Ensuring the Safety of Classical Biological Control for Cabbage Seedpod Weevil in Canada: Assessment of the Ecological Host Range of Candidate Ectoparasitoids in Europe and Clarification of their Taxonomic Status

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Summary

Classical biological control of insect pests and weeds can lead to potential conflicts, particularly if the insect pest and weed agents are closely related. Such a conflict may occur in biological control of the Cabbage Seedpod Weevil (CSPW), *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)] (Coleoptera: *Curculionidae*), which belongs to the same subfamily, *Ceutorhynchinae*, as a number of agents introduced or proposed for introduction against invasive alien weed species in North America. The CSPW is a serious pest of canola and rapeseed in North America. Some of the known European parasitoids such as *Trichomalus perfectus* Walker and *Mesopolobus morys* Walker (both Hym.; Pteromalidae) show the greatest potential for incorporation into an IPM programme in North America and are currently being considered for introduction. Prior to importation, several issues have to be assessed, and results are presented here:

Taxonomy of the European species of the genus *Trichomalus* and *Mesopolobus* associated to Ceutorhynchinae hosts were revised and illustrated keys provided to ensure up to date information is available to biological control practitioners. A three years field survey has been carried out in five European countries to field collect Ceutorhynchinae hosts, rear their ectoparasitoids and identify them. Furthermore, implications of the host-parasitoid associations recovered by the surveys are discussed relative to introduction of species to North America for classical biological control of cabbage seedpod weevil.

The host specificity of the candidate ectoparasitoids has to be determined in their native cultivated and non-cultivated habitats. A three year field survey has been carried out to document the European parasitoid assemblages associated with several Ceutorhynchinae species either already introduced in North America as classical weed biological control agents (*Microplontus edentulus* (Schultze) against scentless chamomile, *Tripleurospermum perforatum* (Mérat) Laínz (=*Matricaria perforata* Mérat) [Asteraceae]; *Hadroplontus litura* (Fabricuius) against Canada thistle, *Cirsium*

arvense (L.) *Scopoli* [Asteraceae]), proposed for introduction (*Ceutorhynchus turbatus* Schultze against hoary cress, *Lepidium draba* L. (=*Cardaria draba*) [Asteraceae]) or of importance in term of conservation (*Ceutorhynchus typhae* (=*C. floralis*) (Paykull) on Shepherd's purse, *Capsella bursa-pastoris* (L.) Medicus [Brassicaceae]). These results are discussed to estimate potential non-target effects on other *Ceutorhynchinae* species and implications for classical biological control of cabbage seedpod weevil.

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Introduction

The Convention on Biological Diversity defines an invasive alien species as follows "an alien species whose introduction and spread threatens ecosystems, habitats or species with socio-cultural, economic and/or environmental harm, and/or harm to human health" (COP, 2002). Biological invasions by non-native species constitute one of the leading threats to natural ecosystems and biodiversity, and impose an enormous cost on agriculture, forestry, fisheries, and other human enterprises, as well as on human health (Wittenberg & Cock, 2001). The ways in which non-native species affect native species and ecosystems are numerous and usually irreversible (Wittenberg & Cock, 2001). Furthermore, the costs of invasions are rising rapidly due partly to increasing human population density, and partly to intensification of production in genetically impoverished agricultural systems (Perrings *et al.*, 2002). The negative effects caused by introduced exotic species on the environment are sometimes massive but often subtle (Wittenberg & Cock, 2001).

The most important pathway of harmful exotics is unintentional importation through international trade (Perrings, 1996). One example illustrating the problem is the cabbage seedpod weevil (CSPW), *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)] (Fig. 1), which is a serious pest of oilseed rape and canola (*Brassica napus* L. and *Brassica rapa* L.) in North America where it was accidentally introduced, presumably with importation of seed stocks, about 75 years ago (McLeod, 1953). The pest was first recovered near Vancouver in British Columbia in 1931 (McLeod, 1953). It became widespread in British Columbia and the Pacific Northwest of North America and is believed to have dispersed throughout most of the United States (Baker, 1936; Hagen, 1946; Crowell, 1952; Walz, 1957; Boyd & Lentz, 1994; Buntin *et al.*, 1995). More recently, *C. obstrictus* was found in southern Alberta in 1995 (Carcamo *et al.*, 2001) and has since spread to central Alberta and western Saskatchewan (Dosdall *et al.*, 2002). In eastern Canada, *C. obstrictus* was found in Quebec in 2000 (Brodeur *et al.*, 2001) and in Ontario in 2001 (Mason *et al.*, 2003a). Based on surveys conducted from 1997 to 2000, Dosdall *et al.* (2002) determined that it is dispersing at approximately 55 Km per year.

Consequently, they predicted that *C. obstrictus* will eventually establish throughout the entire canola-growing region of western Canada. Furthermore, Olfert and Weiss (Olfert & Weiss, 2006) predicted that with increases in temperature due to climate change the risks associated with *C. obstrictus* will become more severe in areas it presently occupies, and be able to establish in new localities.

Its discovery immediately raised concern within Canada's canola industry because *C. obstrictus* is usually considered the most economically important pest infesting oilseed rape during pod development (Walters & Lane, 1994). The potential for yield loss in northern Idaho, USA, was estimated at 15-35% in untreated winter rape and 3-6 adults per sweep can cause economic losses (McCaffrey *et al.*, 1986). Populations of *C. obstrictus* remained relatively low in southern Alberta from 1995 to 1998, but in 1999 and 2000 outbreak densities occurred over an area of about 100,000 ha of canola (Dosdall *et al.*, 2002).

Fig. 1. Three trophic levels a) the crop : *Brassica napus* L., b) the pest, *Ceutorhynchus obstrictus* Marsham, and c) its principal parasitoid species in Europe, *Trichomalus perfectus* Walker.

The biology of *C. obstrictus* has been studied in detail in Europe (Bonnemaison, 1957; Dmoch, 1965; Ni *et al.*, 1990; Kjaer-Pedersen, 1992). The species is univoltine. Adults overwinter in soil or leaf litter outside agricultural fields and wild crucifers serve as important food for them in spring. Oilseed rape and other brassicaceous seed crops are colonized with the onset of flowering. The life cycle is better synchronised with winter than spring crops. Adults feed for 3 – 4 weeks on buds, flowers, pods and stem tips before mating (Williams & Free, 1979). This causes a delay in oviposition on winter rape, but oviposition starts immediately on spring rape. Females bore a hole through the pod wall with their rostrum and then insert a

single egg into the pod, which is subsequently marked with an oviposition deterrent pheromone emitted from the abdomen (Kozlowski *et al.*, 1983; Mudd *et al.*, 1997). Females lay a total of 25-240 eggs, larvae hatch after 6-10 days and feed within the pods for 14-21 days (Bonnemaison, 1957). Each larva consumes about five seeds and mature larvae leave through an exit hole, drop to the soil, and pupate within a cocoon, usually before the crop is harvested (Fox & Dosdall, 2003; Dosdall & Moisey, 2004). Adults of the next generation emerge after 15-19 days and feed on any remaining crop pods or wild hosts during July and August before dispersing to overwintering sites (Bonnemaison, 1957). In spring, overwintered adults feed and females oviposit until June when they begin to die.

Ceutorhynchus obstrictus is currently controlled through the use of broad-spectrum chemical insecticides in North America (Dosdall *et al.*, 2001; Carcamo *et al.*, 2005). In Europe, studies have shown that chemical insecticides have a negative impact on parasitoids associated with the pest in canola fields (Alford, 1995).

Compared to other control methods, classical biological control is considered as the most appropriate option to manage invasive alien species; if successful, it is often highly cost-effective, permanent, and self-sustaining (Wittenberg & Cock, 2001). Classical biological control provides an opportunity to partially reconstruct the natural enemy complex of an invading alien pest (Mills, 1994), and can be integrated with other control methods in a sustainable integrated pest management approach. In Europe, populations of the seed-feeder *C. obstrictus* are suppressed by a wide range of natural enemies, some of which might have the potential to be considered as biological control agents for *C. obstrictus* in North America. Among them, the pteromalid wasps *Trichomalus perfectus* (Walker) and *Mesopolobus morys* (Walker) are the most abundant and together account for up to 80-90% parasitism of *C. obstrictus* in Europe, even at low pest densities (Buntin, 1998; Murchie & Williams, 1998; Williams, 2003). Given the importance of parasitism in Europe (Harmon & McCaffrey, 1996; Williams, 2003) to control *C. obstrictus*, there is potential for classical biological control agents to reduce *C. obstrictus* populations in recently infested areas.

In a first attempt at biological control of cabbage seedpod weevil, three European pteromalid parasitoid species were released in British Columbia, Canada in 1949 (McLeod, 1953). In their review of the scientific literature relating to parasitoids

of the cabbage seedpod weevil, Murchie and Williams (1998) stated that "the study of literature is hampered by synonymy and misidentification". In fact, recent work by Gibson et al (2005) illustrated these words by stating that the species introduced as *Trichomalus fasciatus* (Thomson), *Xenocrepis pura* Mayr, and *Habrocytus* sp. for classical biological control of *C. obstrictus* were misidentifications of *T. perfectus*, *M. morys* and *Stenomalina gracilis* Walker (Hym.: Pteromalidae) respectively. In addition, Gibson *et al.* (2005; in press) determined that species reared subsequently from *Brassica* spp. seedpods in British Columbia and reported in the literature under the first three names were misidentifications of *Trichomalus lucidus* (Walker), *Mesopolobus moryoides* Gibson and one or both of *Pteromalus puparum* (Linnaeus) and *T. lucidus*, respectively. Therefore, neither *T. perfectus* nor *M. morys* are established in North America, but *S. gracilis* is newly recorded from British Columbia (Gibson *et al.*, in press).

In a renewed classical biological control initiative against *C. obstrictus*, both *T. perfectus and M. morys* are being considered for introduction to Canada (Kuhlmann *et al.*, 2002). In this context of misidentifications, false synonymies and lack of taxonomic information, it became critical to revise the species of *Mesopolobus* and *Trichomalus* associated with Ceutorhynchinae hosts in Europe. The work presented in the first two chapters of this thesis aims at clarifying the taxonomic status of the species involved, and providing up-to-date identification keys.

Prior to any introduction, field surveys are needed in the area of origin of candidate biological control agents to assess their ecological host range. The assessment of the host specificity in their area of origin facilitates accurate prediction of potential non-target host impacts in the area of introduction as proposed by Kuhlmann *et al.* (Kuhlmann & Mason, 2002; Kuhlmann *et al.*, 2006b). Based on current knowledge, host associations of candidate biological control agents of *C. obstrictus* in its area of origin are probably restricted to the genus *Ceutorhynchus* but certainly to the subfamily Ceutorhynchinae (Graham, 1956a; Graham, 1969; Yu *et al.*, 2005; Noyes, 2006). Prior the assessment of the ecological host range of *M. morys* and *T. perfectus*, a list of non-target host species was selected under the principle that parasitoids of *C. obstrictus* might parasitize other Ceutorhynchinae hosts in the same feeding niche (seeds), in other above-ground feeding niches (stem and leaf), as well as

in a feeding niche below ground (roots) (Kuhlmann *et al.*, 2006a). In addition, Kuhlmann *et al.* (2006a) proposed that a phenological and geographical overlap of *C. obstrictus* (target) with closely related Ceutorhynchinae species (non-target) would lead towards a higher risk that parasitoids of the target species could parasitize non-target species. In this context it is essential to know that several species of Ceutorhynchinae have been introduced, or are being considered for introduction, as classical biological control agents of weed species in North America. Those Ceutorhynchinae released or considered for introduction to control weeds in cultivated crops will be at great risk of potential attack if agents released for biological control of *C. obstrictus* are not specific to that species. This is the case for (1) *Hadroplontus litura* (Fabricius) released for the control of Canada thistle, *Cirsium arvense* (L.) *Scopoli* [Asteraceae]; (2) *Microplontus edentulus* (Schultze) for the control of scentless chamomile, *Tripleurospermum perforatum* (Mérat) Laínz (=*Matricaria perforata* Mérat) [Asteraceae], and (3) *Ceutorhynchus turbatus* (Schultze), considered for introduction against hoary cress, *Lepidium draba* L. (=*Cardaria draba*) [Asteraceae]. In addition, a fourth species, *Ceutorhynchus typhae* (=*C. floralis*) (Herbst), which is of conservation interest as it is part of the natural enemy complex associated with a weed in cultivated habitats, shepherd's purse, *Capsella bursa-pastoris* (L.) Medicus [Brassicaceae] has also been considered in this study. Results presented in the second part of this thesis document the parasitoid assemblages associated with these four species, with the aim of verifying whether or not the candidate biological control agents occur in these parasitoid assemblages and if safety issues apply or not for the classical biological control of *C. obstrictus* in Canada.

❑ *Chapter 1* provides a review of the species belonging to the genus *Trichomalus* associated with Ceutorhynchinae hosts in Europe. In order to provide taxonomic clarifications for this genus, an illustrated identification key has been developed including description and revision of the taxonomic status of each species involved.

❑ In *Chapter 2* species belonging to the genus *Mesopolobus* associated with Ceutorhynchinae hosts in Europe are reviewed and a species new to science

discovered during this study is described. In order to provide taxonomic clarifications for the genus *Mesopolobus*, an illustrated identification key has been developed together with descriptions of the species and including a revision of the taxonomic status of each species involved.

❑ ***Chapter 3*** deals with the potential conflicts that could occur between classical biological control of *C. obstrictus* and classical biological control of weeds. The objectives of this study were to: 1) describe the phenologies of *H. litura* and *M. edentulus* to determine if there is phenological overlap with *C. obstrictus*; 2) assess the parasitism levels, particularly by ectoparasitoids; and 3) determine the parasitoid assemblages associated with *M. edentulus* and *H. litura*.

❑ **Chapter 4** presents the case of two Ceutorhynchinae species closely related to *C. obstrictus* that are part of the herbivore complex associated with two weed species. The objectives of this study were to: 1) describe the phenology of *C. typhae* and *C. turbatus* to determine if there is a phenological overlap with that of *C. obstrictus*; 2) assess the parasitism levels of *C. typhae* and *C. turbatus*, particularly by ectoparasitoids; and 3) determine the parasitoid assemblage associated with these two Ceutorhynchinae species.

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A Review of the Species of *Trichomalus* (Chalcidoidea: Pteromalidae) Associated with *Ceutorhynchus* (Coleoptera: Curculionidae) Host-Species of European Origin

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Abstract

Six species of *Trichomalus* Thomson, 1878, were reared as parasitoids of Ceutorhynchinae hosts in Europe during surveys in 2000‒2004. *Trichomalus rusticus* (Walker, 1836) is treated as a valid species, resurrected from synonymy under *T. lucidus* (Walker, 1835), and *T lyttus* (Walker, 1848) is transferred from synonymy under *T. lucidus* and newly placed in synonymy with *T. rusticus*. An illustrated key is given to differentiate the six species (*T. bracteatus* (Walker, 1835), *T. campestris* (Walker, 1834), *T. gynetelus* (Walker, 1835), *T. lucidus* (Walker, 1835), *T. perfectus* (Walker, 1835) and *T. rusticus*) except for males of *T. bracteatus* and *T. gynetelus*. *Trichomalus campestris* is newly recorded as a parasitoid of *Ceutorhynchus cardariae*. Implications of the host-parasitoid associations recovered by the surveys are discussed relative to introduction of species to North America for classical biological control.

Introduction

Colonnelli (2004) stated that Ceutorhynchinae (Coleoptera: Curculionidae) contained 1,316 species, making it one of the most speciose subfamilies of Curculionidae, which itself is the most speciose family of Coleoptera. Some species of Ceutorhynchinae are used worldwide as natural enemies for classical biological control of weeds to reduce their impact in crop and non-crop habitats (Julien & Griffiths, 1998), but several species are known to be herbivorous pests of high economic importance in agricultural crops (Dieckmann, 1972; Mason & Huber, 2002). One such species, the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)], is a pest of *Brassica napus* L. (oilseed rape) in Europe and was accidentally introduced to western North America in the early 1930's (McLeod, 1953). It is now widespread in North America (Kuhlmann *et al.*, 2002), though only recently was found in the Canadian provinces of Alberta (Carcamo *et al.*, 2001), Saskatchewan (Dosdall *et al.*, 2002), Quebec (Brodeur *et al.*, 2001) and Ontario (Mason *et al.*, 2003b). In the major canola (oilseed rape) production areas in Alberta and Saskatchewan, crop losses due to *C. obstrictus* have been reduced through registration of broad-spectrum insecticides (Mason & Huber, 2002).

As an invasive alien species for North America, *C. obstrictus* was considered for classical biological control after its establishment, and in 1949 three species of larval ectoparasitoids were introduced from Europe to British Columbia, Canada (McLeod, 1962). McLeod (1953) reported one of these species as *Trichomalus perfectus* (Walker) (Hymenoptera: Chalcidoidea: Pteromalidae). *Trichomalus perfectus* is the most important parasitoid responsible for reducing populations of the cabbage seedpod weevil in Europe (Williams, 2003) and is one of several species of the genus known to be important members of parasitoid complexes associated with some Ceutorhynchinae species (Murchie & Williams, 1998). Although follow up studies to prove establishment were not carried out, *T. perfectus* was considered as established in North America until very recently. Gibson *et al.* (2005) verified from voucher material that *T. perfectus* was indeed introduced into BC in 1949, but showed that all subsequent reports of *T. perfectus* in North America were based on misidentifications of another European species, *Trichomalus lucidus* (Walker).

Classical biological control of *C. obstrictus* is again being considered for North America to provide a reduced-risk strategy alternative to insecticide applications. Accurate identification of natural enemies is the cornerstone of biological control and is essential when exotic species are introduced, especially when morphological differentiation among species is slight, such as for the species of *Trichomalus* associated with Ceutorhynchinae hosts. It is crucial to clarify the taxonomic status of closely related *Trichomalus* species to: 1) accurately document ecological data on the species associated with Ceutorhynchinae hosts in Europe, the area of origin of *C. obstrictus*, and 2) address safety issues in classical biological control initiatives.

In this paper we provide: 1) illustrated keys to differentiate females and most males of *Trichomalus* species known from Ceutorhynchinae hosts in Europe; 2) a list of type material examined together with notes on the concordance with specific characters of voucher specimens; 3) a complete list of all voucher specimens examined; 4) a short diagnosis for females and males or, if required, a more comprehensive description of the species for which taxonomic status is being changed.

Material and Methods

Our study is based primarily on surveys in Switzerland, Germany, France, Austria, Hungary, Romania and Ukraine during 2000–2004, which were made to obtain specimens of relevant species of *Trichomalus* associated with *Ceutorhynchus*. When stated as "individually reared", the specimens were obtained by dissecting host plants for *Ceutorhynchus* hosts having a larval ectoparasitoid and then rearing these individually to the adult stage (for detailed rearing methods see Chapter 3). "Mass collected" specimens were obtained by collecting and placing host plants in emergence boxes. Adult parasitoids emerged into glass vials and were collected, killed, air-dried, pinned, labelled and curated for later identification. All voucher specimens stated as collected by F. Muller, B. Klander, M. Grossrieder and M. Cripps were obtained during the 2000–2004 field surveys and are deposited in the Natural History Museum in Bern, Switzerland (NMBE). Additional material, including type specimens of relevant species, was obtained either from the NMBE or from the British Museum of Natural History (BMNH) in London, United Kingdom.

Descriptions are based on observations made using a Leica MZ16 binocular microscope coupled to a Leica CLS 150 x incandescent light source and a light diffuser placed over the specimen to reduce the effects of glare. Several images of a specimen were taken through the binocular microscope at different focal planes using a JVC KY-F70BU triple CCD digital camera and processed using the Syncroscopy Auto-Montage™ software suite. This enabled production of a single, composite, focused image, which allowed us to overcome the problems historically associated with inadequate depth of field for 3-dimensional imaging of tiny specimens. Images obtained from the Syncroscopy Auto-montage™ software suite were retouched using Adobe Photoshop CS™ to enhance clarity of the illustrations.

Terms for morphological features and sculpture follow Gibson *et al.* (1997) and Goulet & Huber (1993). Terms for colours of various body parts are taken from Graham (1969). Measurements for each species were taken from about 6–10 air dried specimens, depending on availability.

The synonymy of most *Trichomalus* species is not quoted here because it often is extensive and is readily accessible electronically in the Universal Chalcidoidea Database (Noyes, 2006); however, for those species where we introduce some nomenclatural change, the complete synonymy is given.

Identification Keys

Females

1   Procoxa testaceous, much paler than dark green body (Fig. 1b). Fore wing often with a dark discal cloud (Fig. 2a), basal fold bare and costal cell setal line widely broken in the middle...*T. campestris* (Walker)

–   Procoxa dark green with metallic tinge, concolorous with body (Fig. 1a). Fore wing hyaline (Fig. 2c, e, g, i), basal fold and costal cell with complete setal line.....
....2

2   Fore wing with marginal vein at most 1.5 x as long as stigmal vein and stigma relatively large (Fig. 2e). Flagellum with first funicular segment distinctly broader than pedicel in lateral view (Fig. 3c)...................................... *T. perfectus* (Walker)

–   Fore wing with marginal vein at least 1.6 x as long as stigmal vein and stigma comparatively smaller (Fig. 2c, g, i). Flagellum with first funicular segment about as broad as pedicel in lateral view (Fig. 3a)..3

3   Metacoxa densely setose dorsobasally (Fig. 4e) .... *T. rusticus* (Walker)

–   Metacoxa sparsely setose dorsobasally (Fig. 4a) 4

4   Gaster at most 2.5 x as long as broad (Fig. 5c). Median area of propodeum 1.25–1.4 x as broad as long .... *T. lucidus* (Walker)

–   Gaster about 2.4–3.4 x as long as broad (Fig. 5g). Median area of propodeum about 1.6–1.95 x as broad as long 5

5   Gaster about 1.6–1.75 x as long as mesosoma; last tergite 1.0–1.6 x as long as its basal breadth (Fig. 5g)..*T. bracteatus* (Walker)

–   Gaster about 1.7–2.0 x as long as mesosoma; last tergite 1.7–2.15 x as long as its basal breadth.. *T. gynetelus* (Walker)

Males

[note: *T. bracteatus* & *T. gynetelus* are keyed out according to features given by Graham (1969)]

1   Procoxa testaceous, much paler than dark green body (Fig. 1b). Antenna (Fig. 3e) with scape long and stout, only about 3 x as long as broad and broadest in middle, but about 1.0–1.2 x as long as eye height; flagellum with funicular segments 4–6 or at least 5 and 6 pale, remaining segments dark. Fore wing with basal fold bare and costal cell setal line widely broken in the middle (Fig. 2b). Gaster with a pale transverse band basally (Fig. 5b)...*T. campestris* (Walker)

–   Procoxa dark green with metallic tinge, concolorous with body (Fig. 1a). Antenna (Fig. 3b, d) with scape short and relatively slender, about 4 x as long as broad and slightly expanded apically, and at most 0.8 x as long as eye height; flagellum dark (Figs. 3b, d). Fore wing with setal lines on basal fold and on lower side of costal cell complete (Fig. 2d, f, h). Gaster at most with a pale spot basally (Fig. 5d, f) 2

2   Gena slightly compressed and with a sharp edge near base of mandible (Fig. 6b). Pronotal collar almost as broad as mesoscutum (Fig. 8b), its anterior margin less strongly curved viewed from behind (Fig. 8b). Marginal vein 1.1–1.4 x as long as stigmal vein, stigma relatively large (Fig. 2f) ....*T. perfectus* (Walker)

–   Gena rounded without sharp edge near base of mandible (Fig. 6a). Pronotal collar distinctly narrower than mesoscutum (Fig. 8a), its anterior margin more strongly curved viewed from behind (Fig. 8a). Marginal vein 1.5–1.8 x as long as stigmal vein, stigma relatively small (Fig. 2d)...3

3   Metacoxa densely setose dorsobasally (Fig. 4e) ....*T. rusticus* (Walker)

–   Metacoxa sparsely setose dorsobasally (Fig. 4b) 4

4   Propodeum medially about half as long as scutellum ....*T. lucidus* (Walker)

–   Propodeum medially slightly less than half as long as scutellum ....
        ....*T. bracteatus* (Walker) & *T. gynetelus* (Walker)

Species treatments

Trichomalus bracteatus (Walker, 1835)

Type material:

Pteromalus bracteatus Walker, 1835, lectotype female (B.M. TYPE HYM. 5.3398) in
BMNH, designated by Graham) (examined by Baur).

