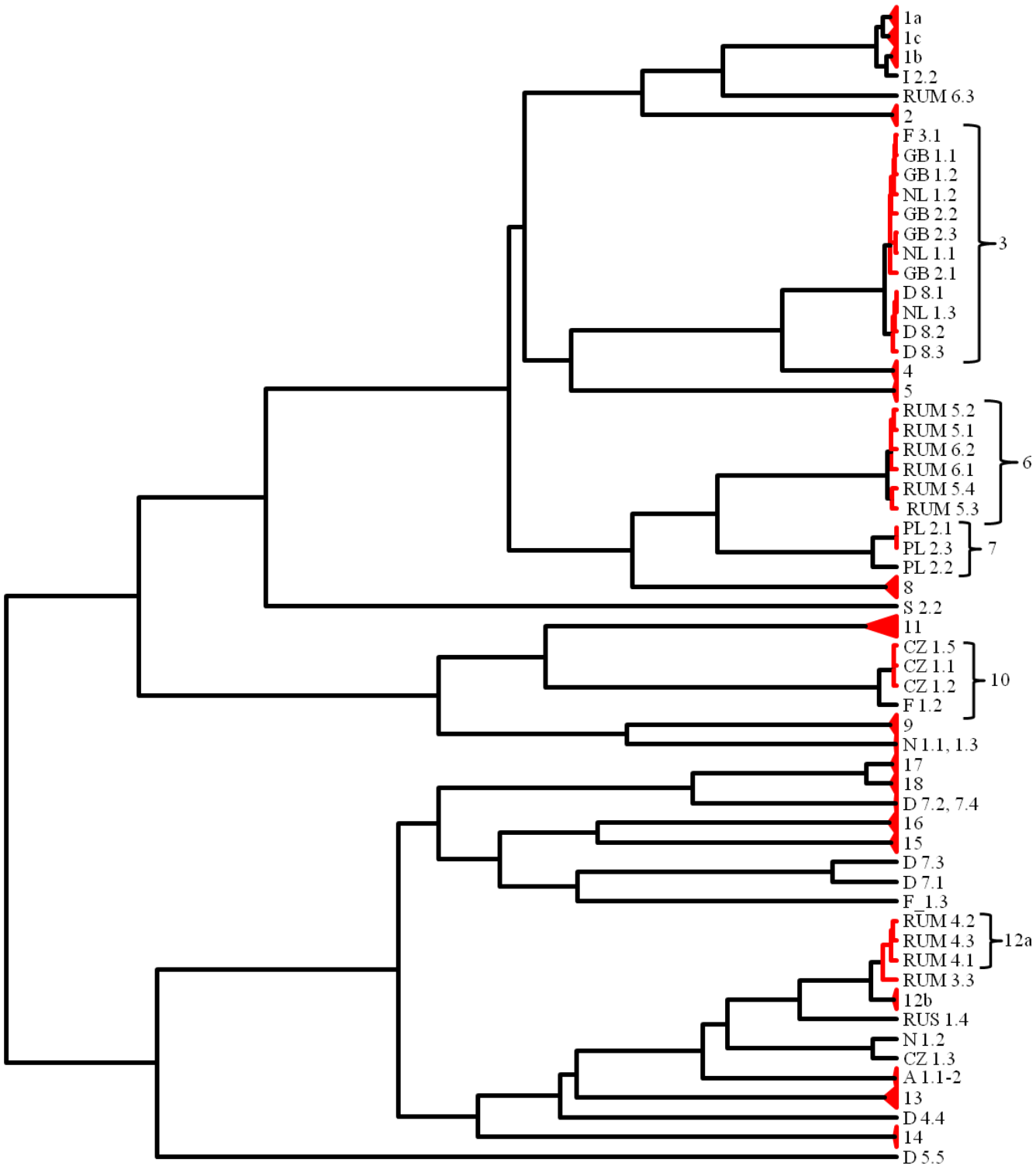

Figure A63: Cluster delimitation of *COI* nucleotide sequences from *Steganacarus magnus* after the multiple method of the generalized mixed Yule coalescent method (GMYC). Red branches are the different distinct clusters. Tip labels are explained in Table 28.

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe. Ich habe noch keinen Promotionsversuch unternommen.

Egelsbach, den 21 Oktober 2010

Martin Julien Rosenberger