Material examined:

ITALY, Como: Olgiate-Com. SW Pare, 721.0/74.0, 370m; coll. 15.VI.1992, leg. H. Baur
(NMBE), collected by sweeping (1♀). **SWITZERLAND, Bern**: Boltigen, Vordere Reldigen,
592/164, 1420m.; coll. 12.VII.1997; leg. H. Baur (NMBE), collected by sweeping (1♀);
Bremgarten, Hoger, 599.4/202.9, 550m.; coll. 21.VI.2001; leg. H. Baur (NMBE); collected by
sweeping (1♀); Rumendingen, 614.9/217.5, 510m.; coll. 26.V.1992; leg. H. Baur (NMBE);
collected by sweeping (1♀). **Jura:** La Chaud-des-Breuleux, tourbière, 569/230, 970m.; coll.
18.VII.1996; leg. H. Baur (NMBE); collected by sweeping (1♀). **Valais**: NE Hohtenn
(subalpline Weide), 625.65/130.75, 1460m.; coll. 30.vi.1992; leg. H. Baur (NMBE); collected
by sweeping (1♀). **Oberwald,** Gonerlital, 671/153, 1500m.; coll. 20.VI.1999; leg. H. Baur
(NMBE); collected by sweeping (1♀). **Vaud**: Le Chenit, Burtignière (emergence trap),
502.3/156.8, 1050m.; coll. 7.–14.VIII.1994; leg. C. Vaucher (NMBE); collected by sweeping
(1♀).

Diagnosis:

Female. Length 2.5–3.1 mm (Fig. 7g). Antenna in lateral view with first funicular segment at
most slightly broader than pedicel. Procoxae dark green with metallic tinge, similar to body.
Forewing hyaline (Fig. 2i); marginal vein 1.7–2.0 x as long as stigmal vein; stigma small;
basal fold and lower side of costal cell with complete setal lines. Metacoxa sparsely setose
dorsobasally (Fig. 4d). Propodeum with median area 1.6–1.85 x as broad as long, from almost
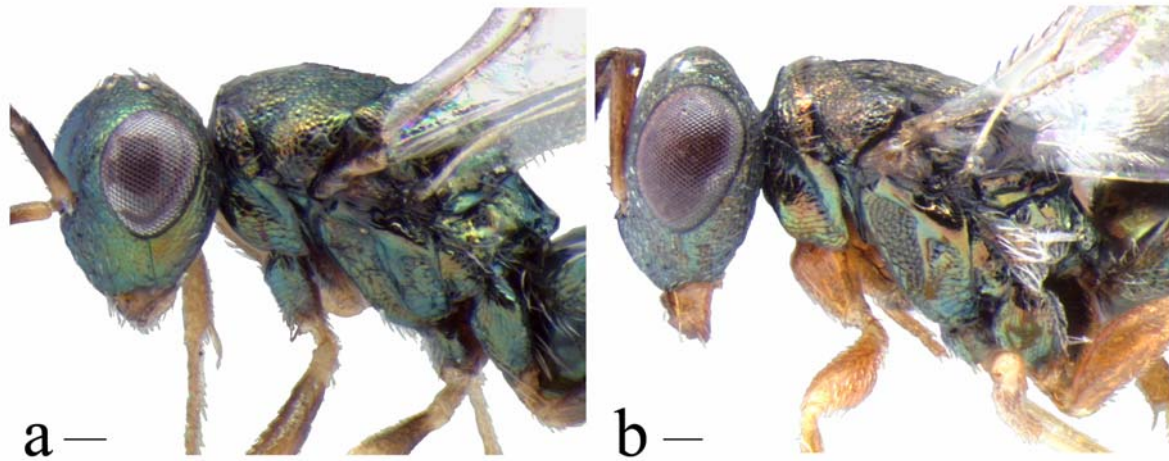
20

Fig. 1. Head and mesosoma (lateral) illustrating differences in procoxae coloration in ♀ of (a) *Trichomalus bracteatus* (Walker, 1835) and (b) *Trichomalus campestris* (Walker, 1834). Scale bars = 100 μm.

smooth to slightly strigose. Gaster 1.6–1.75 x as long as mesosoma and 2.4–3.0 x as long as broad; length of last tergite about 1.0–1.6 x its basal breadth.

Male. See characters given in the key.

Remarks:

We reared only females of *T. bracteatus* and *T. gynetelus* in our surveys. Females of theses two species are differentiated only by relatively subtle differences in gastral shape as given in the key. The differences mentioned by Graham (Graham, 1969) concerning the size of the fore wing stigma were actually not diagnostic for our specimens.

Trichomalus campestris (Walker, 1834)

Type material:

Amblymerus campestris Walker, 1834, lectotype female in BMNH, designated by Graham (Graham, 1956a) (not examined).
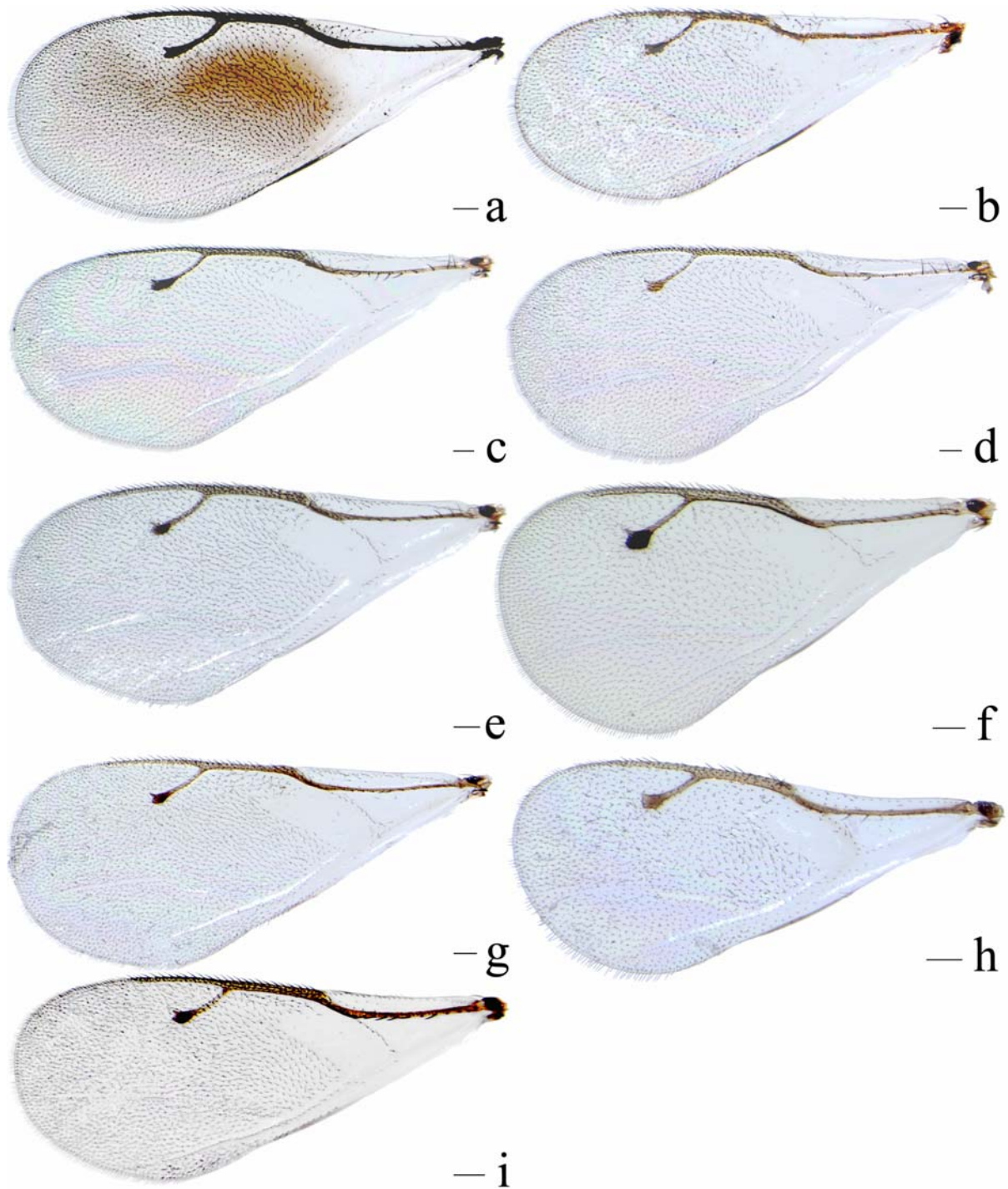
Fig. 2. Forewings of (a) ♀ *Trichomalus campestris* (Walker, 1834); (b) ♂ *T. campestris*; (c) ♀ *Trichomalus lucidus* (Walker, 1835); (d) ♂*T. lucidus*; (e) ♀ *Trichomalus perfectus* (Walker, 1835); (f) ♂ *T. perfectus*; (g) ♀ *Trichomalus rusticus* (Walker, 1836), (h) ♂ *T. rusticus*, (i) ♀ *Trichomalus bracteatus* (Walker, 1835). Scale bars = 100 μm.

Material examined:

FRANCE. Hérault: S-Guilhem-le-Désert, Les Lavagnes; coll. 30.III.2002; leg. H. Baur (1♀).
ITALY. Novara: 0.9 km E of Orta; 678.0/72.4, coll. 24.6.1993; leg. H. Baur (1♀; 1♂).
Varallo, 0.5 km S of Monte Novesso; 668.8/77.0, coll. 27.6.1993; leg. H. Baur (3 ♂).
ROMANIA. Lasi, RO12 N47°10.139 E027°28.035; coll. 12.V.2003, em. 2.–4.VII.2003; leg
M. Cripps; indiv. reared from *Ceutorhynchus cardariae* in root-crown galls of *Lepidium
draba* L. (2♂; 1♀). **SWITZERLAND. Berne:** Berne, Eymatt, 596.7/201.4; coll. 20.V.2004;
leg. H. Baur (3 ♀); Bremgarten, Hoger, 599.4/202.9; coll. 12.V.1992; leg. H. Baur (1♀); coll.
26.V.2004; leg. H. Baur (3♀). **Solothurn:** Rickenbach, 631.2/244.2; coll. 10.X.1995; leg. H.
Baur (1♀); Trimbach, Miesernbach, 632.9/246.2; coll. 25.4.2002; leg. H. Baur (2♀). **Valais.**
Baltschieder–Kumme; coll. 3.4.1999; leg. B. Merz (1♀); Simplon, Laggintal, 548.5/113.3;
coll. 21.9.1998; leg. H. Baur (1♂). **UKRAINE. Crimea:** Shchebetovka, N44°57.180
E035°07.936; coll. 17.V.2003, em. 8.VI.2003; leg. M. Cripps; indiv. reared from
Ceutorhynchus cardariae in root–crown galls of *Lepidium draba* L. (1♀)

Diagnosis:

Female. Length 1.8–2.3 mm (Fig. 7a). Antenna in lateral view with first funicular segment
about as broad as pedicel. Procoxa testaceous (Fig. 1b). Fore wing often with a dark discal
cloud (Fig. 2a); marginal vein 1.1–1.5 x as long as stigmal vein (Fig. 2a); stigma small; basal
fold bare; costal cell with setal line on lower surface widely interrupted medially. Metacoxa
densely setose dorsobasally (see Fig. 4e). Propodeum with median area 1.45–1.6 x as broad as
long, weakly sculptured. Gaster about 1.4–1.6 x as long as mesosoma and 1.7–2.1 x as long as
broad; length of last tergite 0.8–0.9 x its basal breadth (Fig. 5a).

Male. Length 1.4–1.7 mm (Fig. 7d). Procoxae testaceous (see Fig. 1b). Antenna with scape
testaceous (Fig. 3e), broadest medially, only about 3 x as long as broad and 1.0–1.2 x as long
as eye height; flagellum dark with funicular segments 4–6 or 5 and 6 testaceous (Fig. 3e).
Gena rounded near base of mandible (see Fig. 6a). Pronotal collar distinctly narrower than
mesoscutum (see Fig. 8a), its anterior margin rather strongly curved viewed from behind.
Fore wing with marginal vein 1.2–1.35 x as long as stigmal vein (Fig. 2b); basal fold bare,
costal cell with setal line on lower surface widely interrupted medially; stigma small.

Metacoxa densely setose dorsobasally. Propodeum about half as long as scutellum. Gaster with a pale transverse band basally (Fig. 5b).
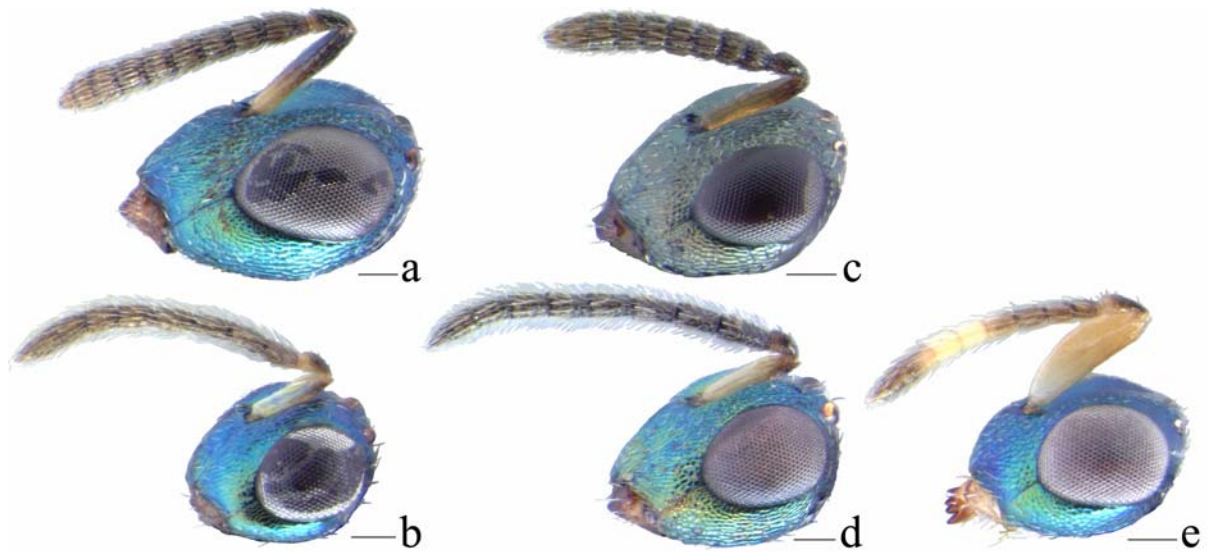

Fig. 3. Head and antennae (front-lateral) of (a) ♀ *Trichomalus lucidus* (Walker, 1835); (b) ♂ *T. lucidus* male; (c) ♀ *Trichomalus perfectus* (Walker, 1835); (d) ♂ *T. perfectus*; (e) ♂ *Trichomalus campestris* (Walker, 1834). Scale bars = 100 μm.

Remarks:

Yellowish procoxae readily differentiate both sexes of *T. campestris* from other *Trichomalus* associated with *Ceutorhynchus* species. Females are often also differentiated by having the forewing infumate behind the marginal vein, and males by structure of their scape, and antennal and gastral colour pattern. Our rearing of *T. campestris* from *Ceutorhynchus cardariae* in root-crown galls of *Lepidium draba* L. represents a new host record for the species.

Trichomalus gynetelus (Walker, 1835)

Type material:

Pteromalus gynetelus Walker, 1835, lectotype female (B.M. TYPE HYM. 5.1767) in BNHM, designated by Graham (Graham, 1956a); paralectotypes 4 female (BMNH) (examined by Baur).
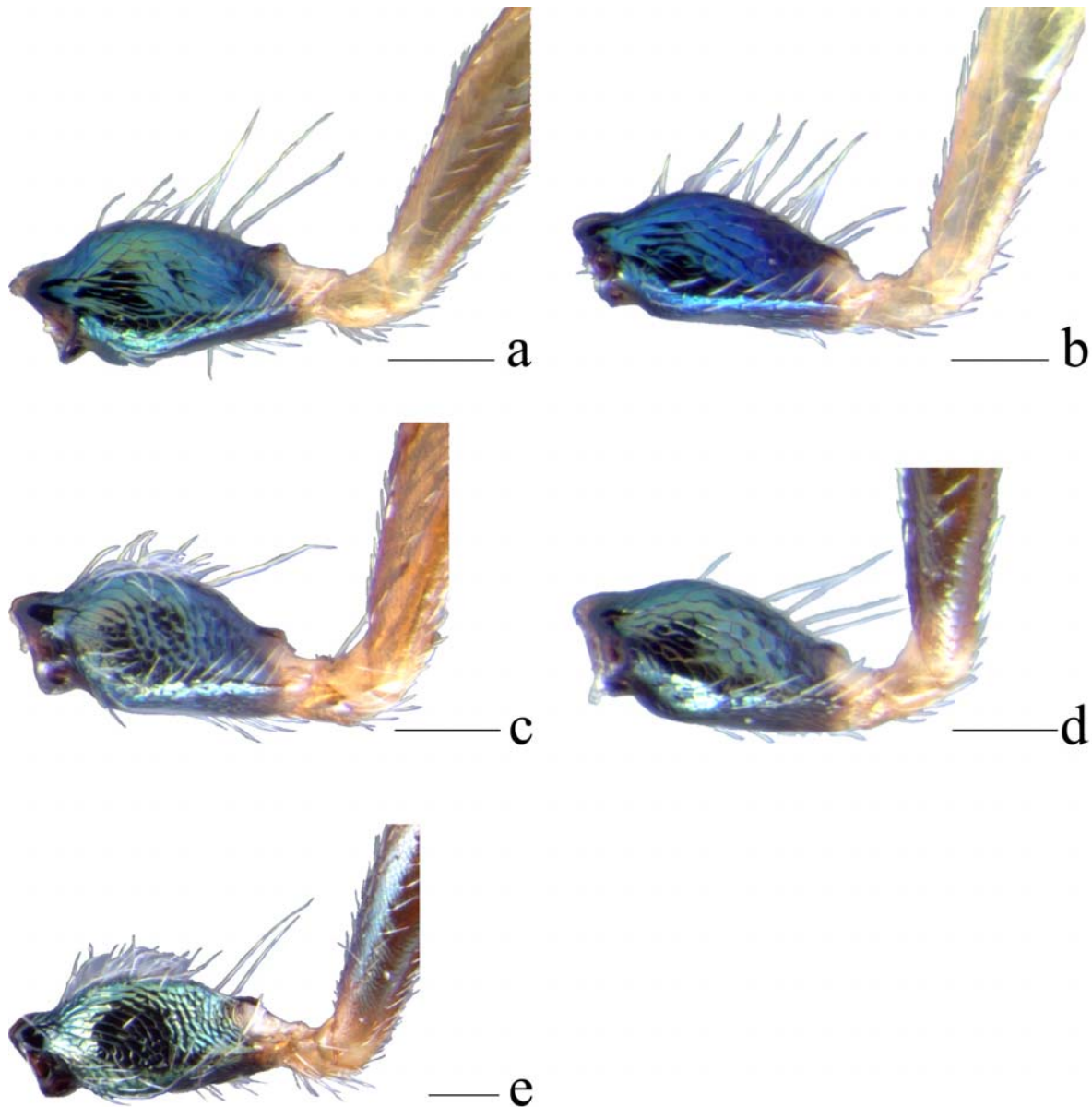
Fig. 4. Metacoxae (lateral) illustrating differences in pilosity for (a) ♀ *Trichomalus lucidus* (Walker, 1835); (b) ♂ *T. lucidus*; (c) ♀ *Trichomalus perfectus* (Walker, 1835); (d) ♀ *Trichomalus bracteatus* (Walker, 1835); (e) ♀*Trichomalus rusticus* (Walker, 1836). Scale bars = 100 μm.

Material examined:

AUSTRIA. Eisenstadt, AUS-EIS2; coll. 15.VI.2003; leg. F. Muller; indiv. reared ex. *Microplontus (Ceutorhynchus) edentulus* Schultze in stems of *Tripleurospermum perforatum* (Mérat) Laínz *(Asteraceae: Anthemideae)* (1♀). **CZECH REPUBLIC. Bohemia**: Praha-Butovice, Prokopskè Udoli, N50.02.609 E014.21.348, 100m; coll. 7.VI.2004; leg. H. Baur (NMBE); collected by sweeping (1♀). **SWITZERLAND. Valais**: NE Hohtenn (subalpline

Weide) 625.65/130.75, 1460m; coll. 30.VI.1992; leg. H. Baur (NMBE); collected by sweeping (1♀). Fully, Les Follatères, 571.6/108.0, 620m; coll. 18.VI.2004; leg. H. Baur (NMBE); collected by sweeping (1♀). **Jura**: La Chaud-des-Breuleux, La Baumatte, 569/230, 1100m; coll. 18.VII.1996; leg. H. Baur (NMBE); collected by sweeping (1♀).

Diagnosis:

Female. Length 2.4–3.1 mm (Fig. 7h). Antenna in lateral view with first funicular segment at most slightly broader than pedicel. Procoxae dark green with metallic tinge, similar to body. Forewing hyaline (see Fig. 2a); marginal vein 1.6–2.0 x as long as stigmal vein; stigma of medium size; basal fold and lower side of costal cell with complete setal lines. Metacoxa sparsely setose dorsobasally (see Fig. 4c). Propodeum with median area 1.65–1.95 x as broad as long, from almost smooth to slightly strigose. Gaster 1.7–1.95 x as long as mesosoma and 2.9–3.4 x as long as broad; length of last tergite about 1.7–2.15 x its basal breadth.

Male. See characters given in the key.

Remarks:

 See *T. bracteatus*.

Trichomalus lucidus (Walker, 1835)

Type material:

Pteromalus lucidus Walker, 1835, lectotype female (B.M. TYPE HYM. 5.3394) in BMNH, designated by Graham (Graham, 1956a) (specimen glued on card rectangle, head and mesosoma with strong coppery tinges; metacoxae covered with glue, hence some setae probably worn off; however, only few pores of setae present) (examined by Baur).

Pteromalus brevicornis Walker, 1835, lectotype female (B.M. TYPE HYM. 5.1772) in BMNH, designated by Graham (Graham, 1956a) (specimen glued on card rectangle, head and mesosoma with strong coppery tinges) (examined by Baur).
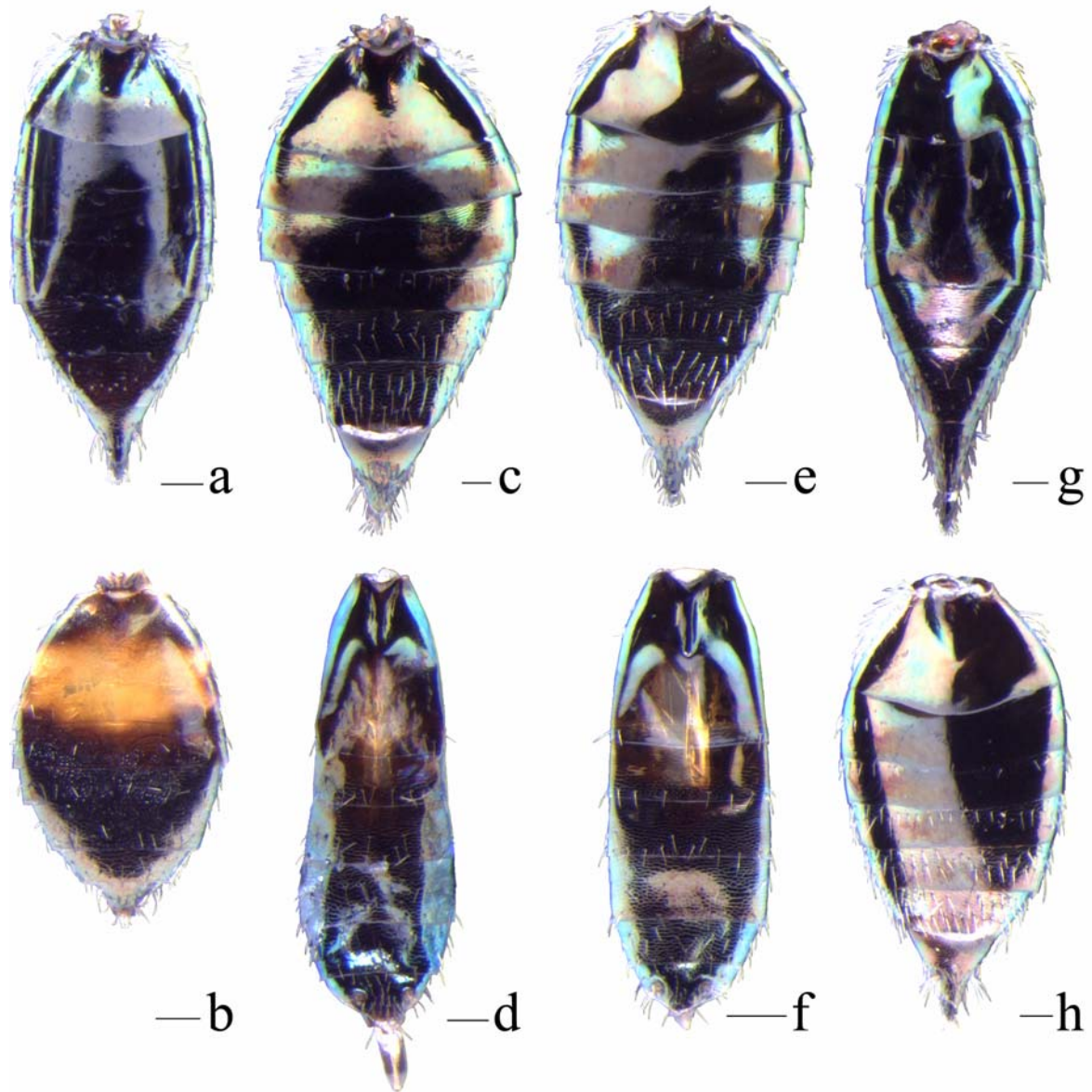
Fig. 5. Metasoma (dorsal) of (a) ♀ *Trichomalus campestris* (Walker, 1834); (b) ♂ *T. campestris*; (c) ♀ *Trichomalus lucidus* (Walker, 1835); (d) ♂ *T. lucidus*; (e) ♀ *Trichomalus perfectus* (Walker, 1835); (f) ♂ *T. perfectus*; (g) ♀ *Trichomalus bracteatus* (Walker, 1835); (h) ♀ *Trichomalus rusticus* (Walker, 1836). Scale bars = 100 μm.

Pteromalus chalceus Walker, 1835, lectotype female (B.M. TYPE HYM. 5. 1770) in BMNH, designated by Graham (Graham, 1956a) (specimen glued on card rectangle) (examined by Baur).

Pteromalus despectus Walker, 1835, lectotype female (B.M. TYPE HYM. 5.1771) in BMNH, designated by Graham (Graham, 1956a) (specimen glued on card rectangle) (examined by Baur).

Pteromalus mundus Förster, 1841, lectotype male in Natural History Museum, Vienna, Austria, designated by Graham (Graham, 1956a) (not examined; primary homonym of *P. mundus* Walker, 1836).

Isocyrtus (Trichomalus) fasciatus Thomson, 1878, lectotype female in Zoological Museum, Lund University, Lund, Sweden, designated by Graham (Graham, 1956a) (not examined).

Pteromalus purus Dalla Torre, 1898, replacement name for *Pteromalus mundus* Förster, 1841, not Walker, 1836.

Material examined:

GERMANY. Baden-Württemberg: Rheintal, Müllheim; coll. 2000, leg. B. Klander; indiv. reared, ex. *Ceutorhynchus roberti* Gyllenhal 1837 in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (1♂); Neuenburg; coll. 31.V.2001 & 19.VI.2001; leg. B. Klander; indiv. reared, ex. *Ceutorhynchus roberti* Gyllenhal 1837 in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (2♂; 2♀); Zienken; coll. 2000; leg. B. Klander; indiv. reared, ex. *Ceutorhynchus roberti* Gyllenhal 1837 in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (1♂). **Brandenburg-Berlin:** Berlin, Schoenebeck; coll. 2.VI.2001; leg. M. Grossrieder; indiv. reared, ex. *Ceutorhynchus alliariae* Gyllenhal in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (1♂; 1♀); coll. 5.VI.2001; leg. M. Grossrieder; indiv. reared, ex. *Ceutorhynchus scrobicollis* Nerensheimen & Wagner in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (1♂; 1♀). **Brandenburg;** coll. 19.–30.V.2001; leg. M. Grossrieder; indiv. reared, ex. *Ceutorhynchus alliariae* Gyllenhal in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (2♂); coll. 19.–25.V.2001; leg. M. Grossrieder; indiv. reared, ex. *Ceutorhynchus scrobicollis* Nerensheimen & Wagner in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (1♂; 1♀). **Schleswig-Holstein:** Griebel; coll. 28.VI.2001; leg. M. Grossrieder; indiv. reared, ex. *Ceutorhynchus alliariae* Gyllenhal in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (1♀). Hotzkaten; coll. 2000; leg. M. Grossrieder; indiv. reared, ex. *Ceutorhynchus alliariae* Gyllenhal in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (1♂). **SWITZERLAND. Baselland**: Grellingen; coll. 30.V.–26.VI.2001, leg. M. Grossrieder; indiv. reared, ex. *Ceutorhynchus roberti* Gyllenhal 1837 in stems of *Alliaria*
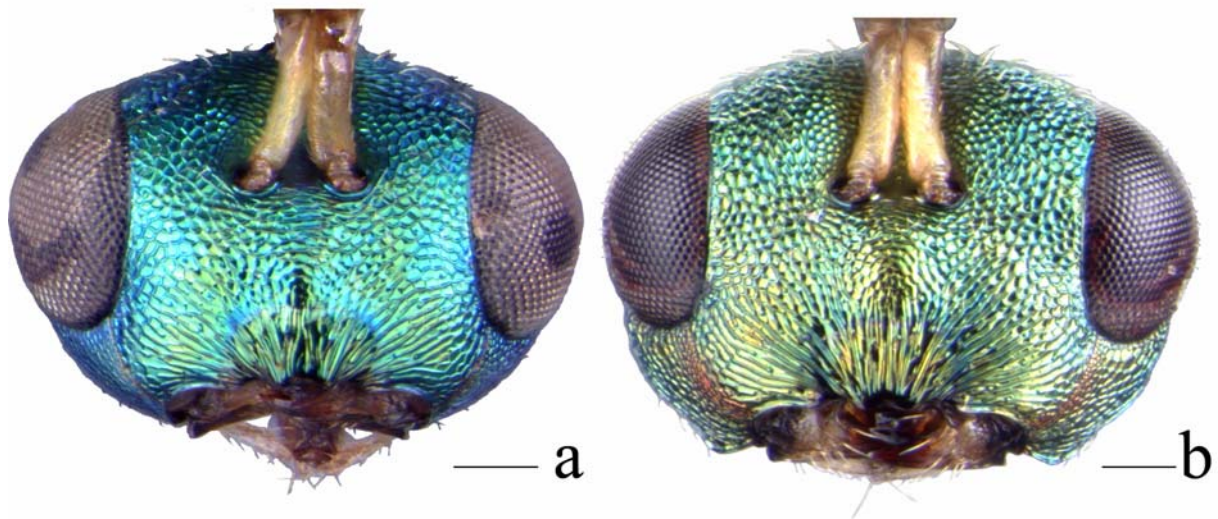
Fig. 6. Head (baso-lateral) illustrating differences in the shape of gena in ♂ of (a) *Trichomalus lucidus* (Walker, 1835) and (b) *Trichomalus perfectus* (Walker, 1835). Scale bars = 100 μm.

petiolata (M. Bieb.) Cavara & Grande (38♂; 19♀). **Fribourg:** Sugiez; coll. 19.VI.2000, leg. M. Grossrieder, ex *Ceutorhynchus quadridens* in cabbage (1♀; 1♂). **Jura:** Delemont; coll. 18.VI.2002, leg. F. Muller; indiv. reared, ex. *Ceutorhynchus alliariae* or *C. roberti* Gyllenhal 1837 in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (1♀); same, coll. 30.V.2002 (1♂) and coll. 3.VI.2002 (1♂); Delemont, Vorbourg; coll. 5.VI.2001, leg. M. Grossrieder; indiv. reared, ex. *Ceutorhynchus roberti* Gyllenhal 1837 in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (2♂); Undevelier; coll. 25.V.2001, leg. M. Grossrieder; indiv. reared, ex. *Ceutorhynchus roberti* Gyllenhal 1837 in stems of *Alliaria petiolata* (M. Bieb.) Cavara & Grande (1♂).

Diagnosis:

Female. Length 2.5–3.1 mm (Fig. 7b). Antenna in lateral view with first funicular segment at most slightly broader than pedicel (Fig. 3a). Forewing hyaline (Fig. 2c); marginal vein 1.7–2.0 x as long as stigmal vein (Fig. 2c); stigma small; basal fold and lower side of costal cell with complete setal lines. Procoxae dark green with metallic tinge, similar to body (see Fig. 1a). Metacoxa sparsely setose dorsobasally (Fig. 4a). Propodeum 1.25–1.4 x as broad as long, median area weakly sculptured. Gaster 1.25–1.4 x as long as mesosoma and 2.1–2.4 x as long as broad; length of last tergite about 0.7–0.9 x its basal breadth (Fig. 5c).

Male. Length 1.9–2.5 mm (Fig. 7e). Procoxae dark (see Fig. 1a). Antenna with scape testaceous, distinctly expanded apically (Fig. 3b), about 4 x as long as broad and about 0.75 x eye height; flagellum dark. Gena rounded near base of mandible (Fig. 6a). Pronotal collar almost as broad as mesoscutum (see Fig. 8b), its anterior margin rather strongly curved when viewed from behind. Fore wing with marginal vein 1.55–1.8 x as long as stigmal vein; basal fold and lower side of costal cell with complete setal lines (Fig. 2d); stigma small. Metacoxa sparsely setose dorsobasally (Fig. 4b). Propodeum about half as long as than scutellum. Gaster with a pale spot basally (Fig. 5d).

Remarks:

Of the lectotypes listed above that we examined, all agree well with the diagnosis of *T. lucidus* and are therefore considered conspecific. We accept the synonymy of Graham (1969) for the other names. Females of *T. lucidus* and *T. rusticus* are very similar, as discussed under the latter species.

Trichomalus perfectus (Walker, 1835)

Type material:

Pteromalus perfectus Walker, 1835, lectotype female (B.M. TYPE HYM. 5.1798) in BMNH, designated by Graham (1956) (examined by Gibson).

Material examined:

FRANCE. Alsace: Faverois, FAV, N47.518947 EO7.053317; coll. 6.VII.204; leg. F. Muller; Mass. Coll, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L.(7♂; 10♀); indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L.(2♂; 3♀); Boron, BRN3, N47.536484 E07.006693; coll. 21.VI.2004; leg. F. Muller, indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L (1♀). **GERMANY. Schleswig-Holstein**: Rastorfer Passau, RP, N54.283 E10.350; coll. 3.– 28.VI.2002 & 1.–10.VII.2002; leg. F. Muller, indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (15♂; 7♀); Kiel, coll. 24.VI.1999, leg. B. Klander ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (1♀); same, coll.
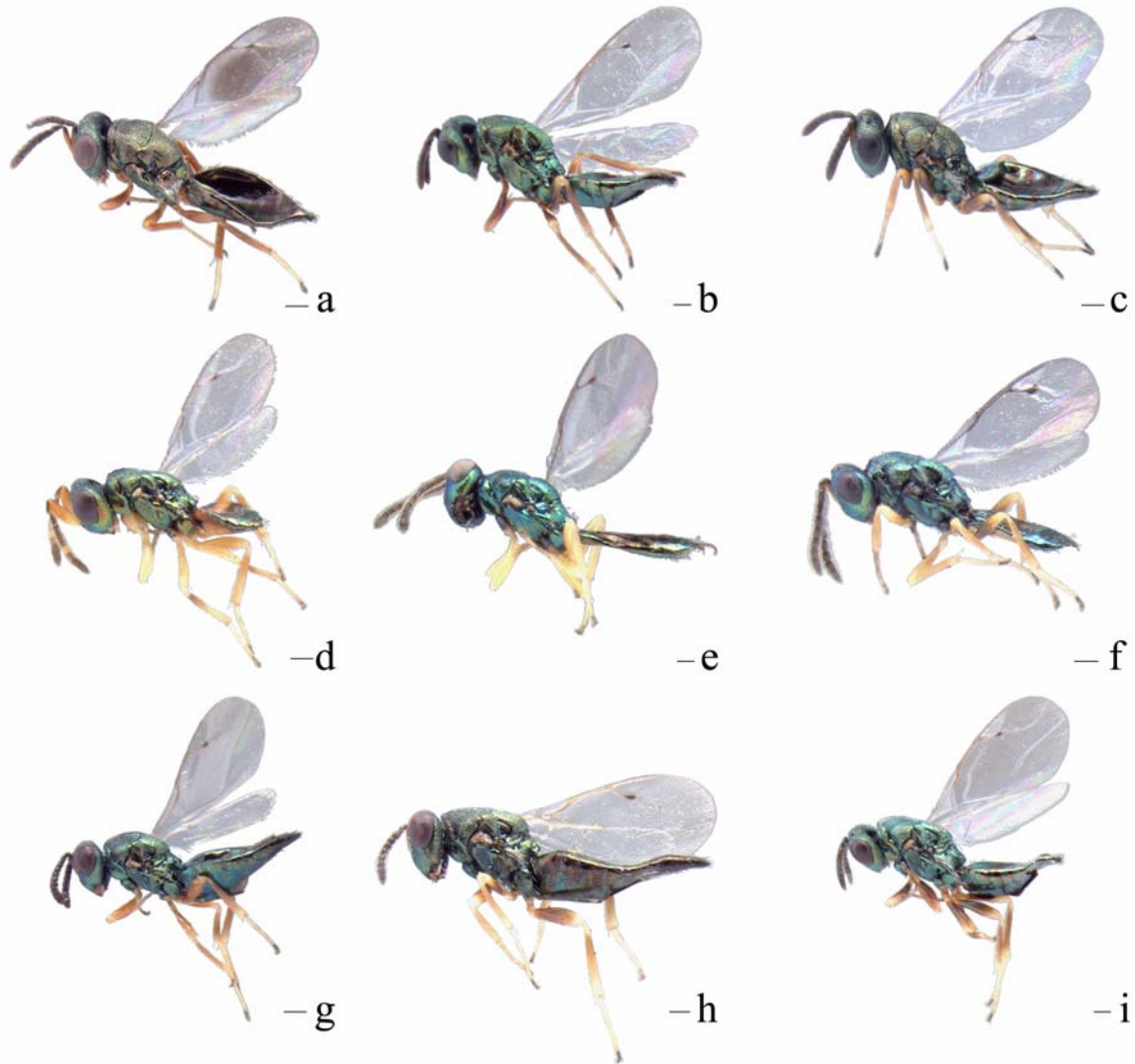
Fig. 7. Habitus of (a) ♀ *Trichomalus campestris* (Walker, 1834); (b) ♀ *Trichomalus lucidus* (Walker, 1835); (c) ♀ *Trichomalus perfectus* (Walker, 1835); (d) ♂ *T. campestris*, (e) ♂ *T. lucidus*, (f) ♂ *T. perfectus*, (g) ♀ *Trichomalus bracteatus* (Walker, 1835); (h) ♀ *Trichomalus Gynetulus* (Walker, 1835), (i) ♀ *Trichomalus rusticus* (Walker, 1836). Scale bars = 100 µm.

5.VI.2000 (2♂). **SWITZERLAND. Jura**: Chatillon, La Prîre, CHA11, N47.334285 E07.332301; coll. 8.VI.2004; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (1♀); CHA12, N47.329621 E07.331510; coll. 22.VI.2004; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (1♂); CHA14, N47.338940 E07.332363; coll. 15.VI.2004; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (2♂);CHA-LP2; coll. 20.VI.2003; leg.

F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. .(1♂; 6♀); coll. 28.VI.2002; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (1♂; 1♀); CHA-LP3; coll. 9.–25.VI.2002 & 1.VII.2002; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (8♂; 3♀); CHA-1, coll. 9., 18.,19., 22., 26. & 28.VI.2002 & 1.VII.2003, leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (7♂; 6♀); Delémont, Le Chavelier DEL-DOM; coll. 10.–13. & 20.VI.2003; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (11♂; 5♀); Courgenay, CGY1, N47.404000 E07.156737; coll. 21.VI.2004, leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (1 ♂). **Fribourg**: Galmiz, Gal, N46.989 E07;100; coll. 28.VII.2004; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (1 ♂; 1♀).

Diagnosis:

Female. Length 1.65–2.9 mm (Fig. 7c). Antenna in lateral view with first funicular segment distinctly broader than pedicel (Fig. 3c). Procoxae dark green with metallic tinge, similar to body (see Fig. 1a). Forewing hyaline (Fig. 2e); marginal vein 1.35–1.5 x as long as stigmal vein (Fig. 2e); stigma of medium size, on average slightly larger than in *T. lucidus* (see Fig. 2c, e); basal fold and lower side of costal cell with complete setal lines. Metacoxa densely setose dorsobasally (Fig. 4c). Propodeum with median area 1.45–1.6 x as broad as long, strigose-reticulate. Gaster 1.2–1.4 x as long as mesosoma and 1.8–2.1 x as long as broad; length of last tergite 0.8–0.95 x its basal breadth (Fig. 5e).

Male. Length 1.6–2.2 mm (Fig. 7f). Procoxae dark (see Fig. 1a). Antenna with scape testaceous, distinctly expanded apically (Fig. 3d), about 4 x as long as broad and about 0.75 x eye height; flagellum dark (Fig. 3d). Gena slightly compressed and with sharp edge near base of mandible (Fig. 6b). Pronotal collar almost as broad as mesoscutum (Fig. 8b), its anterior margin only weakly curved when viewed from behind. Fore wing with marginal vein 1.1–1.4 x as long as stigmal vein; basal fold and lower side of costal cell with complete setal lines (Fig. 2f); stigma relatively large. Metacoxa densely setose dorsobasally. Propodeum about half as long as scutellum. Gaster with a pale spot basally (Fig. 5f).

Remarks:

Individuals of *T. perfectus* are differentiated from other species of *Trichomalus* associated with *Ceutorhynchus* species by a combination of features as given in the key.
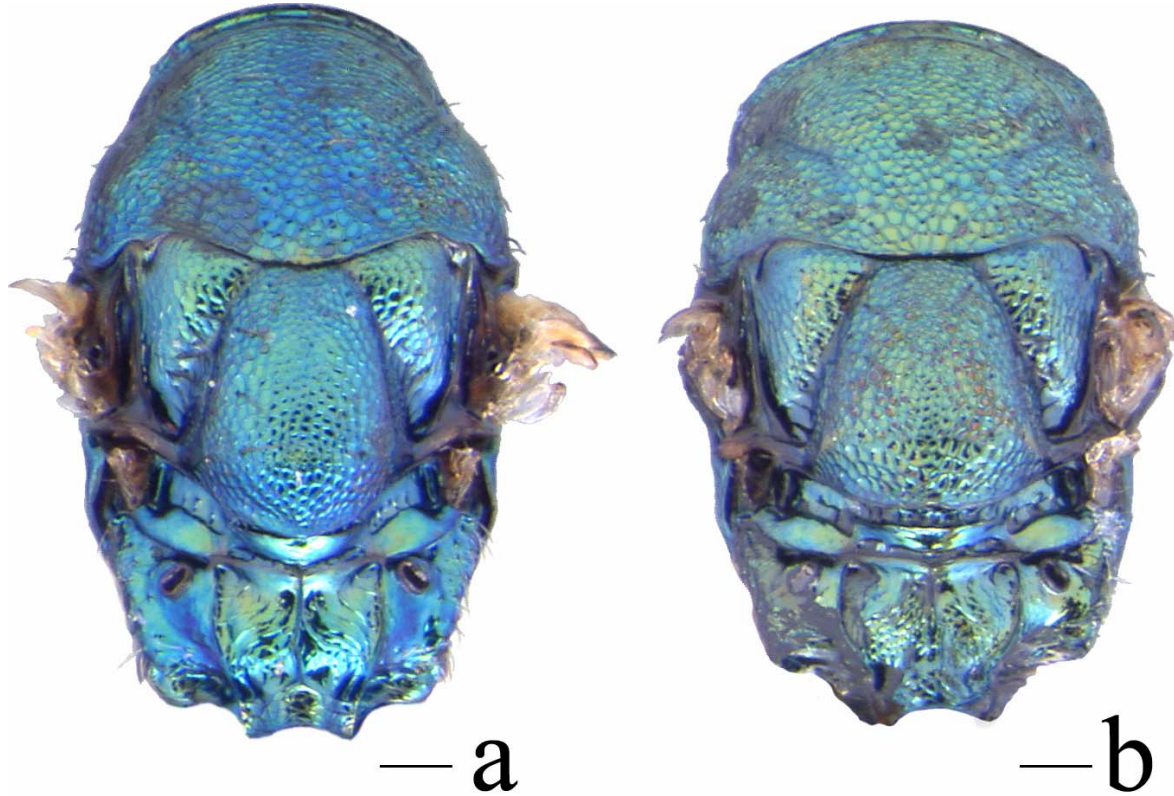
Fig. 8. Mesosoma (dorsal) illustrating differences in pronotal collars in ♂ of (a) *Trichomalus lucidus* (Walker, 1835) and (b) *Trichomalus perfectus* (Walker, 1835). Scale bars = 100 µm.

Trichomalus rusticus (Walker, 1836) revised status

Type material:

Pteromalus rusticus Walker, 1836, lectotype female (B.M. TYPE HYM. 5.3396) in BMNH, **present designation**; labelled "Pteromalus rusticus Walker; Stood under this name in old B. M. Coll. C. Waterhouse. [back side of former label]; Pteromalus rusticus W. LECTOTYPE M. de V. Graham 1956; B.M. TYPE HYM. 5.3396" (specimen glued on card rectangle, the right metacoxa and gaster lacking; badly covered with dust, color of legs and venation partly darkened and certainly an artefact) (examined by Baur).

Pteromalus lyttus Walker, 1848, lectotype female (B.M. TYPE HYM. 5.1773) in BMNH, designated by Graham (Graham, 1956a) (specimen glued on card rectangle, body with rather strong coppery tinges) (examined by Baur) **syn. n.**

Material examined:

SWEDEN. Akarp; coll. 17.I.1962; leg. H. Von Rosen (NMBE); collected by sweeping (1♀); **Lomma;** coll. 17.I.1962 & 1.I.1964; leg. H. Von Rosen, collected by sweeping (5♀). **SWITZERLAND. Neuchatel**: St-Blaise; coll. 10.IV.1978 & 13.–16.IV.1979; leg. J. Casas (NMBE); collected by sweeping (6♀); Sugiez; coll. 19.VI.2000; leg. B. Klander (NMBE); found in cabbage, ex. *Ceutorhynchus quadridens*? (1♀). **Solothurn:** Hüniken, S Tannfeld; 20.IX.1993; leg. H. Baur (NMBE) (1 ♀).

Diagnosis:

Female. Length 2.3–2.9 mm (Fig. 7i). Antenna with scape dark in apical three quarters, pedicel and flagellum dark; scape 0.75–0.9 x eye height; pedicel about 1.8 x as long as broad in lateral view; pedicel plus flagellum 0.85–1.0 x as long as head breadth; flagellum slender, slightly clavate; first funicular segment rather slender, about 1.25 x as long as broad, only slightly broader than pedicel in lateral view; last funicular segment distinctly transverse, about 0.8 x as long as broad. Head 2.1–2.2 x as broad as long, 1.2–1.35 x as long as mesoscutum, finely reticulate with minute and high meshes on vertex; POL 1.6–1.8 x OOL; eye 1.3–1.4 x as high as broad, separated by 1.25–1.4 x their height; malar space 0.44–0.53 x as long as eye height; clypeus striate, weakly emarginate medially. Body dark metallic green (Fig. 7i); legs testaceous except coxae concolorous with body and femora slightly dark. Mesosoma 1.6–1.7 x as long as broad. Pronotal collar about one seventh as long as mesoscutum length. Mesoscutum 1.45–1.55 x as broad as long, reticulate with sculpture consisting of small meshes anteriorly and larger ones posteriorly; scutellum 0.9–0.95 x as broad as long, reticulate. Fore wing hyaline (Fig. 2g); marginal vein 1.6–1.8 x as long as stigmal vein; stigma small, separated by about 2.5–3.1 x its height from hind margin of postmarginal vein; basal fold and lower side of costal cell with complete setal lines; speculum open below. Metacoxa densely setose dorsobasally (Fig. 4e); metafemur about 3.8–4.4 x as long as broad. Propodeum with complete plicae, median area finely alutaceous, about 1.2–1.4 x as long as broad and 0.5–0.6 x as long as scutellum; costulae indicated laterally, sometimes complete; nucha weakly reticulate; callus moderately hairy. Gaster (1.3–) 1.5–1.9 x as long as broad

(Fig. 5h) and 0.9–1.17 x as long as mesosoma, ovate and acuminate; length of last tergite about 0.55–0.8 x its basal breadth; sides of basal tergite with conspicuous patch of whitish hairs basally.

Male. Unknown.

Remarks:

Pteromalus rusticus was listed by Graham (1969) among the synonyms of *T. lucidus*, but the lectotype designation was not published in that or any other paper (see Delucchi & Graham, 1956; Graham, 1969). Hence the specimen is regarded as a syntype that is herewith designated as lectotype. As suggested by Graham (Graham, 1956a), *T. rusticus* is very similar to *T. lucidus*, but we consider it is a distinct species. Although males are unknown, females of *T. rusticus* can be differentiated from those of *T. lucidus* by the following features (features of *T. lucidus* in brackets): Femora, especially metafemur, infuscate in basal half [mostly testaceous]. Sculpture on vertex, mesoscutum and scutellum relatively strong, with high meshes [sculpture finer]. Marginal vein usually shorter, 1.6–1.8 x as long as stigmal vein [1.7–2.0 x]. Base of metacoxa with thick batch of setae, which extend somewhat to outer aspect of coxa [setae sparse and confined to dorsal aspect]. Gaster normally 1.5–1.9 x as long as wide, however one specimen has a ratio of only 1.3 [about 2–2.4 x].

Discussion

Results of our study have direct implications for documenting ecological baseline data of parasitoid-Ceutorhynchinae host associations, which is essential to a renewed classical biological initiative against *C. obstrictus* in Canada. *Trichomalus lucidus* is confirmed as a parasitoid of *C. alliariae* and *C. roberti*, two candidate classical biological control agents against *Alliaria petiolata* (garlic mustard) (Gerber *et al.*, 2003), which poses a severe threat to biodiversity of forest habitats (Blossey *et al.*, 2002) in much of the eastern and Midwestern U.S.A. We did not rear *T. lucidus* from *C. obstrictus* in our surveys, though Klander (2001) reported that 1.7% of the parasitoids she reared from *C. obstrictus* on *B. napus* from Schleswig-Holstein, Germany, in 2000, were *T. lucidus*, and this species is purportedly one of the major parasitoids of *C. obstrictus* in North America (Gibson et al., 2005). Based on this

apparent ecological difference, molecular analyses are warranted for specimens identified as *T. lucidus* in Europe and North America in order to test whether the populations actually represent two cryptic species. Also, for the first time we record *T. campestris* as a parasitoid of the gall-forming weevil *C. cardariae*, a potential agent for classical biological control of *Lepidium draba* (whitetop) in North America. We reared *T. perfectus* only from *C. obstrictus*, which is its main host; it has only rarely been recorded from other *Ceutorhynchus* spp. hosts in Europe (Klander, 2001). Consequently, this species could be considered for introduction to North America for classical biological control of *C. obstrictus*.

The number of concerns regarding potential non-target effects of invertebrate biological control agents of arthropods has risen during the last decade and an increasing number of studies have since dealt with this topic (Babendreier *et al.*, 2005). However, understanding the population dynamics of Ceutorhynchinae species of economic importance is still hampered by the insufficient knowledge of the natural enemy complexes that may play an important role in the regulation of these herbivorous insects (Vidal, 2003). Clarification of the taxonomy of *Trichomalus* species presented here provides a sound basis for understanding these dynamics, leading towards the safe use of *Trichomalus* species in future biological control approaches against Ceutorhynchinae pest species.

Acknowledgments

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A Review of the Species of *Mesopolobus* (Chalcidoidea: Pteromalidae) Associated with *Ceutorhynchus* (Coleoptera: Curculionidae) Host-Species of European Origin.

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Abstract

Four species of *Mesopolobus* Westwood, 1833, were reared as parasitoids of Ceutorhynchinae hosts in Europe during surveys in 2000─2004. An illustrated key is given to differentiate the four species (*M. gemellus* Baur&Muller sp. nov., *M. incultus* (Walker, 1834), *M. morys* (Walker, 1848), and *M. trasullus* (Walker, 1848)) except for males of *M. trasullus*. *Mesopolobus morys* is for the first time accurately associated with the seed weevil *Ceutorhynchus turbatus* (Schultze), a potential agent for classical biological control of hoary cress, *Lepidium draba* L. [Asteraceae] in North America. *Mesopolobus gemellus* is for the first time associated with another seed weevil, *Ceutorhynchus typhae* (=*C. floralis*) (Herbst). Furthermore, implications of the host-parasitoid associations recovered by the surveys are discussed relative to introduction of species to North America for classical biological control of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)].

Introduction

In the last two decades increasing concerns have been expressed regarding potential non-target effects of invertebrate biological control agents of arthropods (Howarth, 1991; Simberloff & Stiling, 1996; Thomas & Willis, 1998; Stiling & Simberloff, 2000; Louda, 2003; Stiling, 2004). This has led to an increasing number of studies investigating non-target effects in many systems and several matters have recently been addressed with the aim of standardizing methods in risk assessment studies worldwide (Babendreier *et al.*, 2005; Simberloff, 2005; Wright *et al.*, 2005; Kuhlmann *et al.*, 2006b). Undoubtedly, understanding the many trophic relationships between parasitic wasps used in classical biological control programmes, the target pest species they are aimed at, and potential non-target species is a key requirement prior to any introduction of candidate parasitoids. One of the many constraints biological control practitioners very frequently encounter is that the taxonomy of the involved groups is often unclear (Van Driesche & Reardon, 2004).

The subfamily Ceutorhynchinae is the most speciose of the family Curculionidae, itself the most diverse family of Coleoptera (Colonnelli, 2004). Ceutorhynchinae contains about 1,316 species among which a certain number is considered to be herbivorous pests of high economic importance in agricultural crops (Dieckmann, 1972; Mason & Huber, 2002). A number of *Ceutorhynchus* species such as the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)], the cabbage stem weevil (*Ceutorhynchus napi* (Marsham)), the cabbage seedstalk curculio (*Ceutorhynchus pallidactylus* (Marsham)), and the turnip gall weevil (*Ceutorhynchus pleurostigma* Marsham) are pests of economic importance in cruciferous crops. Simultaneously, a number of Ceutorhynchinae species are used worldwide for classical biological control of weeds in crop and non-crop habitats (Julien & Griffiths, 1998). In North America, Ceutorhynchinae released to reduce the impact of weed species include *Mogulones crucifer* (Pallas) for houndstongue *Cynoglossum officinale* (L.) [Boragincaceae] (De Clerck-Floate & Schwarzlaender, 2002), *Hadroplontus litura* (Fabricius) for Canada thistle, *Cirsium arvense* (L.) Scopoli [Asteraceae] (McClay *et al.*, 2002a), and *Microplontus edentulus* (Schultze) for scentless chamomile, *Tripleurospermum perforatum* (Merat) Lainz [Asteraceae] (McClay *et al.*, 2002b). The

existing interrelationships of pests and beneficial agents belonging to the same subfamily add one level of complexity to the work of biological practitioners.

The Chalcidoidea, one of the most important Hymenoptera groups, contains over 800 different species that have been associated with biological control programmes in one or another way (Noyes, 2006). A number of *Mesopolobus* (Chalcidoidea: Pteromalidae) species are known to be important members of parasitoid assemblages associated with several Ceutorhynchinae species (Murchie & Williams, 1998). For instance, *Mesopolobus morys* Walker is one of the most important parasitoids involved in the cabbage seedpod weevil natural enemy complex in Europe (Williams, 2003). *Ceutorhynchus obstrictus* is a pest of oilseed rape, *Brassica napus* L., in Europe and was accidentally introduced in North America where it is now widespread (Kuhlmann *et al.*, 2002). As an invasive alien species *C. obstrictus* has always been considered as a prime target for classical biological control (McLeod, 1962). In a first attempt at classical biological control of *C. obstrictus,* several larval ectoparasitoids, including *M. morys* were released into British Columbia in 1949 (McLeod, 1953). Later, McLeod (1962) reported that *M. morys* might have already been present, presumably accidentally introduced with *C. obstrictus*. Until very recently this species was considered to be established in North America although follow up studies were never conducted. Recently, the pest has been reported from the Canadian provinces of Alberta (Carcamo *et al.*, 2001) Saskatchewan (Dosdall *et al.*, 2002), Quebec (Brodeur *et al.*, 2001), and Ontario (Mason *et al.*, 2003b). *C. obstrictus* is currently controlled through the use of broad-spectrum chemical insecticides (Dosdall *et al.*, 2001; Carcamo *et al.*, 2005). Classical biological control has recently been reconsidered with the aim to reduce the use of chemical cultural control methods. Gibson *et al.* (2005) recently reviewed the parasitoid – *C. obstrictu*s associations in Canada and determined that data on *M. morys* recoveries after its initial introduction in 1949 were actually based on successive misidentifications of a previously undescribed species, *Mesopolobus (Xenocrepis) moryoides* Gibson, presumably of North American origin (Gibson *et al.*, 2005). Thus, in contrast to previous beliefs, *M. morys*, one of the most important parasitoid species regulating *C. obstrictus* in Europe, is not present in North America.

The importance of systematics in relation to pest management, environment, ecology and quarantine and regulatory activities has been reported by several authors and many studies relate its importance in particular on the relationship of taxonomy to biological control (Knutson & Murphy, 1988; Huber *et al.*, 2002; Bigler *et al.*, 2005). Indeed, accurate identification of natural enemies is the cornerstone of biological control and essential when

exotic biological control agents are introduced, especially when morphological variation among species in the genus *Mesopolobus* associated with Ceutorhynchinae hosts is slight. To decide whether to introduce European parasitoids of *C. obstrictus* into North America, it is crucial to provide clarification of the taxonomic status of these *Mesopolobus*. Taxonomic clarification has: 1) implications for providing accurate ecological baseline data on parasitoid species associated with Ceutorhynchinae hosts in Europe, the area of origin: and 2) applications towards ensuring safety of classical biological control initiatives.

In this paper we provide: 1) illustrated keys to identify females and males of all *Mesopolobus* species known from *Ceutorhynchinae* hosts in Europe; 2) a list of type material examined together with notes on the concordance with specific characters of voucher specimens; 3) a complete list of all voucher specimen examined; and 4) a short diagnosis for females and for males or, if required, a more comprehensive description of the new species or those for which taxonomic status is being changed. Most important characters included in the determination keys and diagnosis are illustrated.

Material and Methods

Our study is based primarily on surveys in Switzerland, Germany, France, Austria, Hungary, Romania and Ukraine during 2000–2004, which were made to obtain specimens of relevant species of *Trichomalus* associated with *Ceutorhynchus*. When stated as "individually reared", the specimens were obtained by dissecting host plants for *Ceutorhynchus* hosts having a larval ectoparasitoid and then rearing these individually to the adult stage (for detailed rearing methods see Chapter 3). "Mass collected" specimens were obtained by collecting and placing host plants in emergence boxes. Adult parasitoids emerged into glass vials and were collected, killed, air-dried, pinned, labelled and curated for later identification. All voucher specimens stated as collected by F. Muller, B. Klander, M. Grossrieder and M. Cripps were obtained during the 2000–2004 field surveys and are deposited in the Natural History Museum in Bern, Switzerland (NMBE). Additional material, including type specimens of relevant species, was obtained either from the NMBE or from the British Museum of Natural History (BMNH) in London, United Kingdom.

Descriptions are based on observations made using a Leica MZ16 binocular microscope coupled to a Leica CLS 150 x incandescent light source and a light diffuser

placed over the specimen to reduce the effects of glare. Several images of a specimen were taken through the binocular microscope at different focal planes using a JVC KY-F70BU triple CCD digital camera and processed using the Syncroscopy Auto-Montage™ software suite. This enabled production of a single, composite, focused image, which allowed us to overcome the problems historically associated with inadequate depth of field for 3-dimensional imaging of tiny specimens. Images obtained from the Syncroscopy Auto-montage™ software suite were retouched using Adobe Photoshop CS™ to enhance clarity of the illustrations.

Terms for morphological features and sculpture follow Gibson *et al.* (1997) and Goulet & Huber (1993). Terms for colours of various body parts are taken from Graham (1969). Measurements for each species were taken from about 6–10 air dried specimens, depending on availability.

The synonymy of most *Mesopolobus* species is not quoted here because it often is extensive and is readily accessible electronically in the Universal Chalcidoidea Database (Noyes, 2006); however, for those species where we introduce some nomenclatural change, the complete synonymy is given.

Identification Keys

Females

[Note: *Mesopolobus moryoides* Gibson also belongs to the group of *Mesopolobus* species treated here, but has only been recorded from North America (Gibson *et al.* 2005)].

1   Tegula dark (Fig. 1g). Median area of propodeum smooth, plica indicated in posterior third only (Fig. 1g) ....*M. trasullus* (Walker)

–   Tegula pale (Fig. 1a – c). Median area of propodeum finely aletaceous, plica more or less complete (Fig. 1 a – c) 2

2(1)   Tip of hypopygium reaching two thirds along the gaster. Gaster 2.0–2.5 x as long as broad (Fig. 2c). Pedicel plus flagellum about 0.85–0.95 x as long as head breadth (Fig. 3c) .... *M. incultus* (Walker)

–   Tip of hypopygium reaching about half way along the gaster. Gaster 1.35–1.9 x as long as broad (Fig. 2a, b). Pedicel plus flagellumabout 0.7–0.8 x as long as head breadth (Fig. 3a, b) 3

3(2)   Speculum extending about to the middle of marginal vein (Fig. 4a, b); basal fold with 2–5 hairs (Fig. 1a, b). Flagellum slightly infuscate (Fig. 3a)............................ ....*M. gemellus* Baur&Muller sp. nov.

–   Speculum extending about to the distal end of marginal vein (Fig. 4d, e); basal fold with 0–1 hair (Fig. 4d, e). Flagellum slightly paler (Fig. 3b) ....*M. morys* (Walker)

Males

[note: *M. trasullus* is keyed out according to features given by Askew *et al.* (1969)]

1   Tegula dark (see Fig. 1g). Median area of propodeum almost smooth, plica indicated in posterior third only ....*M. trasullus* (Walker)

–   Tegula pale except sometimes apically (Fig. 1d – f). Median area of propodeum finely reticulate, plica more or less complete (Fig. 1 d – f) 2

2(1)   Marginal vein inflated and only about 4.5 x as long as broad (Fig. 4f). Head more transverse in dorsal view, occiput less concave. Gaster with pale spot medially (Fig. 2f)..*M. morys* (Walker)

–   Marginal vein not inflated more than 6 x as long as broad (Fig. 4c, h). Head less transverse in dorsal view, occiput more concave. Gaster without pale spot medially (Fig. 2e, g) 3

3(2)   Head in frontal view 1,23–1.31 times as broad as high (Fig. 5a), gena curved ....
....*M. gemellus* Baur&Muller sp. nov.

–   Head in frontal view 1,14–1.21 times as broad as high (Fig. 1c), gena rather straight ....*M. incultus* (Walker)

Species treatments

Mesopolobus gemellus **Baur & Muller sp. nov.**

Type material:

Holotype male labelled: "ALE 2 coll. 28.VI.2004/61(1) found as larval parasitoid; Alle/ALE2 Jura (Ajoie)/Switzerland GPS lat. N47.436608 long. E07.141965; Ex.: Ceuto. floralis in: C. bursa-pastoris leg. F. Muller/CABI-CH; Holotype ♂ *Mesopolobus gemellus* sp. n. det. Baur & Muller 2005".

Paratypes: **GERMANY. Ostholstein:** Nähtkamp; coll. 14.vi.2000; leg. B. Klander; indiv. reared from larval parasitoids, ex *Ceutorhynchus typhae* (=*C. floralis*) in fruits of *Capsella bursa pastoris* (L.) Medik. (17♂; 15♀). **Schleswig-Holstein:** Kiel, Scharnhagen; coll. 16–22.vi.2001; leg. B. Klander; indiv. reared from larval parasitoids, ex *Ceutorhynchus typhae* (=*C. floralis*) in fruits of *Capsella bursa pastoris* (L.) Medik. (13♂; 25♀). **SWITZERLAND. Canton Jura:** Alle, Les Genavrats, 490 m, N47°26'11.79" E7° 8'31.07", 28.vi.2004, leg. F. Muller, ex *Ceutorhnchus typhae* (Herbst) (Coleoptera: Curculionidae) in fruits of *Capsella bursa-pastoris* (L.) Medik. (23♂; 15♀). Mass collected material from various sites in: **FRANCE. Alsace**: Faverois, FAV, N47°31'8.21" E7°3'11.94" & **SWITZERLAND. Valais:** valley from Martigny to Sion; coll. 9–29. vi. 2004; leg. F. Muller; mass collected adults emerged from pods of *Capsella bursa pastoris* (L.) Medik. placed in emergence boxes (41♂; 31♀).

Material:

SWITZERLAND. Valais: Martigny, N46°6'36.49" E7°6'12.82"; coll. 29.VI.2004; leg. F. Muller, ex *Ceutorhynchus turbatus* Schulze in fruits of *Lepidium draba* L. (1♀).

Etymology

The specific name is formed after the latin noun "gemellus", meaning twin. It refers to the close relationship of this species with *M. morys*.
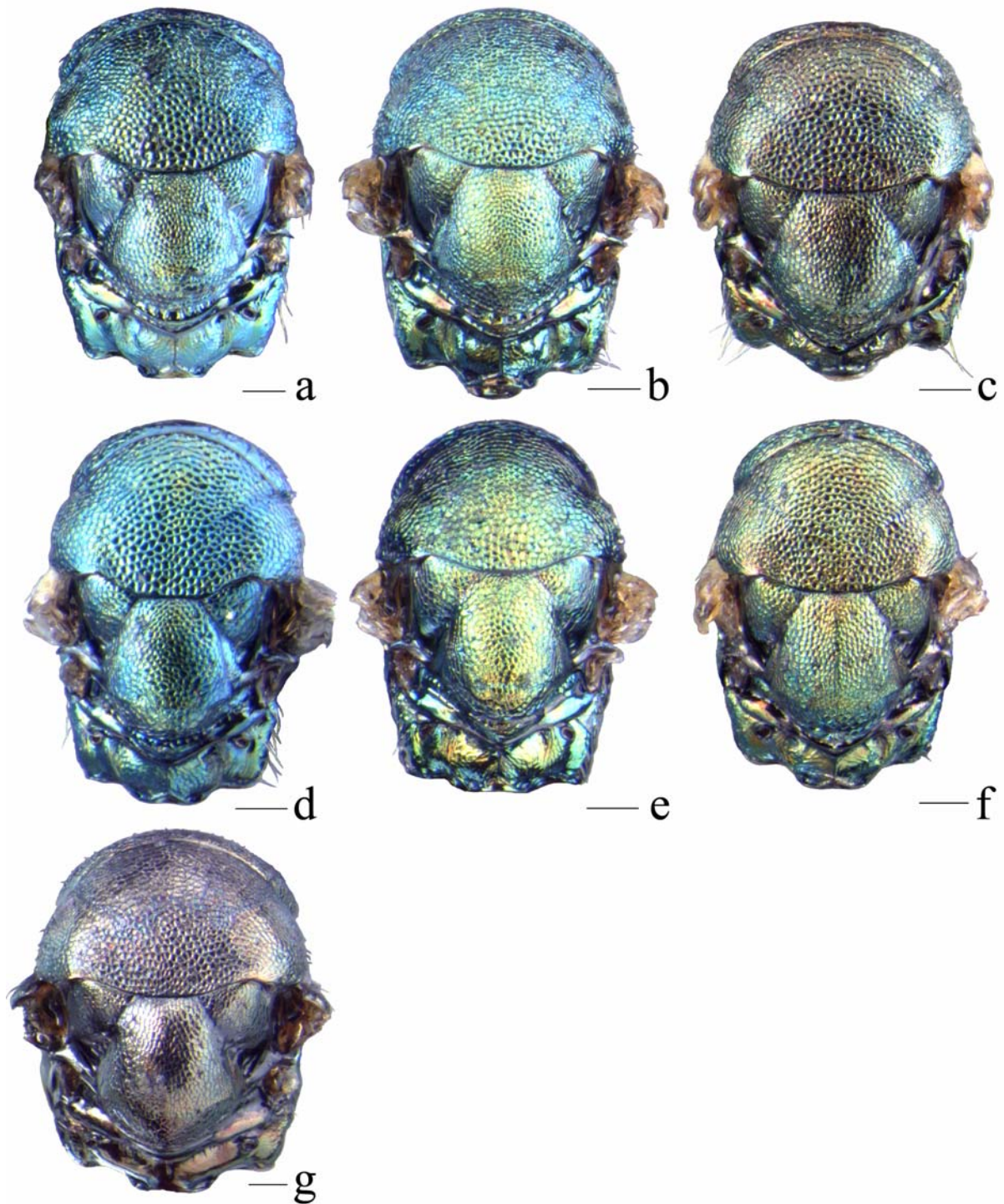
Fig. 1. Mesosoma (dorsal) of (a) ♀ *Mesopolobus gemellus* Baur & Muller sp. nov.; (b) ♀ *Mesopolobus morys* (Walker, 1848); (c) ♀ *Mesopolobus incultus* (Walker, 1834); (d) ♂ *M. gemellus*; (e) ♂ *M. morys*; (f) ♂ *M. incultus*. Scale bars = 100 μm.

Diagnosis

Female. Length: 1.5–2 mm (Fig. 6a). Flagellum weakly infuscate (Fig. 3a). Pedicel plus flagellum about 0.75–0.8 x as long as head breadth. Tegulae pale (Fig. 1a). Speculum

extending to distal end of marginal vein (Fig. 4a, b); basal fold with 2–5 hairs (Fig. 4a, b). Median area of propodeum finely reticulate, plica more or less complete. Gaster 1.3–1.6 (to 1.9) x as long as broad (Fig. 2a); tip of hypopygium reaching about half way along the gaster.

Male. Length: 1.55–1.85 mm (Fig. 6d). Head in frontal view 1.23–1.31 x as broad as high (Fig. 5a), genae curved; in dorsal view about 1.95–2.05 x as broad as long, occiput rather strongly excaavate. Marginal vein inflated and only about 4.5 x as long as broad (Fig. 4f); tegula pale (Fig. 1d). Median area of propodeum finely reticulate, plica more or less complete. Gaster with indistinct pale spot at base (Fig. 2e).

Description

Holotype male.

Length: 1.85 mm.

Head bright green with blue tinge in some lights; scape citron yellow; pedicel and flagellum slightly infuscate dorsally, paler below. Mesosoma bright green with bluish tinge. Tegulae yellow. Veins light testaceous. Legs citron yellow except for dark apical tarsal segments. Gaster green with indistinct pale spot at base.

Head in dorsal view 1.97 x as long as broad, 1.27 x as broad as high; occiput rather strongly excavate; POL 2.07 x OOL; malar space 0.63 x eye height; eye 1.23 x as high as broad; face finely reticulate, clypeus reticulate with anterior margin truncate. Antenna with lower edge torulus inserted very slightly above level of lower ocular line; antennal formula 111363; scape not quite extending to ventral margin of anterior ocellus, about 5 x as long as broad and 0.91 x eye height; pedicel in dorsal view 1.8 x as long as broad, slightly longer than anelli plus first funicular segment; combined length of pedicel plus flagellum 0.92 x as long as head breadth; flagellum distinctly clavate; anelli strongly transverse; first funicular slightly transverse and distinctly smaller than second funicular segment; second funicular segment slightly longer than broad, fifth transverse; clava 2.1 x as long as broad, slightly shorter than combined length of 3 apical segments (collapsed in holotype), funicular and claval segments with single row of longitudinal setae.

Mesosoma 1.5 x as long as broad. Pronotal collar differentiated but rounded anteriorly, about one ninth as long as mesoscutum, reticulate with a smooth stripe along postrerior margin; mesoscutum 0.6 x as long as broad, finely reticulate with meshes only very slightly smaller in anterior part; scutellum about as long as broad, 0.92 x as long as mesoscutum, finely reticulate
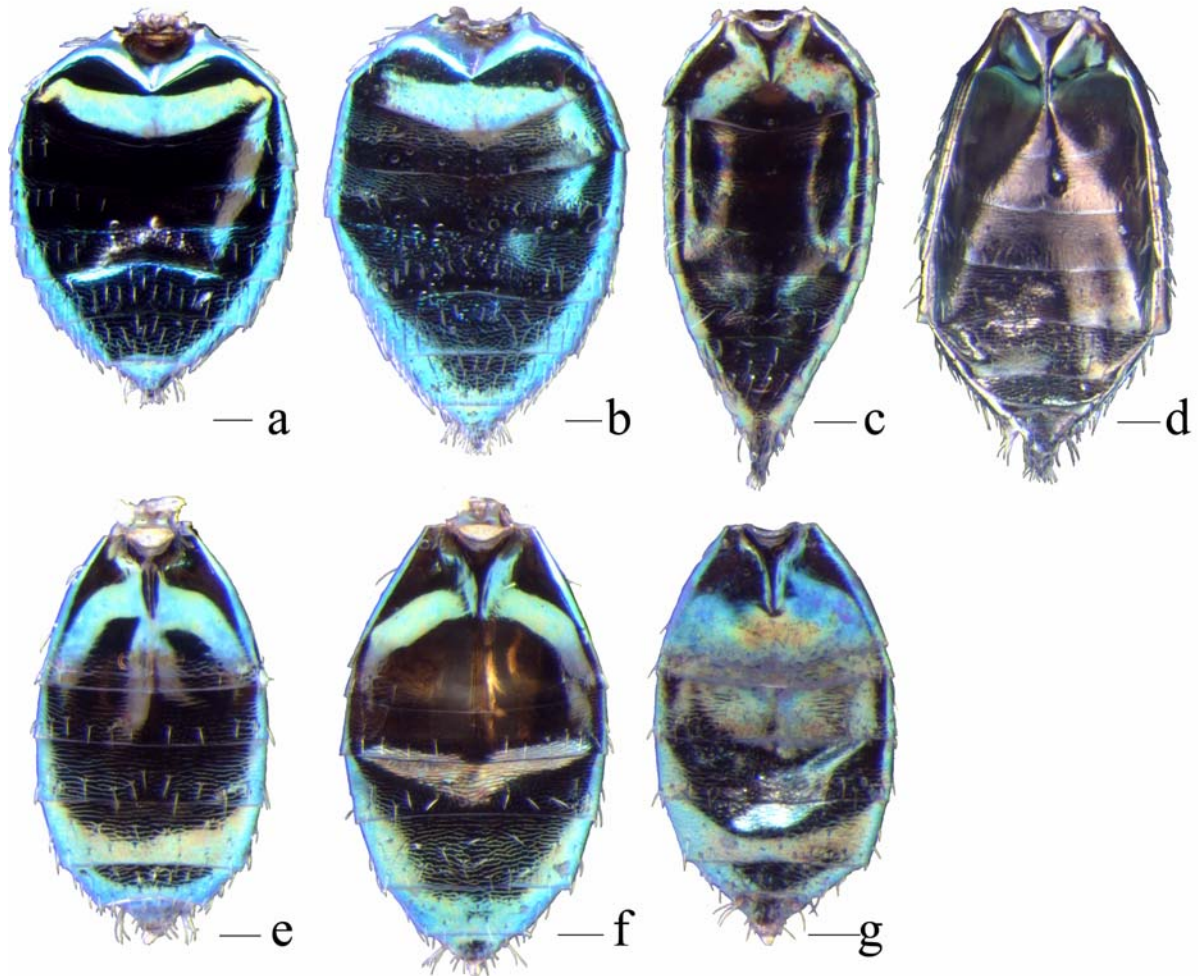
Fig. 2. Metasoma (dorsal) of (a) ♀ *Mesopolobus gemellus* Baur & Muller sp. nov.*;* (b) ♀ *Mesopolobus morys* (Walker, 1848); (c) ♀ *Mesopolobus incultus* (Walker, 1834); (d) ♀ *Mesopolobus trasullus* (Walker, 1839); (e) ♂ *M. gemellus*; (f) ♂ *M. morys*; (g) ♂ *M. incultus*. Scale bars = 100 µm.

with minute meshes along median line and larger meshes on frenum; frenal line indicated laterally; dorsellum alutaceous. Forewing with basal cell with 1–2 setae, basal setal line lacking; costal cell with complete setal line on lower side, upper side bare; speculum extending to middle of marginal vein, open below; marginal vein about 6.3 x as long as broad, not inflatted, 1.33 x as long as stigmal vein and 0.95 x as long as postmarginal vein. Propodeum with superficial but complete plica, median area superficially reticulate, 1.7 x as broad as long, 0.47 x as long as scutellum; median carina distinct, straight; nucha angulate medially; spiracular sulci superficial; right spiracle deformed and tooth like raised in holotype.

Gaster slightly ovate, 1.78 x as long as broad; tergite 1 smooth, tergites 2–6 alutaceous; first tergite occupying two fifths of length of gaster.

Female differs from male as follows:

Length 1.8 mm.

Head bright green to blue green; scape testaceous; pedicel and flagellum infuscate. Mesosoma bright blue-green. Tegulae yellow. Veins light testaceous. Legs with coxa concolorous with body, femora broadly fuscous in middle, rest of legs testacoeous. Gaster blue-green.

Head in dorsal view more transverse 2.17 x as long as broad, 1.3 x as broad as high; occiput less strongly excavate; POL 2.25 x OOL; malar space 0.5 x eye height; eye 1.24x as high as broad; face finely reticulate, clypeus reticulate with anterior margin truncate. Antenna with lower edge torulus inserted distinclty above level of lower ocular line; scape not extending to ventral margin of anterior ocellus, about 4.9 x as long as broad and 0.75 x eye height; pedicel in dorsal view about as long as anelli plus first funicular segment; combined length of pedicel plus flagellum 0.77 x as long as head breadth; first funicular segment about as long as broad and of same size as second.

Gaster ovate pointed, 1.52 x as long as broad; tergite 1 smooth, tergites 2–6 alutaceous; first tergite occupying one third of length of gaster; hypopygium extending to about middle of the gaster.

Remarks:

The male of *M. gemellus* sp. nov. is readily separated from all other *Mesopolobus* species treated here by the combination of characters given in the key and the diagnosis. However, the female is very close to *M. morys* from which it can only be separated by the slightly reduced fore wing speculum. Because the male sex is much more distinct, it was chosen for the holotype.

The female reared from *C. turbatus* on *Lepidium draba* fits the type series quite well, except for a slightly longer gaster. Therefore, it was not included in the type series.
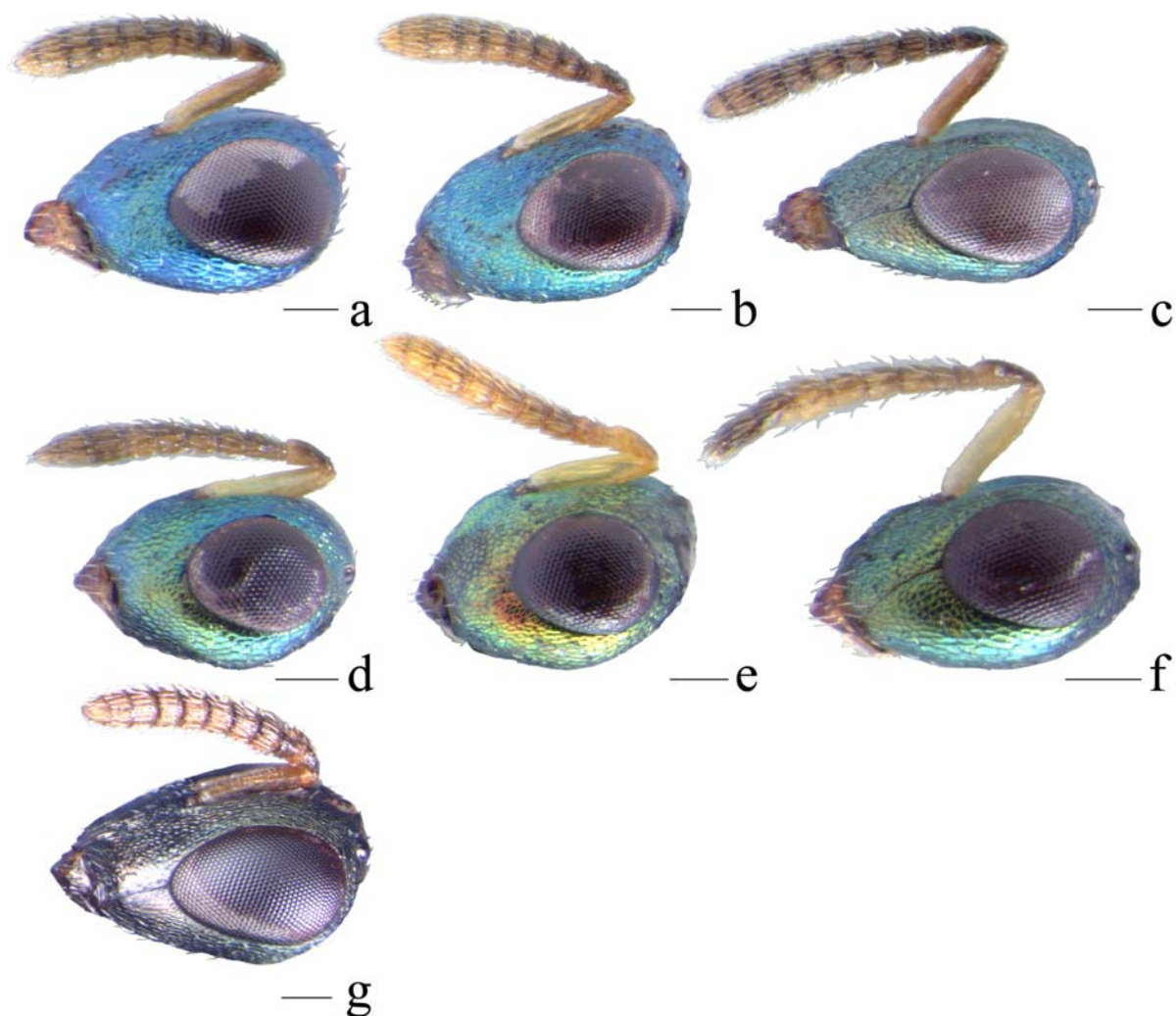
Fig. 3. Head and antennae (front-lateral) of (a) ♀ *Mesopolobus gemellus* Baur & Muller sp. nov.; (b) ♀ *Mesopolobus morys* (Walker, 1848); (c) ♀ *Mesopolobus incultus* (Walker, 1834); (d) ♂ *M. gemellus*; (e) ♂ *M. morys*; (f) ♂ *M. incultus*; (g) ♀ *Mesopolobus trasullus* (Walker, 1839). Scale bars = 100 μm.

Mesopolobus incultus (Walker, 1834)

Type material:

Platyterma incultum Walker, 1834, lectotype male in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Amblymerus stupidus Walker, 1834, lectotype female in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Platyterma femorale Walker, 1834, lectotype female in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Pteromalus ergias Walker, 1839, lectotype male in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Pteromalus leodocus Walker, 1839, lectotype male in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Pteromalus amyntor Walker, 1845, lectotype female in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Pteromalus urgo Walker, 1845, lectotype female in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Pteromalus belesis Walker, 1848, lectotype male in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Pteromalus berecynthos Walker, 1848, lectotype male in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Pteromalus lissos Walker, 1848, lectotype female in Zoological Museum, Lund University, Lund (Sweden), designated by Graham (Graham, 1956a) (not examined).

Eutelus (*Amblymerus*) *crassicornis* Thomson, 1878, lectotype female in BMNH, designated by Graham (Graham, 1956a) (examined by Gibson).

Material examined:

GERMANY. Ostholstein: Nähtkampf; coll. 14.vi.2000; leg. B. Klander; indiv. reared ex *C. bursa-pastoris* (1♀). **ITALY. Novara**: Antronaplana, V. Loranco, W.Rif Andolla, 648.1/105.0, 2300m.; coll. 6.viii.1992; leg. H. Baur (NMBE); collected from sweeping (3♀); **L. d'Orta**, 678.72.4, 310m.; coll. 24.vi.1993; leg. H. Baur (NMBE); collected from sweeping (2♀). **MOROCCO. Talasoltane**, Rif. 1850m.; Coll. 4–11.vii.1961; leg. V. Delucchi (NMBE); collected from sweeping (4♀). **SWEDEN. Akarp**; coll. 4.vii.1961; leg. H. Von Rosen (NMBE); collected by sweeping (1♂; 1♀). **SWITZERLAND. Bern**: Bremgarten, 599.4/202.9,550M.; coll. 12.v–18.vii.1992; leg. H. Baur (NMBE); collected from sweeping (6♀); Diemtigen, SW Griemialp, 602.9/157.1, 1240m.; coll. 9.vi.1992; leg. H. Baur (NMBE); collected from sweeping (1♀); Eymatt, 596.7/201.45, 510m.; coll. 20.v.2004; leg. H. Baur (NMBE); collected from sweeping (1♀); Kandersteg, Undere Allme, 616.025/149.175, 1790m.; coll. 28.viii.1991; leg. H. Baur (NMBE); collected from sweeping (3♀);
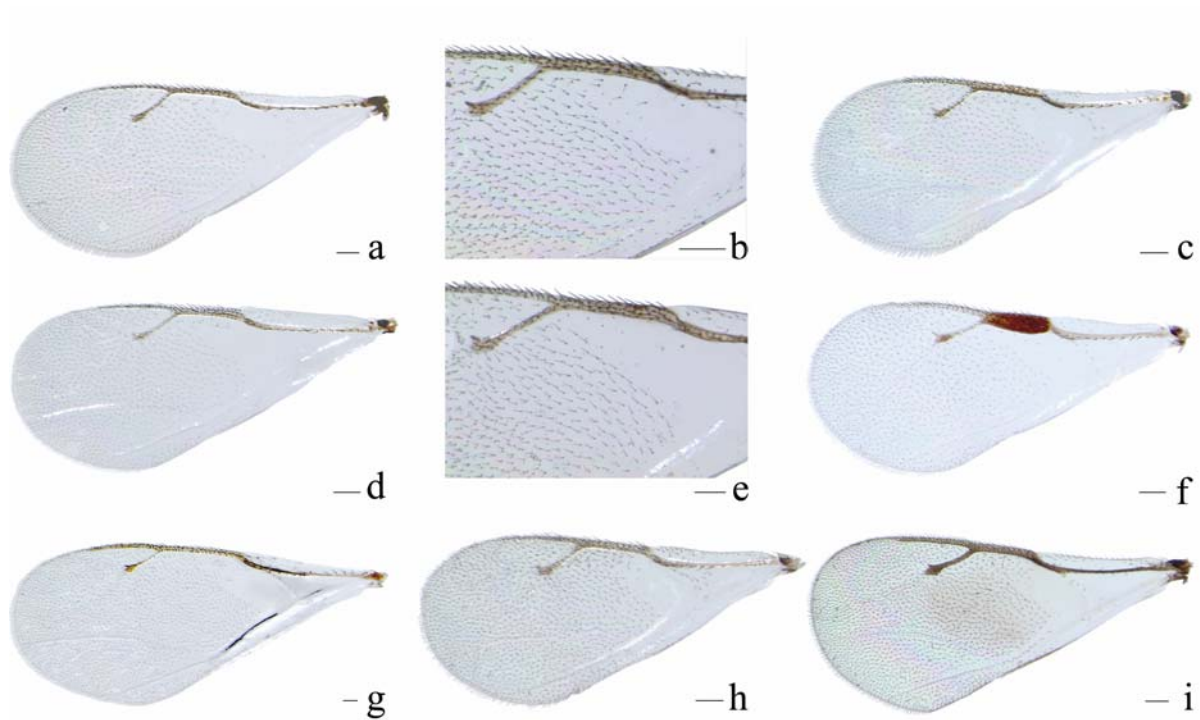
Fig. 4. Forewings of (a) ♀ *Mesopolobus gemellus* Baur & Muller sp. nov.; (b) ♀ *M. gemellus* : detail of speculum; (c) ♂ *M. gemellus*; (d) ♀ *Mesopolobus morys* (Walker, 1848); (e) ♀ *M. morys* : detail of speculum; (f) ♂ *M. morys*; (g) ♀ *Mesopolobus incultus* (Walker, 1834); (h) ♂ *M. incultus*; (i) ♀ *Mesopolobus trasullus* (Walker, 1839). Scale bars = 100 μm.

Ruemendingen, 614.9/217.5, 510m.; coll. 26.v.1992; leg. H. Baur (NMBE); collected from sweeping (1♀); Vauffelin, 591/226, 720m.; coll. 16.vii.1996; leg. H. Baur (NMBE); collected from sweeping (1♀); Wohlen b. B., Schürhubel, 588.9/202.35, 520m.**;** coll.14.v.1992; leg. H. Baur (NMBE); collected from sweeping (3♀); **Neuchatel**: La Brévine, SW Le Maix Rochat, 539.4/205.0, 1050m.; coll. 3–10.viii.1992; leg. C. Vaucher (NMBE); collected from sweeping (1♀); **Valais**: Bitsch, Schwarzes-Flesh, 643.3/133.25, 1620m.; coll. 17.viii.1992; leg. H. Baur (NMBE); collected from sweeping (1♀); Ferden, Torbu, 623.55/138.7, 2010m.; coll. 2.viii.2003; leg. H. Baur (NMBE); collected from sweeping (1♀); NE Hohtenn, 625.65/130.75, 1460m.; coll. 30.vi.1992; leg. H. Baur (NMBE); collected from sweeping (3♂; 2♀); **Vaud**: Burlignère, Le Chenit, 502.3/156.8, 1050m.; coll. 17–24vii.1994; leg. C. Vaucher (NMBE); collected from sweeping (1♀); **Zurich**: Maschwanden, Rüss-Spiez-Ried, 388m.; coll. 21.viii.1989; leg. Rezbanyai-reser (NMBE); collected from sweeping (1♀);

Diagnosis:

Female. Length: 1.5–2.4 mm (Fig. 6c). Flagellum infuscate (Fig. 3c). Pedicel plus flagellum about 0.85–0.95 x as long as head breadth. Tegulae pale (Fig. 1c). Speculum extending to distal end of marginal vein (Fig. 4g); basal fold with 0–2 hairs. Median area of propodeum finely reticulate, plica more or less complete. Gaster 2.0–2.45 x as long as broad (Fig. 2c); tip of hypopygium reaching about two thirds along the gaster.

Male. Length: 1.4–1.8 mm (Fig. 6f). Head in frontal view 1.13–1.21 x as broad as high (Fig. 3c), genae rather straight; in dorsal view about 2.2 x as broad as long, occiput rather strongly concave. Marginal vein not inflated, more than 6 x as long as broad (Fig. 4h); tegula pale (Fig. 1f). Median area of propodeum finely reticulate, plica more or less complete. Gaster without pale spot medially (Fig. 2g).

Remarks:

The male of *M. incultus* is closest to *M. gemellus* sp. nov., but can be separated by a less transverse head in frontal view (see Fig. 5a, c). From the female it is differentiated by the longer gaster and hypopygium which extends about two third along the gaster.

Mesopolobus morys (Walker, 1848)

Type material:

Pteromalus morys Walker, 1848, lectotype male in BMNH, designated by Graham (Graham, 1956a) (not examined).

Encyrtus ceutorhynchi Rondani, 1872, lectotype female in Zoological Museum "La Specola" in Florence (Italy), designated by Boucek (Graham, 1956a) (examined by Baur).

Disema pallipes Förster, 1878, syntype male in Museum für Naturkunde, Berlin

(Germany); synonymized with *M. morys* by Rosen (1961) (not examined).

Xenocrepis pura Mayr, 1904, holotype male in Naturhistorisches Museum, Vienna (Austria); synonymized with *M. morys* by Graham (1957) (not examined).
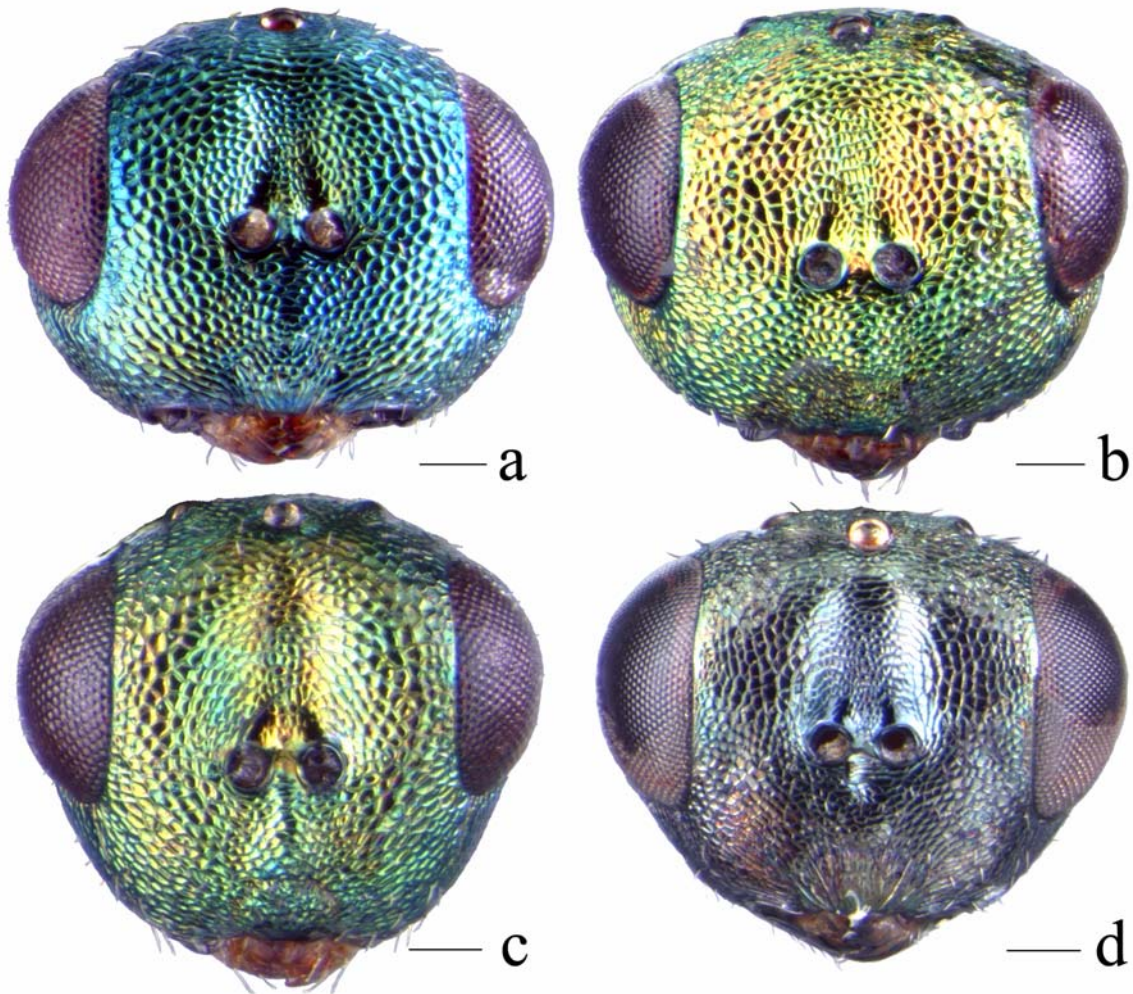
Fig. 5. Head (frontal) of (a) ♂ *Mesopolobus gemellus* Baur & Muller sp. nov.; (b) ♂ *Mesopolobus morys* (Walker, 1848); (c) ♂ *Mesopolobus incultus* (Walker, 1834); (d) ♀ *Mesopolobus trasullus* (Walker, 1839). Scale bars = 100 μm.

Material examined:

FRANCE. Alsace: Boron, BRN3, N47°32'11.34" E7°0'24.09"; coll. 21.vi.2004; leg. F. Muller, indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (1♀). Faverois, FAV, N47°31'8.21" E7° 3'11.94"; coll. 6.vii.2004; leg. F. Muller; mass. coll, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L.(7♂; 3♀); coll. 28.vi.2004; Leg F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L.(3♂; 5♀). **GERMANY. Schleswig-Holstein**: Rastorfer Passau, RP, N54°16'58.80" E10°20'60.00"; coll. 24–28.vi.2002 & 1.vii.2002; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L (4♂; 1♀). **SWITZERLAND. Jura**: Chatillon, La Prîre CHA1, N47°20'3.43" E7°19'56.28"; coll. 9–27.vi.2002 & 01.vii.2002; leg. F. Muller; indiv. reared from larval parasitoids, ex.

Ceutorhynchus obstrictus in pods of *Brassica napus* L.(21♂; 17♀); CHA-LP2; coll. 9–26.vi.2002, em. 23–27.vi.2003 & 1–12.vii.2002; Leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (7♂; 6♀); coll. 10–22.vi.2003, em. 16.vi–27.vii.2003; Leg. F. Muller, indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L.(15♂; 32♀); CHA-LP3; coll. 10–25.vi.2002, em. 25–27.vi.2003 & 1–20.vii.2002; Leg. F. Muller, indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L (9♂; 14♀); Coeuve, COE1, N47°27'55.00" E7°6'40.01"; coll. 21.vi.2004; leg. F. Muller, indiv. reared from Larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L (1♂); Courrendlin, COUR1, N47°20'47.55" E7°17'36.21"; coll. 15.vi.2004; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (1♂; 1♀); Courroux, CRX11, **N**47°22'1.64" E7°22'3.90"; coll. 21.vi.2004; leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L (2♂; 1♀); Delémont, Le Chavelier DEL-DOM; coll. 10–20.vi.2003, em. 21.vi–11.vii.2003; Leg. F. Muller; indiv. reared from larval parasitoids, ex. *Ceutorhynchus obstrictus* in pods of *Brassica napus* L. (11♂; 22♀); **Valais**: Martigny, MAR1A, N46° 6'25.60" E7° 4'19.33"; coll. 29.vi.2004 & 5.vii.2004; Leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (9♂; 4♀). MAR2, N46° 6'47.04" E7°6'54.24"; coll. 15.vi–5.vii.2004; Leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (7♂; 11♀). Ecône-Riddes, VS-Eco, N46°10'28.87" E7°12'54.19"; coll. 17.vi.2004, em. 3.vii.2003; Leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (2♀); Saxon, VS-Sax; coll. 17&22.vi.2004, em. 27.vi–11.vii.2003; Leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (14♂; 20♀); Sion, SIN11, N46°13'11.80" E7°20'43.09"; coll. 29.vi.2004 & 5.vii.2004; Leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (14♂; 7♀); Sion VS-SW1; coll. 17&22.vi.2004, em. 3–11.vii.2003; Leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (2♂; 2♀). **HUNGARY. Csongrad**: Lep-HU36, N46°24'50.80" E20°0'3.24"; coll. 24.vi–11.vii.2004; Leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (8♂; 6♀); Hödmezövazarhely, Lep-HU61, N46°15'41.62" E20°12'24.52"; coll. 22.vi–11.vii.2004; leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (4♂; 4♀). **Pest**: Apaj, Lep-HU65,

N47°12'9.12" E19°13'50.22"; coll. 24.vi.2004 & 11.vii.2004; leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (1♂; 1♀). **Kiskunlachaza**, Lep-HU66, N47°11'48.19" E19° 6'36.40"; coll. 19.vi.2004; Leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (1♂). **Budapest**, Lep-HU7B, N47°12'30.24" E19°13'6.06"; coll. 30.vi.2004 & 11.vii.2004; leg. F. Muller; indiv. reared from larval parasitoids, ex *Ceutorhynchus turbatus* Schultze in pods of *Lepidium draba* L. (2♂).

Diagnosis:

Female. Length: 1.6–2.5 mm (Fig. 6b). Flagellum weakly infuscate (Fig. 3b). Pedicel plus flagellum about 0.7–0.8 x as long as head breadth. Tegulae pale (Fig. 1b). Speculum extending to distal end of marginal vein (Fig. 4f); basal fold with 0–1 hairs. Median area of propodeum finely reticulate, plica more or less complete. Gaster 1.4–1.8 x as long as broad (Fig. 2b); tip of hypopygium reaching about half way along the gaster.

Male. Length: 1.4–2.1 mm (Fig. 6e). Head in frontal view 1.22 – 1.33 x as broad as high, genae curved; in dorsal view about 2 x as broad as long, occiput weakly concave. Marginal vein inflated and only about 4.5 x as long as broad (Fig. 4f); tegula pale (Fig. 1e). Median area of propodeum finely reticulate, plica more or less complete. Gaster without pale spot medially (Fig. 2f).

Remarks:

The male of *M. morys* is readily recognized by the inflated marginal vein (Fig. 4f), which is unique among species of *Mesopolobus*. It was therefore not necessary to re-examine the male types of the synonyms cited above. However, the female is very close to *M. gemellus* sp. nov., from which it is separated only by a slightly larger fore wing speculum (Fig. 4e).
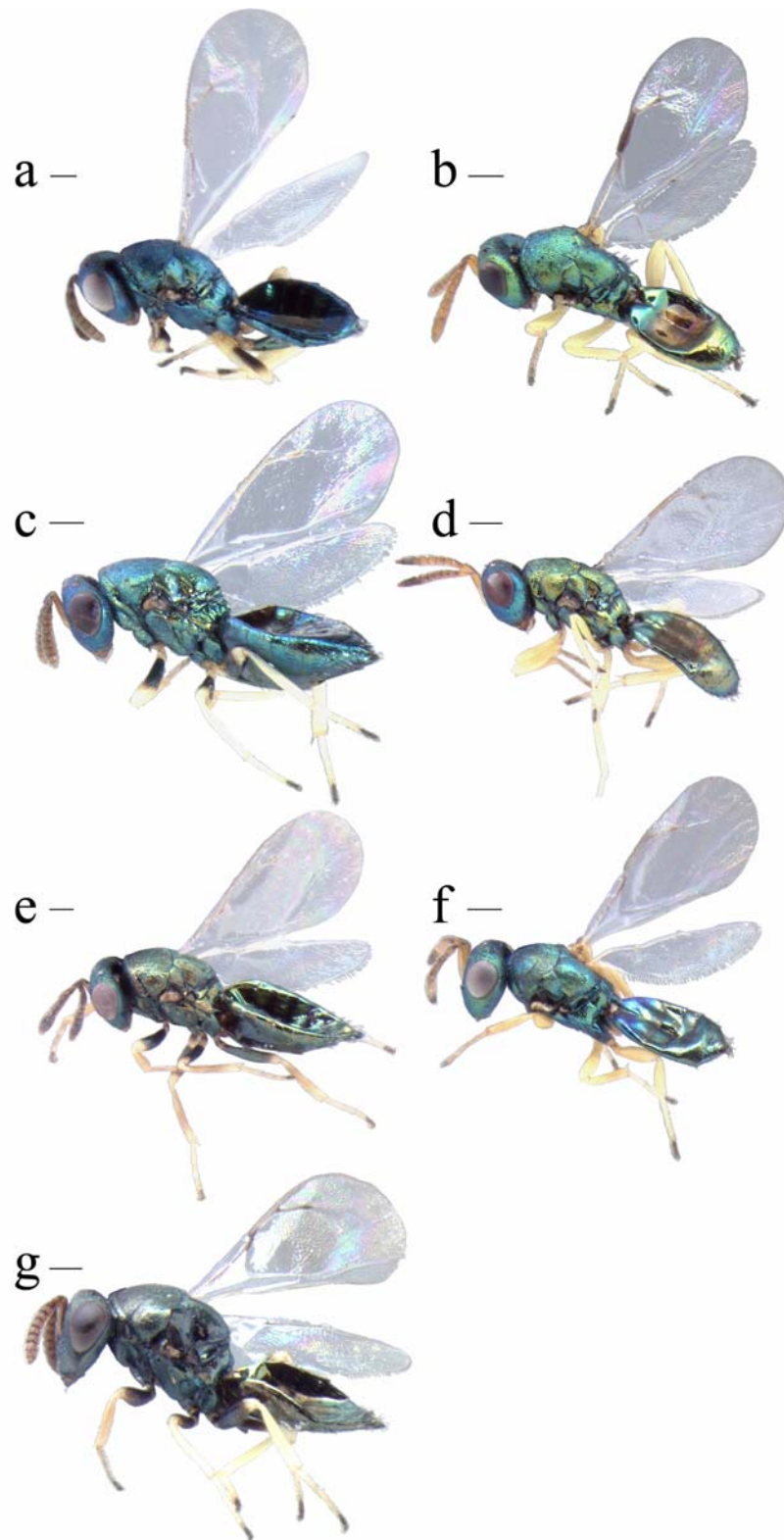
Fig. 6. Habitus of (a) ♀ *Mesopolobus gemellus* Baur & Muller sp. nov.; (b) ♀ *Mesopolobus morys* (Walker, 1848); (c) ♀ *Mesopolobus incultus* (Walker, 1834); (d) ♂ *M. gemellus*; (e) ♂ *M. morys*; (f) ♂ *M. incultus*; (g) ♀ *Mesopolobus trasullus* (Walker, 1839). Scale bars = 200 µm.

Mesopolobus trasullus (Walker, 1839)

Type material:

Ormocerus trasullus Walker, 1839, lectotype female in BMNH, designated by Graham (examined by Gibson).

Mesopolobus roseni Graham, 1984, holotype female in BMNH (examined by Gibson).

Material examined:

CZECH REPUBLIC. Bohemia: Praha-Butovice, Prokopskè Udoli, lat. N50°02.609'' long. E014°21.348', 100m; coll. 7.vi.2004; leg. H. Baur (NMBE); collected by sweeping (1♀). **SWITZERLAND. Basel**: Bottmingen; coll. 22.vii.1935; leg. W. Wittmer (NMBE) (1♀).

Diagnosis:

Female. Length: 2.1–2.3 mm (Fig. 6g). Flagellum infuscate dorsally, slightly paler on lower side (Fig. 3g). Pedicel plus flagellum about 0.77 x as long as head breadth. Tegulae dark (Fig. 1g). Speculum extending about to the middle of the marginal vein (Fig. 4d); basal fold with 2–5 hairs. Median area of propodeum smooth, plica indicated in posterior third only. Gaster 1.7 x as long as broad (Fig. 2d); tip of hypopygium reaching about half way along the gaster.

Remarks:

Gibson & Baur (2005) pointed out that *M. trasullus* was erroneously synonymised under *M. incultus* by Graham (1956b) and recognized it as a senior synonym of *Mesopolobus roseni* Graham, 1984. Because males of this species were not available for study, the characters given in the key were taken from Askew *et al.* (2001).

Discussion

All *Mesopolobus* species previously known as parasitoids of *Ceutorhynchinae* hosts in Europe were described and included in the key. Features are provided to differentiate *Mesopolobus morys*, *M. incultus* and *M. trasullus* from *Mesopolobus gemellus* sp. nov. Results of this study will have direct implications on allowing us to provide ecological

baseline data for correctly identify parasitoid-Ceutorhynchinae host associations, which is essential for a renewed classical biological initiate against *C. obstrictus* in Canada. In this context, *M. morys* with its main host *C. obstrictus*, was thought to occur on *C. typhae* (=*floralis*) in Europe (Klander, 2001). We show here that these records were based on misidentifications of *M. gemellus*, undescribed at the time of those studies. However, *M. morys* is for the first time accurately associated with the seed weevil *C. turbatus*, a potential agent for classical biological control of hoary cress, *Lepidium draba* in North America.

Concern regarding potential non-target effects of invertebrate biological control agents of arthropods has risen over the last decade and an increasing number of studies have since dealt with this topic (Babendreier *et al.*, 2005). The taxonomic clarification reported in this paper provides a sound basis for accurate documentation of *Ceutorhynchus* spp. – *Mesopolobus* spp. host-parasitoid associations. This information is of significant interest to the scientific community involved in the classical biological control of *C. obstrictus* in North America (Kuhlmann *et al.*, 2006a) because biological control practioners now have to assess potential non-target impacts of invertebrate biological control agents to justify release.

It should be noted that in general the understanding of the population dynamics of Ceutorhynchinae species of economic importance is still hampered by the insufficient knowledge of natural enemy complexes that may play an important role in regulating these herbivorous insects (Vidal, 2003). Clarification of the taxonomy of *Mesopolobus* species presented here provides a sound basis for understanding these dynamics, leading towards the safe use of *Mesopolobus* species against *Ceutorhynchinae* pest species.

Acknowledgments

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European Ectoparasitoid Assemblages of Two Classical Weed Biological Control Agents Released in North America: Ensuring the Safety of Biological Control Agents for Cabbage Seedpod Weevil

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Abstract

Among the species of the curculionid subfamily Ceutorhynchinae, two stem miners *Hadroplontus litura* (Fabricius) and *Microplontus edentulus* (Schultze), have been released and are established as biological control agents for two weeds of economic importance, Canada thistle, *Cirsium arvense* (L.) Scopoli and scentless chamomile, *Tripleurospermum perforatum* (Mérat) Laínz (=*Matricaria perforata* Mérat), respectively. Canada thistle and scentless chamomile occur in the same regions and same kind of habitats as the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)], a major pest of canola (*Brassica napus* L.) in North America. Several chalcid parasitoid species such as *Mesopolobus morys* Walker, *Trichomalus perfectus* Walker and *Stenomalina gracilis* Walker are being evaluated as candidate classical or inundative biological control agents. Those Ceutorhynchinae released to control weeds in cultivated crops will be at great risk of potential attack if agents released for biological control of *C. obstrictus* are not specific to that species. A three-year field survey was carried out to collect *H. litura* and *M. edentulus* and their ectoparasitoids in France, Germany, Austria and Hungary. The ectoparasitoids were reared to adults and identified with the aim of verifying whether or not the candidate ectoparasitoids occur in the assemblages associated with these

two non-target species. Despite phenological overlap of *M. edentulus* and *H. litura* with *C. obstrictus*, and sympatric distributions in Europe, *M. morys* did not parasitize *M. edentulus* or *H. litura*. Furthermore, we found no evidence that *T. perfectus* attacks these species. In contrast, *S. gracilis* parasitized *M. edentulus* at several sites surveyed during two years, in Austria and Hungary.

Introduction

Classical biological control, i.e. the introduction and establishment of exotic insects, mites, or pathogens to give permanent control, is the predominant strategy used for biological control of weeds (McFadyen, 1998). Its application is highly recommended to control established non-indigenous invasive weed populations (Wittenberg & Cock, 2001). Review of classical biological control agents released in Canada, United States, Australia and New Zealand against weeds indicated that more than 50% of these 85 agents belong to the Curculionidae, Chrysomelidae (Coleoptera), and Tephritidae (Diptera) (Gassmann, 1995; Mason & Huber, 2002; Coombs, 2004). A total of 13 species of the curculionid subfamily Ceutorhynchinae have been released in Canada for biological control of 10 weeds. Among these are *Hadroplotus litura* (Fabricius) (McClay *et al.*, 2002a) and *Microplontus edentulus* (Schultze) (McClay *et al.*, 2002b).

Microplontus edentulus, a stem mining weevil, was released to control scentless chamomile, *Tripleurospermum perforatum* (Mérat) Laínz (=*Matricaria perforata* Mérat) [Asteraceae], a weed of cultivated land (Hinz & McClay, 2000). Scentless chamomile forms dense, semi-permanent stands in periodically disturbed sites such as slough margins, field depressions or roadsides, from which seeds disperse into adjacent fields. Biological control targets these populations in uncultivated areas because chemical and cultural control methods are impractical in these habitats (Hinz & McClay, 2000). Adult *M. edentulus* overwinter and in early summer lay eggs into the upper parts of scentless chamomile stems. Larval feeding reduces both seed output and biomass of scentless chamomile (Bacher, 1993). Hinz *et al.* (1996) confirmed that *M. edentulus* has a narrow host range in its area of origin in south-eastern Europe. According to Julien and Griffiths (1998), *M. edentulus* is established only in Alberta.

Hadroplontus litura (Fabricius), a stem- and root-mining weevil, was released to control Canada thistle, *Cirsium arvense* (L.) Scopoli [Asteraceae] in Canada (McClay *et al.*, 2002a). Canada thistle is one of the most widespread and competitive European weeds in United States and Canada (Skinner *et al.*, 2000) causing extensive crop yield losses through competition (Stachon & Zimdahl, 1980) but it is also a major problem in disturbed sites in non-crop areas (White *et al.*, 1993). As large-scale chemical control is detrimental to the environment and not economic on low-value land, classical biological control is an important alternative management strategy early on (McClay *et al.*, 2002a). *Hadroplontus litura* oviposits into the midveins of Canada thistle rosette leaves in early spring and larvae mine down through the vein into the stem base and upper part of the tap root. Mature larvae emerge from the stem and pupate in the soil, and adults emerge to feed on *C. arvense* foliage in late summer (Zwölfer & Harris, 1966; Peschken & Wilkinson, 1981). Adults overwinter in the soil. According to Freese (1994), Canada thistle is the only known field host plant in Europe; however, Zwölfer & Harris (1966) noted three records from *Carduus defloratus* L. (Asteraceae). *Hadroplontus litura* is established in Canada (Julien & Griffiths, 1998) and, although the efficacy of *H. litura* is inconsistent (McClay *et al.*, 2002a), the insect appears to give acceptable control once populations reach high enough levels and when its use is combined with other control methods (Donald, 1990; Rees, 1990).

Scentless chamomile and Canada thistle are major weed species in or near fields of rapeseed, *Brassica napus* L., in Europe (Schroeder *et al.*, 1993). In Canada, both weeds are widespread on the Canadian prairies (Manitoba, Saskatchewan and Alberta), the major canola production area (Donald, 1990; Woo *et al.*, 1991). In this same region as well as the eastern provinces of Ontario and Québec, the invasive alien cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)] is a serious pest of canola. Adults feed on flower buds causing their destruction and larvae feed within seedpods, causing economic losses (Kuhlmann *et al.*, 2002). In Europe, the parasitoid assemblage and its impact on *C. obstrictus* have been well documented (Williams, 2003) and several species are being evaluated for possible introduction into Canada as classical biological control agents. Those Ceutorhynchinae released to control weeds in cultivated crops will be at great risk of potential attack if agents released for biological control of *C. obstrictus* are not specific to that species (Kuhlmann *et al.*, 2006a). Both *M. edentulus* and *H. litura* belong to the Ceutorhynchinae and have therefore been included in the proposed non-target species test list for candidate biological control agents of *C. obstrictus* (Kuhlmann *et al.*, 2006a). Despite a number of studies on *M. edentulus* and *H. litura*, host-parasitoid association records are rather

limited and it is unknown if parasitoid species attacking *C. obstrictus* will also attack the taxonomically related *H. litura* and *M. edentulus*.

To assess the potential risks of *C. obstrictus* parasitoids attacking related weed biological control agents, surveys are necessary in the area of origin to determine the host-parasitoid associations. These field studies provide guidance on ecological attributes of weevils that are important in selecting non-target species for host-specificity testing. Furthermore, it will facilitate better prediction of potential risks associated with introduction of candidate biological control agents of *C. obstrictus* into Canada. The objectives of this study were to: 1) describe the phenologies of *H. litura* and *M. edentulus* to determine if there is phenological overlap with *C. obstrictus*; 2) assess the parasitism levels, particularly by ectoparasitoids; and 3) determine the parasitoid assemblages associated with *M. edentulus* and *H. litura*.

Material & Methods

Field collected material

All scentless chamomile and Canada thistle plants were collected in fallow fields, at field margins or along road sides in regions where canola fields, *C. obstrictus* and its common parasitoids co-occurred. Surveys for scentless chamomile and *M. edentulus* were carried out from May to July in 2003 and 2004 at eight sites in eastern Austria and three distinct regions in Hungary (Figure 1). Surveys for Canada thistle and *H. litura* were carried out in May and June in 2002, 2003 and 2004 at seven sites located in eastern France and three separate regions of Germany (Figure 1). As Canada thistle is a pioneer species, it very often grows along road sides or field margins. Preliminary studies showed that *H. litura* occurs more frequently in stable sites where it has had time to build up a population; thus, sites sampled were in wastelands or abandoned fields because they represented more stable habitats.

Phenology of hosts

Samples of 20 to 50 host plants were collected and dissected as often as every two days and at a maximum of 10-day intervals. For Canada thistle the whole plant was dissected, and for scentless chamomile the main shoot and three randomly chosen side-shoots were dissected to detect Ceutorhynchinae eggs and larvae which were determined to instar. The late third instar

larvae of both *M. edentulus* and *H. litura* chew an exit hole through the parenchyma tissue to exit the plants and drop down into the soil where they pupate. Thus, the exit holes were
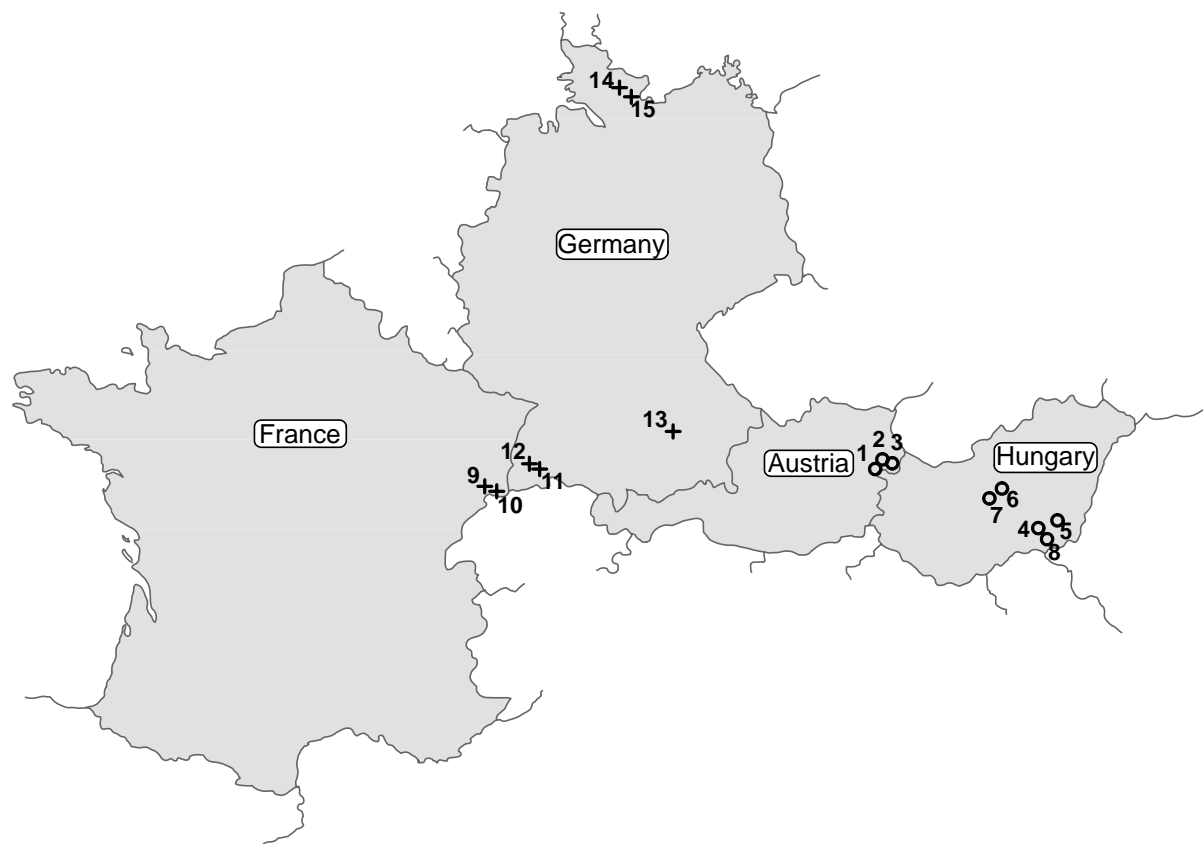
Host plant / Ceutorhynchinae species	Country	Region / Town	Site #	Geographic coordinates		Elevation (m.)
				Lat. N	Long. E	
Tripleurospermum perforatum / *Microplontus edentulus*	Austria	Burgenland / Eisenstadt	1	47°49'22.57"	16°32'18.77"	148
		Burgenland / Eisenstadt	2	47°50'01.50"	16°34'01.95"	138
		Burgenland / Eisenstadt	3	47°48'54.06"	16°32'16.45"	149
	Hungary	Csongrad / Hodmezovasarhely	4	46°22'01.13"	20°13'17.51"	74
		Bekes / Kardoskut	5	46°27'45.83"	20°32'37.14"	79
		Pest / Bugyi	6	47°11'28.20"	19°04'20.10"	93
		Pest / Kiskunlachaza	7	47°11'15.66"	19°02'38.58"	96
		Csongrad / Maroslele	8	46°19'07.44"	20°17'23.10"	85
Cirsium arvense / *Hadroplontus litura*	France	Alsace / Lucelle	9	47°26'23.93"	07°18'06.15"	480
		Alsace / Lucelle	10	47°26'21.93"	07°19'12.99"	525
	Germany	Southern Rhine valley / Grissheim	11	47°52'02.78"	07°36'33.27"	213
		Southern Rhine valley / Grissheim	12	47°52'28.23"	07°34'51.26"	205
		Bayern / Scheyern	13	48°30'00.32"	11°28'01.02"	492
		Schleswig Holstein / Stolpe	14	54°09'00.00"	10°46'58.08"	19
		Schleswig Holstein / Stolpe	15	54°08'49.93"	10°47'31.67"	21

Figure 1: Location of field collection sites of *Tripleurospermum perforatum* (Mérat) Laínz and *Cirsium arvense* (L.) Scopoli surveyed for the occurrence of *Ceutorhynchus edentulus* (Schultze) [o] and *Hadroplontus litura* (Fabricius) [+], respectively, in 2002, 2003, and 2004.

recorded and taken into account to estimate the number of healthy third instar larvae that had left the plants. In cases of high larval infestation, several third instar larvae can exit the plant using the same hole, which might have led to an under-estimation of these numbers.

Parasitism by Ectoparasitoids

For each plant dissected, ectoparasitoid eggs and larvae found on parasitized host weevil larvae as well as ectoparasitoid pupae found near the remains of a consumed host body were recorded to estimate the parasitism level on each collection date for every selected field site. Mortality due to host feeding by ectoparasitoid adults was considered as an indirect effect of parasitism and recorded independently. Parasitism was calculated for each site as follows:

$$\% Parasitism = \frac{\sum_1^n paras.}{\sum_1^n avail.hosts} \times 100 = \frac{\sum_{larv}^{par} + \sum_{larv}^{\dagger} + \sum_{par}^{alone}}{\sum_{larv}^{healthy} + \sum_{larv}^{par} + \sum_{larv}^{\dagger} + \sum_{holes} + \sum_{par}^{alone}} \times 100$$

where "$\sum_1^n paras.$" is the total number of parasitoids found in the plant; "$\sum_1^n avail.hosts$" is the total number of hosts available for parasitism (second and third instar larvae) found in the plant; Σ_{larv}^{par} is the number of larvae found parasitized by an ectoparasitoid; $\Sigma_{larv}^{\dagger}$ is the number of larvae found dead with traces of host feeding like brownish punctures on the tegumen; Σ_{par}^{alone} is the number of parasitoids found alone near the remains of a consumed host, either as a pupa, or as a newly emerged adult which had not yet exited the plant; $\Sigma_{arv}^{healthy}$ is the number of healthy host larvae; Σ_{hole} is the number of exit holes (made by healthy third instar larvae that have left the plant), found on the shoots during dissections.

A representative parasitism level was estimated for each site. The date on which the parasitism level was considered representative was that date on which the maximum number of healthy larvae still available for parasitism were present in the plant, i.e., just before the number of exit holes started to increase.

Host density was determined by considering the maximum number of hosts parasitized or available for parasitism during the season. Thus, only second and third instar larvae were used for calculations. Exit holes were recorded as equivalent to a single third instar larva that had exited the plant.

Parasitoid assemblages

Parasitoid eggs and larvae were reared on the host larvae they had been found parasitizing, and pupae were reared individually on a disc of filter paper in 5.5 cm diameter Petri dishes.

Insects were reared in a climate chamber at 20 ± 2°C, 70% ±10 RH, and 16h L: 8h D. The insects were checked daily until emergence of the adult parasitoid. Some groups emerged the same summer season that they were collected. Other groups, such as the Braconidae, overwintered in cocoons and emerged the following spring. Mortality was rather high over rearing and overwintering periods. Identification was possible only for adults which, directly after they had emerged in their individual rearing chamber, were killed using ethyl acetate. Every specimen was card-mounted, labelled with individual information, and stored for further identification purposes. All taxonomic identifications were made with the help of Hannes Baur at the Natural History Museum of Berne (NMBE), Switzerland, except for the Braconidae. These specimens were identified by Dr. C. van Achterberg, National Natural History Museum of Leiden, Netherlands. Where possible, Pteromalidae and Braconidae specimens were identified to species while other families were identified to genus.

Available information on life cycle, primary host association, hyperparasitism records, if existing, and plant host – weevil – parasitoid associations were obtained from the literature. Relevant references were obtained from the Taxapad programme (Yu *et al.*, 2005) and the Universal Chalcidoidea Database (Noyes, 2006). Hannes Baur, NMBE, Switzerland, and Dr. Gary Gibson, Canadian National Collection of Insects, Ottawa, Canada were consulted for information on Chalcidoidea. Information on Ichneumonidea was obtained from Dr. K. Van Achterberg, Department of Entomology, National Natural History Museum of Leiden, Netherlands and Dr. Klaus Horstmann, University of Würzburg, Germany. Eurytomidae specimens were identified by Michael Gates, USDA-ARS, National Museum of Natural History, Washington DC, United States.

Results

Microplontus edentulus on scentless chamomile

Phenology of hosts

Sites surveyed in Hungary in 2004 all yielded similar results (Table 1). First instar larvae of *M. edentulus* were present in scentless chamomile shoots until the last week of June 2004 at all sites studied (Figure 2). Second instar larvae were observed from the second week of May to the first week of July. Third instar larvae appeared only for a short period (10 days to 2

weeks) before the first mature larvae started to leave the plants very early in the season, beginning the first week of June. Weevil infestation levels were rather high, with densities between 4.0±0.6 and 7.5±0.9 larvae per plant in Austria in 2003, and between 1.9±0.5 and 7.1±0.8 larvae per plant in Hungary in 2004 (Table 1).

Table 1. Mean number (±SE) of second and third instar larvae of *Microplontus edentulus* (Schultze) available for parasitism, and percentage parasitism observed in Austria in 2003 and Hungary in 2004.

	Location	Site code	Mean # (±SE) of hosts (L2/L3) per plant			% Parasitisim
Austria	Burgenland / Eisenstadt	1	4.9	±	0.60	23.0
	Burgenland / Eisenstadt	2	7.5	±	0.92	52.3
	Burgenland / Eisenstadt	3	4.0	±	0.57	1.9
Hungary	Csongrad / Hodmezovasarhely	4	1.9	±	0.46	3.1
	Bekes / Kardoskut	5	5.2	±	0.63	11.6
	Pest / Bugyi	6	3.7	±	0.30	17.8
	Pest / Kiskunlachaza	7	4.7	±	0.51	26.3
	Csongrad / Maroslele	8	7.1	±	0.82	4.7

Phenology of ectoparasitoids

Ectoparasitoids were active from the third week of May and for a period of about one month (Figure 2). Parasitism was highest near Eisenstadt, Austria in 2003. Parasitism reached a maximum of 26.3% at the site #7 near Kiskunlachaza on the 24 June 2004. All healthy third instar larvae had left the shoots by the first week of July. Parasitism levels ranged from 1.9 to 52.3% in Austria in 2003 and from 3.1 to 26.3% in Hungary in 2004.

Parasitoid assemblages

Parasitoids associated with weevils collected from scentless chamomile belonged to the hymenopteran families Pteromalidae, Eulophidae, Eurytomidae (Chalcidoidea), Braconidae, and Ichneumonidae (Ichneumonoidea) (Table 3). In Austria in 2003, seven species from four families and in Hungary in 2004 nine species from five families were represented. Regarding species identification, some parasitoid taxa are poorly known and taxonomic expertise is not available to determine their species identities. Available information such as life cycle, primary host association and hyperparasitism, and plant association are provided for all members of the parasitoid assemblage of *M. edentulus* in alphabetic order:

Bracon atrator Nees (Braconidae):

Life cycle : Very little is known of the life cycle of this species. In this study we found *B. atrator* as a larval ectoparasitoid of *M. edentulus*. Adults laid eggs in spring and larvae built cocoons from which adults of the new generation emerged during the following spring.

Primary host associations: *B. atrator* has been recorded as a primary parasitoid of various hosts including Diptera (Agromyzidae, Tephritidae), Lepidoptera (Coleophoridae) and Coleoptera (Apionidae, Curculionidae) (Yu *et al.*, 2005).
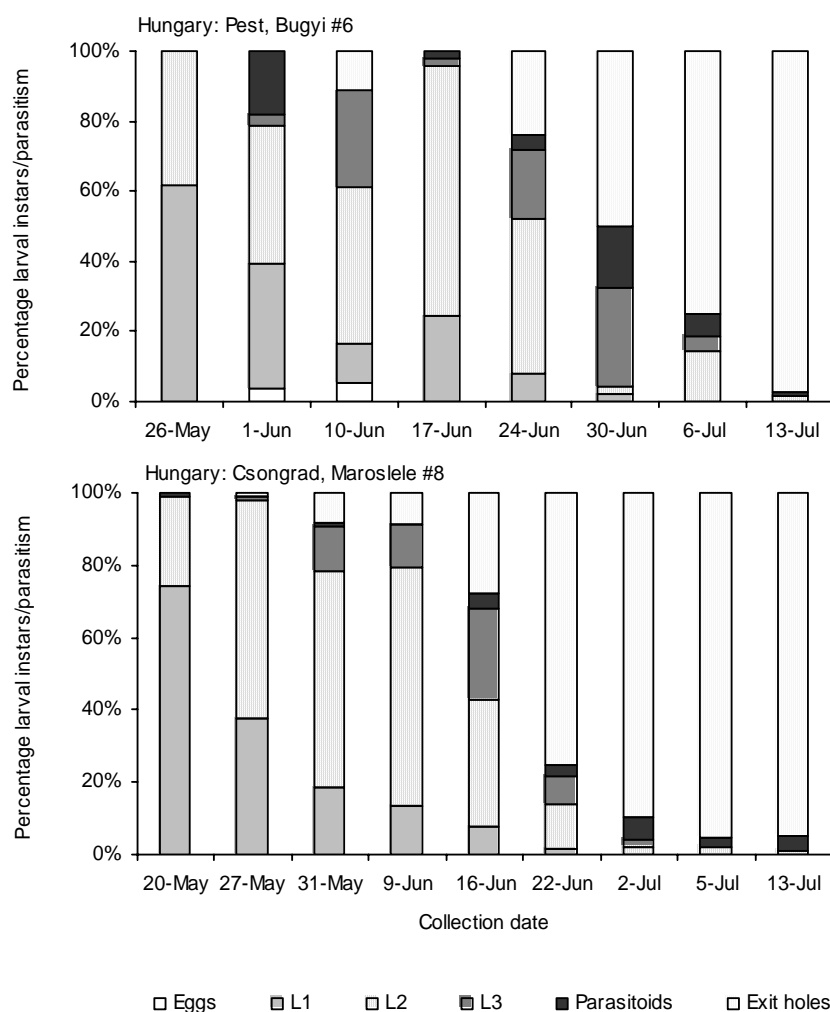
Figure 2. Proportion of eggs, larval instars 1-3 of *Microplontus edentulus* (Schultze), parasitism, and exit holes at Pest/Bugyi (site 6) and Csongrad/Maroslele, Hungary (site 8) between May and July in 2004.

Plant association: The species is associated with hosts feeding on *Daucus carota* L. (Apiaceae) and *Listera ovata* (L.) R.Br. (Orchidaceae) (Yu *et al.*, 2005). In this study the species was found on *M. edentulus* in shoots of scentless chamomile (Asteraceae).

Bracon intercessor Nees (Braconidae):

Life cycle: Bracon intercessor is a solitary ectoparasitoid (Georgiev, 2000; Yu *et al.*, 2005) that sometimes oviposits in galls formed by species such as sawflies (Hymenoptera: Tenthredinidae) in *Salix* species (Kopelke, 2003).

Primary host associations: Hosts of *B. intercessor* comprise species of Coleoptera (Cerambycidae, Curculionidae, Rhynchitidae), Hymenoptera (Tenthredinidae, Eurytomidae) and Lepidoptera (Gelechiidae, Tortricidae, Sesiidae) (Yu *et al.*, 2005).

Plant association: Bracon intercessor develops in hosts associated with Poaceae, Umbellifereae, Fabaceae, Apiaceae and Asteraceae (Yu *et al.*, 2005).

Chlorocytus sp. (Pteromalidae):

Life cycle: Species of the genus *Chlorocytus* are known to be primary larval or pupal ectoparasitoids, and in some cases hyperparasitoids (Graham, 1969). Adults appear in the field from May to July and occasionally August (Graham, 1969).

Primary host association and hyperparasitism if known: These species have a relatively diverse host range, parasitizing Coleoptera species such as Cerambycidae and Curculionidae (Yu *et al.*, 2005). Records are known from *Ceutorhynchus rapae* Gyllenhal and *C. adspersus* Dietz hosts (Tremblay, 1968; Charlet *et al.*, 2002). Diptera (Agromyzidae, Tephritidae) and Hymenoptera (Cephidae and non-gall forming Eurytomidae) are also hosts of *Chlorocytus* spp. (Noyes, 2006). In this study Eurytomidae species parasitized *M. edentulus* larvae at all sites where *Chlorocytus* spp. were present.

Plant association: Species of *Chlorocytus* develop in hosts associated with Asteraceae species (Graham, 1969; Charlet *et al.*, 2002); however, the record here is the first association with *T. perforatum*. *Chlorocytus* spp. are also associated with plants from a wide range of families like Apiaceae, Fabaceae, Poaceae and Urticaceae (Graham, 1969; Boucek, 1977; Berg *et al.*, 1995; Askew *et al.*, 2001).

Closterocerus sp. (Pteromalidae):

Species of the genus *Closterocerus* have a very broad host range (Hansson, 1990; Hansson, 1994; Gumovski, 2001; Yu *et al.*, 2005). Some species are primary parasitoids of leaf-miners and gall-formers, and others are also reported from Symphyta eggs (Argidae, Diprionidae), armored scales, and psyllids (Schauff, 1991; Hansson, 1996; Yu *et al.*, 2005).

Eupelmidae sp. (Pteromalidae):

The vast majority of Eupelmidae species are parasitoids and facultative hyperparasitoids on the immature stages of other insects, with hosts recorded in the orders Lepidoptera, Homoptera, Hymenoptera, Coleoptera, Neuroptera and Orthoptera (Noyes, 2006). A small number of species are predators on the eggs or larvae of various insects, or on the eggs of spiders and a few are solitary, primary endoparasitoids of eggs of Lepidoptera, Orthoptera and Hemiptera (Yu *et al.*, 2005). Most eupelmids are solitary, but some species are gregarious (Clausen, 1940). Most are ectoparasitoids, including some that develop gregariously on dipterous pupae within puparia. A few species are solitary endoparasitoids of Coccoidea (Noyes, 2006).

Eurytoma curculionum Mayr (Pteromalidae):

Life cycle: The species is known to be a larval ectoparasitoid of concealed Coleoptera hosts (Fischer, 1965). It occurs less frequently as a hyperparasitoid attacking late instar larvae of chalcid wasps (Fischer, 1965). *Eurytoma curculionum* is bivoltine; adults emerge in late May and June whilst a partial second generation occurs in August (Fischer, 1965).

Primary host association: Eurytoma curcuilonum is known to be associated with species of Coleoptera such as Apionidae (Vidal, 1997), Buprestidae (Boucek, 1977) and Curculionidae (Dmoch, 1975a; Boucek, 1977). In this study, *E. curculionum* was found to be associated with *M. edentulus*.

Plant association: Records can be found of *Eurytoma curcuilonum* has been associated with hosts developing in Brassicaceae, Polygonaceae, Rubiaceae and Scrophulariaceae (Yu *et al.*, 2005).

Shpegigaster sp. (Pteromalidae):

Life cycle: *Sphegigaster* sp. larval or pupal primary- or hyper- parasitoids are associated with gall-forming hosts (Graham, 1969). Adults fly from May to July and some species have more than one generation per year (Graham, 1969).

Primary host association and hyperparasitism: Primary hosts of *Sphegigaster* spp. comprise families of Coleoptera (Curculionidea), Diptera (Agromyzidae, Anthomyiidae, Chloropidae, Drosophilidae and Tephritidae) and Lepidoptera (Lyonetiidae) (Yu *et al.*, 2005). Some *Sphegigaster* spp. are hyperparasitoids of Hymenoptera (Braconidae) (Graham, 1969).

Plant association: *Sphegigaster* spp. are known to be associated with hosts developing in Aquifoliaceae, Brassicaceae, Fabaceae, Rubiaceae and Theaceae (Kumar, 1984; Potter & Gordon, 1985).

     Stenomalina gracilis Walker (Pteromalidae):

Life cycle: *Stenomalina* spp. are solitary primary larval or pupal ectoparasitoids. Adults can be observed in the field from June to October (Graham, 1969).

Primary host association: Stenomalina gracilis is associated with Coleoptera species in the weevil subfamily Ceutorhynchinae, such as *C. obstrictus* (Dmoch, 1975a), *H. litura* (Vidal, 1997), *C. napi* Gyllenhal, *C. pallidactlylus* (Marsham) and *C. roberti* Gyllenhal (Kuhlmann & Mason, 2002). In this study, *S. gracilis* was associated with *M. edentulus*. Other known hosts of *S. gracilis* include Diptera (Agromyzidae, Calliphoridae, Cecidomyiidae, Chloropidae and Tephritidae), Hymenoptera (Cynipidae) and Lepidoptera (Tortricidae) (Noyes, 2006).

Plant association: *Stenomalina gracilis* has been associated with hosts developing on other Asteraceae, Brassicaceae, Cupressaceae and Fagaceae (Noyes, 2006) .

     Syntomopus sp. (Pteromalidae):

Life cycle: *Syntomopus* species are primary pupal ectoparasitoids (Graham, 1969). Results obtained in this study indicate that *Syntomopus* sp. is a primary larval ectoparasitoid. Adults appear in the field in May and July-August, and there are probably two generations per year (Graham, 1969).

Primary host association and hyperparasitism if existing: Hosts of *Syntomopus* sp. include Diptera (Agromyzidae) and Lepidoptera (Pterophoridae) (Yu *et al.*, 2005). The records from this study indicate that *Syntomopus* sp. is an ectoparasitoid of *M. edentulus*.

Plant association: Whereas *Syntomopus* spp. are known to develop in hosts associated with Asteraceae, no species had ever previously been reared from scentless chamomile. Other hosts of *Syntomopus* spp. feed on Apiaceae, Fabaceae, Solanaceae and Urticaceae (Graham, 1969; Vidal, 1993; Andriescu & Mitroiu, 2001).

     Triaspis sp. (Braconidae):

Life cycle : Species of the genus *Triaspis* are known to be larval or pupal endoparasitoids (Yu *et al.*, 2005). In our study a single specimen was found, as a cocoon near the remains of a consumed larva of *M. edentulus*.

Primary host associations: *Triaspis* species are known to be associated with hosts that include Coleoptera (Anobiidae, Attelabidae, Bostrichidae, Brentidae, Buprestidae, Cerambycidae, Chrysomelidae, Curculionidae), Diptera (Chlorophidae, Lonchaeidae, Tephritidae), and Lepidoptera (Choreutidae, Tischeridae, Tortricidae) (Yu *et al.*, 2005). Graham (1991) recorded *B. atrator* as a hyperparasitoid on *Baryscapus endemus* (Walker) (Hymenoptera: Chalcidoidea: Eulophidae : Tetrastichinae).

Plant association : Records have been made of *Triaspis* species associated with hosts in *Aesculus hippocastanum* L. (Hippocastanaceae) and *Poa pratensis* L. (Poaceae) (Yu *et al.*, 2005).

      Trichomalus cf. *gynetelus* Walker (Pteromalidae):

Note: Identifications of *T.* cf. *gynetelus* and *T.* cf *perfectus* are difficult to confirm because single specimens of each were collected and a series of each species is needed to study variations in several morphological characters that differentiate the species

Life cycle: The related species, *Trichomalus gynetelus* is considered to be a solitary pupal ectoparasitoid (Graham, 1969). Little is known of its biology. In this study, a single specimen of a species near *Trichomalus gynetelus* was reared from a larva of *M. edentulus*.

Primary host association: This species is considered to have a host range restricted to the family Apionidae (Coleoptera). Our single record on *M. edentulus* is the first for Curculionidae.

Plant association: Associations have been recorded of *T. gynetelus* on hosts living in Asteraceae, Cupressaceae, Polygonaceae and Tamaricaceae (Vidal, 1993; Vidal, 1997; Askew *et al.*, 2001).

      Trichomalus cf. *perfectus* Walker (Pteromalidae):

Note: see *Trichomalus* cf. *perfectus*.

Life cycle: The related species, *Trichomalus perfectus* is a solitary larval ectoparasitoid; adults emerge from diapause in spring and fly from May until August (Graham, 1969). There is evidence of a possible partial second generation if acceptable hosts are present later in the season (Graham, 1969).

Primary host association: This species is restricted to species of Ceutorhynchinae (Coleoptera) (Noyes, 2006). However, a few records are known of *T. perfectus* reared from Diptera (Cecidomyiidae) (Vidal, 1993) and Hymenoptera (Torymidae) (Mitroiu, 2001).

Plant association: *Trichomalus perfectus* is associated with hosts that develop in plant species among the families Asteraceae, Brassicaceae, Cupressaceae, Poaceae and Resedaceae (Dmoch, 1975a; Vidal, 1997; Kuhlmann & Mason, 2002).

Hadroplontus litura on Canada thistle

Phenology of Ceutorhynchinae hosts

All three *H. litura* larval instars were observed in shoots of Canada thistle during the first week of May and at each sampling site (Figure 3). Healthy third instar larvae left the plant beginning in the second week of May. Infestation levels were rather low, with densities never reaching three larvae per plant at any of the sites studied. Although densities observed at the sites in France and Germany in 2004 were similar to those observed in 2003, the average temperatures observed in 2004 were in the normal seasonal range (compared to 2.0-3.5°C higher temperatures observed in 2003) and the phenology of *H. litura* was delayed by one week in 2004 at the three sites studied (Figure 3). Infestation levels were rather low, with densities between 1.2±0.4 and 2.4±0.8 larvae per plant in France, and between 0.4±0.2 and 1.6±0.4 larvae per plant in Germany over the three-year survey (Table 2).

Table 2. Mean number (±SE) of second and third instar larvae *Hadroplontus litura* (Fabricius) available for parasitism, and percentage parasitism observed at sites in France and Germany in 2002, 2003 and 2004.

	Location	Site #	Mean # (±SE) of hosts (L2/L3) per plant			% Parasitisim
France	Alsace / Lucelle	9	1.21	±	0.41	12.5
		9	1.90	±	0.63	6.9
		9	2.35	±	0.81	47.1
	Alsace / Lucelle	10	1.25	±	0.33	15.4
		10	0.85	±	0.38	43.8
Germany	Southern Rhine valley / Grissheim	11	0.40	±	0.22	37.5
	Southern Rhine valley / Grissheim	12	0.65	±	0.23	35.3
	Bayern / Scheyern	13	1.55	±	0.44	9.3
	Schleswig Holstein / Stolpe	14	0.56	±	0.18	18.8
	Schleswig Holstein / Stolpe	15	0.48	±	0.20	15.4

Phenology of ectoparasitoids

Ectoparasitoids were already present when collections were begun in the second week of May 2003 (Figure 3). However, parasitoid activity increased from the third week of May to reach maximum levels by the second week of June. Parasitism levels were between 6.9 and 47.1%

in France and between 9.3 and 37.5% in Germany during the survey (Table 2). These parasitism levels might be slightly overestimated because healthy host larvae had already started to leave the plant when sampling was begun, and only the parasitized larvae and a low number of unparasitized third instar larvae were still present in the shoots.
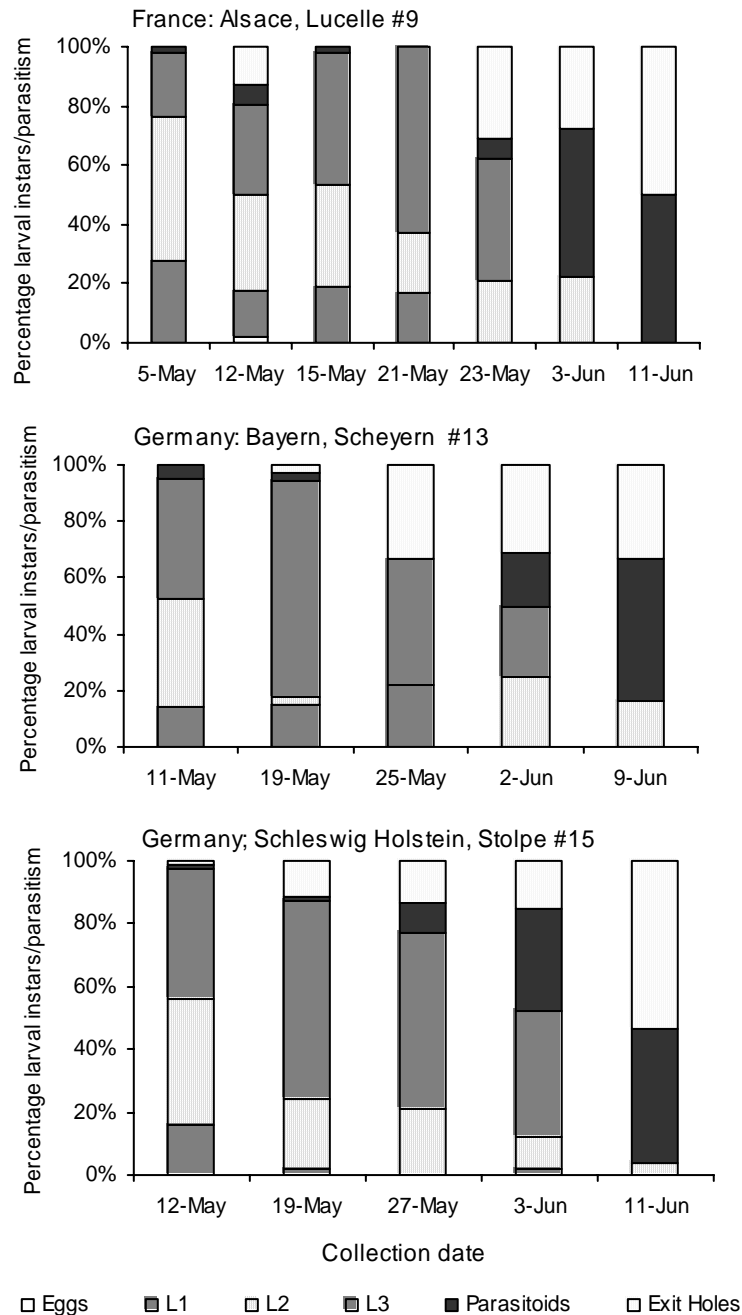

Figure 3. Proportion of larval instars 1-3 and pupae of *Hadroplontus litura* (Fabricius), exit holes, and parasitism at Alsace/Lucelle (site 9) in France, Bayern/Scheyern (site 13) and Schleswig Holstein/Stolpe (site 15) in Germany between May and June in 2003.

The ectoparasitoids that were found in the shoots on the last sampling dates (the beginning of June) in 2003 and 2004 had already built cocoons in the shoots. Twenty-five and 15 adults emerged over a one month period from these cocoons in April of 2003 and 2004, respectively.

Parasitoid assemblage

Bracon intercessor Nees was the only species found parasitizing *H. litura* larvae at all sites in 2003 (n=25) and 2004 (n=15). These data contrast with the diverse parasitoid complex associated with *M. edentulus* in scentless chamomile stems.

      Bracon intercessor Nees (Braconidae):

Life cycle: *Bracon intercessor* is an ectoparasitoid that sometimes oviposits in root galls made by Cleoninae weevils (Coleoptera: Curculionidae) (Volovnik, 1994; Yu *et al.*, 2005). Georgiev (2000) stated that the species has a gregarious life style. In this study *B*. *intercessor* was reared as a solitary larval ectoparasitoid of *H. litura.*

Primary host associations: The primary hosts of *B*. *intercessor* comprise Coleoptera (Cerambycidae, Curculionidae, Rhynchitidae), Hymenoptera (Tenthredinidae, Eurytomidae) and Lepidoptera (Gelechiidae, Tortricidae, Sesiidae) (Yu *et al.*, 2005).

Plant association: *Bracon intercessor* develops in hosts associated with Poaceae, Umbellifereae, Fabaceae, Apiaceae and Asteraceae (Yu *et al.*, 2005).

Discussion

The phenology of the Ceutorhynchinae hosts *H. litura* and *M. edentulus* observed in this study confirms the findings reported by Zwölfer & Harris (1966), Hinz *et al.* (1996) and McClay *et al.* (2002b). *Microplontus edentulus* oviposits in stems of scentless chamomile in May, and larvae develop through three larval instars between May and July, exiting the stems to pupate in the soil. *Hadroplontus litura* lays eggs in stems of Canada thistle, and larvae develop during May and June. Larvae of *C. obstrictus* also develop in canola pods during May to July (Bonnemaison, 1957) confirming that its phenology overlaps with those of *M. edentulus* and *H. litura* in the field. The geographical distributions of these three species also overlap in Europe (Bonnemaison, 1957; Zwölfer & Harris, 1966; Peschken & Wilkinson, 1981; Hinz *et al.*, 1996; McClay *et al.*, 2002b; Dosdall & Moisey, 2004). The phenological and geographical overlap of target (*C. obstrictus*) and non-target (*H. litura* and *M. edentulus*)

species clearly indicates a high risk that parasitoids of the target species could attack the two non-target species based on the criteria proposed by Kuhlmann *et al.* (2006a). *Trichomalus perfectus* and *M. morys* are the two most common parasitoids of *C. obstrictus*, and combined with the less common *S. gracilis*, these three species account for up to 90% of parasitism in Europe (2003). Of these parasitoids, *T. perfectus* and *M. morys* are potential candidates for introduction as biological control agents against *C. obstrictus* in North America (Kuhlmann *et al.*, 2002).

Gibson *et al.* (2005) demonstrated that *T. perfectus* and *M. morys* are not present in North America. *Stenomalina gracilis*, however, occurs on *C. obstrictus* in low numbers in British Columbia (Gibson *et al.*, in press). Prior to introduction of *T. perfectus* and *M. morys* and before considering *S. gracilis* for inundative biological control, it is essential to document their host ranges so that any actions taken are only with those parasitoids that are specific to ensure potential non-target effects are avoided. Assessment of the host specificity of these candidate parasitoids in their area of origin facilitates accurate prediction of potential non-target host impacts in the area of introduction as proposed by Kuhlmann *et al.* (2006a).

Our results determined several new host-parasitoid associations. *Chlorocytus* spp. were associated with *M. edentulus* for the first time and *E. curculionum* was associated with *M. edentulus* larvae at all sites where *Chlorocytus* spp. were present. Although *Chlorocytus* spp. are known to be hyperparasitoids of Eurytomidae (Graham, 1969) they were found only as primary larval ectoparasitoids of *M. edentulus*. Species of *Chlorocytus* develop in hosts associated with Asteraceae species; however, the association with hosts feeding on scentless chamomile reported here is new.

Stenomalina gracilis is associated with Ceutorhynchinae species such as *C. obstrictus* (Dmoch, 1975), *H. litura* (Vidal, 1997), *C. napi*, *C. pallidactylus*, and *C. roberti* (Kuhlmann & Mason, 2002). In this study, *S. gracilis* was associated with *M. edentulus* for the first time.

Our results indicated that *Syntomopus* sp. is a primary larval ectoparasitoid, whereas *Syntomopus* spp. have previously only been recorded as primary pupal ectoparasitoids (Graham, 1969). Furthermore, *Syntomopus* spp. hosts recorded in the literature include Diptera (Agromyzidae) and Lepidoptera (Pterophoridae) (Yu *et al.*, 2005). The records of *Syntomopus* sp. parasitizing *M. edentulus* comprise a new association.

Trichomalus gynetelus had been recorded as parasitizing weevils in the genus *Apion* (Vidal, 1993; Askew *et al.*, 2001). The single specimen of *T.* sp. nr. *gynetelus* reared from a larva of *M. edentulus* may represent a new family (Curculionidae) association for *T. gynetelus* or *T.* sp. nr. *gynetelus* may simply be another species that attacks a different host group of

Coleoptera. Identifications of *T.* sp. nr. *gynetelus* and *T.* cf *perfectus* are difficult to confirm because single specimens of each were collected and a series of each species is needed to study variations in several morphological characters that differentiate the species.

Table 3. Species and their proportions of the parasitoid assemblages associated with *Microplontus edentulus* (Schultze) for each sample site in 2003 and 2004.

Year	Site	Parasitoid species	# Individuals	%
2003	Austria Eisenstadt-Site 1	*Stenomalina gracilis* Walker[1]	2	25,0
		Eupelmus sp.[4]	2	25,0
		Bracon intercessor Nees[5]	2	25,0
		Trichomalus cf. perfectus Walker[1]	1	12,5
		Eupelmus sp.[4]	1	12,5
	Austria Eisenstadt-Site 2	*Stenomalina gracilis* Walker[1]	14	66,7
		Eurytoma curculionum Mayr [3]	5	23,8
		Trichomalus cf. gynetelus Walker[1]	1	4,8
		Bracon intercessor Nees[5]	1	4,8
	Austria Eisenstadt-Site 3	*Eurytoma curculionum* Mayr [3]	3	50,0
		Stenomalina gracilis Walker[1]	1	16,7
		Syntomopus sp. [1]	1	16,7
		Triaspis sp.[5]	1	16,7
2004	Hungary Bekes-Site 5	*Chlorocytus* sp.1[1]	7	36,9
		Eurytoma curculionum Mayr [3]	5	26,3
		Bracon intercessor Nees[5]	2	10,5
		Chlorocytus sp.2 [1]	2	10,5
		Ichneumonidae[6]	2	10,5
		Closterocerus sp.[2]	1	5,3
	Hungary Pest- Site 6	*Chlorocytus* sp.1[1]	2	22,2
		Chlorocytus sp.2 [1]	2	22,2
		Sphegigaster sp. [1]	2	22,2
		Eurytoma curculionum Mayr [3]	1	11,1
		Stenomalina gracilis Walker1	1	11,1
		Syntomopus sp. [1]	1	11,1
	Hungary Pest- Site 7	*Eurytoma curculionum* Mayr [3]	5	33,3
		Bracon intercessor Nees[5]	3	20,0
		Bracon atrator Nees[5]	2	13,3
		Stenomalina gracilis Walker[1]	2	13,3
		Syntomopus sp. [1]	2	13,3
		Sphegigaster sp. [1]	1	6,7
	Hungary Csongrad-Site 8	*Bracon atrator* Nees[5]	2	22,2
		Chlorocytus sp.1[1]	2	22,2
		Eurytoma curculionum Mayr [3]	2	22,2
		Syntomopus sp. [1]	2	22,2
		Sphegigaster sp. [1]	1	11,1

Chalcidoidea : 1Pteromalidae, 2 Eulophidae, 3Eurytomidae, 4Eupelmidae;
Ichneumonoidea: 5Braconidae, 6Ichneumonidae

Overall parasitism levels were equivalent between *M. edentulus* and *H. litura*, on average respectively 17.6% (1.9 – 53.3) and 24.2% (6.9 – 57.1) of all the host larvae available to parasitoids were parasitized. Among the thirteen species that represent the parasitoid assemblage of *M. edentulus*, 75% of the composition is represented by six species, *E. curculionum* (24%), *S. gracilis* (23%), *Braconidae* spp. (15%) and *Chlorocytus* sp. 1 (13%) respectively, the other 25% being shared among nine species. Our results for parasitism of *H. litura* are confirmed by results of Freese (1997) who found 24% parasitism at various sites in three regions of Germany. Furthermore, the braconid wasp *B. intercessor* is one of the four most common species in the parasitoid assemblage of *M. edentulus*, and was found to be the only species associated with *H. litura* in our study, a new record for that species. In fact, very few studies have dealt with parasitism of *H. litura*. However, Freese (1997) found another species of Braconidae, *Bracon immunator* Nees, attacking the weevil, and found *B. intercessor* in stems of *C. arvense* as well, but associated with *Mordellistema* sp. (Coleoptera: Mordellidae) and not with *H. litura*.

Exclusively idiobiont parasitoids were found on both *M. edentulus* and *H. litura* parasitizing larval instars or pupae in our study. Breadth of host range is very closely correlated with the koinobiont / idiobiont characterization (1986). The parasitoid complex of a given host appears to be reasonably constant over much of the host's geographic range (Askew & Shaw, 1986), and our findings confirm this for the parasitoid assemblages associated with *M. edentulus* and *H. litura* in four European countries: at least three of the four most represented species parasitizing *M. edentulus* (*Eurytoma curculionum*, *S. gracilis*, *Braconidae spp.*, *Chlorocytus* spp.) were always represented, independently of the country and survey sites, and the single species, *B. intercessor*, associated with *H. litura*, occurred at all sites where parasitism was observed. Furthermore, Askew & Shaw (1986) also stated that similar ectoparasitoid complexes attack taxonomically and ecologically related hosts in widely separated geographic areas. We could not confirm this because we looked only at two Ceutorhynchinae species, although *M. edentulus* was associated with a much broader parasitoid assemblage (thirteen species) than *H. litura* (a single parasitoid species). Nevertheless, Mills (1994) gives a partial answer, stating that the major correlate of species richness in the parasitoid communities of phytophagous insects is the host larval feeding site. It has been postulated that plant species of high exposure are subject to highest levels of colonization by herbivorous insects, and it would follow that parasitoids should find the greatest range of hosts on such plants (Askew & Shaw, 1986). This hypothesis is well illustrated in our study where: 1) *H. litura* is a stem and root feeder and mines into the thistle

rosette, at ground level, in spring and feeds downward into the root (i.e. it is subterranean and difficult to find); and 2) *M. edentulus* mine the aerial stems of scentless chamomile plants and move upward to the seed heads (i.e. it is easy to find). Furthermore, Hawkins (1994) demonstrates that when hosts are ranked on a scale of increasing concealment within the food-plant or soil, parasitoid species richness is dome-shaped, with borers supporting in general more parasitoid species than root-feeders. Therefore, Hawkins (1994) argues that there is evidence that host feeding niche can be used as a template against which to examine other forces that influence community size and structure, such as feeding biology or host taxonomy. As a matter of fact, the only representative of the family Braconidae, *B. intercessor*, was the only species found parasitizing both host species, which could lead to the hypothesis that some braconid wasps might be more adept at finding subterreanean hosts than pteromalids.

Our results show that at least three (*E. curculionum*, *Chlorocytus* spp., *S. gracilis*) and possibly two more (*T.* sp. nr. *gynetelus* and *T.* cf. *perfectus*) species parasitizing *M. edentulus* in shoots of scentless chamomile do occur in the parasitoid assemblages associated with *C. obstrictus* in Europe as shown by Williams (2003). In contrast, the parasitoid assemblage associated with *H. litura* does not share any species with that of *C. obstrictus*. These findings indicate the importance of considering feeding niche as an important criterion when developing a non-target species test list for host range testing of potential biological control agents as proposed by Kuhlmann *et al.* (2006a; 2006b).

Despite phenological overlap of *M. edentulus*, *H. litura*, and *C. obstrictus*, and overlapping geographical distributions in Europe, *M. morys* did not parasitize non-target hosts. Furthermore, we also found no evidence that *T. perfectus* attacks these species. In contrast, *S. gracilis* parasitized *M. edentulus* at five of the seven sites surveyed during two years, in Austria and Hungary.

The concerns regarding potential non-target effects of invertebrate biological control agents of arthropods has risen during the last decade and an increasing number of studies have addressed this topic (Babendreier *et al.*, 2005). Moreover, understanding the population dynamics of species of economic importance is still hampered by the insufficient knowledge of the natural enemy complexes that may play an important role in the regulation of herbivorous insects (Vidal, 2003). In the case of biological control of *C. obstrictus* in Canada, our studies, apart from providing ecological baseline data on parasitoid complexes of non-target species, show that, in their area of origin, *T. perfectus* and *M. morys* do not attack *M. edentulus* or *H. litura*. These results constitute a first step at understanding the risks associated

with an introduction of these potential agents to North America for biological control of *C. obstrictus*. In the event that these two parasitoids are introduced to Canada in the future, it appears that they would pose no risk to either *M. edentulus* or *H. litura*. However, *S. gracilis* poses some risk to *M. edentulus*, and therefore should not be further considered because its release or redistribution would lead to conflicting situations with weed biological control.

Acknowledgments

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European Ectoparasitoids of Two Ceutorhynchinae Associated with Shepherd's Purse and Hoary Cress: Assessing Safety Requirements for Classical Biological Control of Cabbage Seedpod Weevil

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Abstract

Two Ceutorhynchinae seed feeders (Coleoptera: Curculionidae) *Ceutorhynchus typhae* (Herbst) [=*Ceutorhynchus floralis* Paykull] and *Ceutorhynchus turbatus* (Schultze) are part of the herbivore complex associated with two weeds in Europe, shepherd's purse, *Capsella bursa-pastoris* (L.) Medicus [Brassicaceae], and hoary cress, *Lepidium draba* L. (=*Cardaria draba*) [Asteraceae] respectively. Both weeds of European origin are present in North America and *C. turbatus* is being considered for introduction as biological control agent against hoary cress, whereas *C. typhae* is adventive on shepherd's purse. Both weeds occur in the same regions and habitats as the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)], a major invasive alien pest of canola (*Brassica napus* L. & *B. rapa* L.) in North America. A three year field survey was carried out in Europe to collect *C. typhae* and *C. turbatus* and their ectoparasitoids, *Trichomalus perfectus* Walker and *Mesopolobus morys* Walker (both Hym: Pteromalidae), with the aim of verifying whether or not the candidate ectoparasitoids considered for introduction as biological control agents against *C. obstrictus* occur in the assemblages associated with these two species. We found no evidence that *T. perfectus* attacks either *C.*

typhae or *C. turbatus*. In contrast, *M. morys* was found to be the most common parasitoid associated with *C. turbatus*.

Introduction

Invasive alien species are recognized as one of the leading threats to biodiversity and they impose enormous costs on agriculture (Wittenberg & Cock, 2001; Wright *et al.*, 2005). North America has to deal with a number of invasive alien species of high economic importance. Pimentel *et al.* (2000) state that exotic species are responsible for approximately $137 billion in damages per year in the USA. In Canada, annual losses are estimated to be $4.2 billion to agriculture alone (Dawson, 2002). Furthermore, invasive species and the ecological and agricultural threats they pose are rapidly increasing with globalization of markets and increased movement of goods and people worldwide (Perrings *et al.*, 2002; Born *et al.*, 2005; Wright *et al.*, 2005). Canada and the USA are currently struggling with a major problem caused by two exotic pest species, one arthropod and one weed, the control measures for which are inextricably linked. The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)] (Col.: Curculionidae), which is a pest of oilseed rape (canola), *Brassica napus* L. [Brassicaceae], in Europe was accidentally introduced to North America where it is now widespread (Kuhlmann *et al.*, 2002). Hoary cress, *Lepidium draba* L. (=*Cardaria draba*) [Asteraceae], which was accidentally translocated from Europe to North America where it is now a weed mainly in wheat and barley (Schwarzlaender *et al.*, 2002), but also occurs in habitats surrounding canola fields (Cripps *et al.*, in press). *Ceutorhynchus obstrictus* is currently controlled through the use of broad-spectrum chemical insecticides (Dosdall *et al.*, 2001; Carcamo *et al.*, 2005). Whereas hoary cress is not only growing in crops but is spreading to natural habitats where the application of herbicides would be neither cost-effective nor feasible. Therefore, alternative control strategies are urgently needed to reduce pesticide use and to overcome difficulties in current management strategies.

In comparison with other methods, classical biological control is a very appropriate option to manage invasive alien species (Wittenberg & Cock, 2001). When it is successful, it is highly cost-effective, permanent, self-sustaining and ecologically safe because of the high specificity of the agents used (Wittenberg & Cock, 2001). In Europe, the seed-feeding *C.*

obstrictus is controlled by a wide range of natural enemies among which are two Pteromalidae species, *Trichomalus perfectus* (Walker) and *Mesopolobus morys* (Walker). These parasitoids are known to reduce the populations of *C. obstrictus* by up to 80% (Williams, 2003) and are being considered for introduction as biological control agents against *C. obstrictus* in Canada (Kuhlmann *et al.*, 2002). Regarding the potential usage of biological control for hoary cress, the seed-feeder *Ceutorhynchus turbatus* Schultze (Col.: Curculionidae) is known to be an important natural enemy due to its success at reducing the impact of the weed in its natural habitats in Europe (Cripps *et al.*, in press). Therefore it is undergoing evaluation as a candidate biological control agent against hoary cress and is being considered for introduction to North America (Cripps *et al.*, in press).

It has been reported that hoary cress is a reservoir for economic pests in the USA, such as *C. obstrictus* (Fox & Dosdall, 2003; Dosdall & Moisey, 2004). Cripps *et al.* (in press) recently confirmed that hoary cress can be used as an alternative food source and/or early season refuge by *C. obstrictus*. Reducing populations of hoary cress would therefore benefit the *C. obstrictus* control programme by decreasing the availability of an alternative host plant that helps maintain populations of *C. obstrictus* outside canola fields, away from insecticide treatments. However, potential conflicts could link these two projects. According to Kuhlmann *et al.* (2006), if *T. perfectus* and *M. morys* are introduced for the control of *C. obstrictus*, the risk of using alternative hosts may be exacerbated if the target and non-targets show an overlap in (1) geographical distribution; (2) time of occurrence; and (3) host feeding niches. These conditions might be combined in the case of both *C. turbatus* and *C. obstrictus*. Interestingly, these overlaps might also apply to another non-target weevil as well, *Ceutorhynchus typhae* (Herbst) [=*Ceutorhynchus floralis* Paykull] (Col.: Curculionidae), which shares the same feeding niche and habitats. This seed-feeder is present in Canada (Bousquet, 1991; Bouchard *et al.*, 2005) and is part of the herbivore complex associated with shepherd's purse *Capsella bursa-pastoris* (L.) Medicus [Brassicaceae], a weed of economic importance in canola, wheat and barley in North America (Moss, 1959; Budd & Best, 1969; Leeson *et al.*, 2005). Consequently, these interactions must be taken into consideration when assessing potential non-target effects of biological control agents of *C. obstrictus*.

Prior to introduction, the potential risks of *C. obstrictus* parasitoids attacking related non-target species have to be assessed; therefore surveys in the area of origin are necessary to determine the parasitoid assemblages associated with *C. turbatus* and *C. typhae*. Furthermore, such studies will provide information on ecological traits of the weevils that is crucial when selecting non-target species for host-specificity testing, allowing more precise prediction of

potential risks associated with introduction of candidate agents for biological control of *C. obstrictus* in Canada. The objectives of this study were to: 1) describe the phenology of *C. typhae* and *C. turbatus* to determine if there is a phenological overlap with that of *C. obstrictus*; 2) assess the parasitism levels of *C. typhae* and *C. turbatus*, particularly by ectoparasitoids; and 3) determine the parasitoid assemblage associated with these two Ceutorhynchinae species.

Material & Methods

Field collected material

All shepherd's purse and hoary cress plants studied were field collected in fallow fields, at field margins or along road sides in May to June in 2003 and 2004. Surveys for *C. typhae* were made at seven sites in Germany, Switzerland and France and for *C. turbatus* at fourteen sites located in Switzerland and two separate regions of Hungary (Figure 1). Because shepherd's purse and hoary cress are pioneer species, they are very often growing at road sides or field margins. Therefore it is difficult to find stable sites as these are regularly mowed. However, it is clear that *C. typhae* and *C. turbatus* occur more frequently in stable sites where they have had time to build up populations. Thus, collections were made in wastelands or abandoned fields because they represented more stable habitats.

Phenology of Ceutorhynchinae hosts

Samples of 10 to 20 host plants were collected and dissected as often as every two days and at a maximum of 10 day-intervals. Twenty pods per plant were dissected to detect Ceutorhynchinae eggs and larvae which were determined to instar. The late third instar larvae of both *C. typhae* and *C. turbatus* chew an exit hole through the pod wall and drop down into the soil where they pupate. Thus, exit holes were recorded and taken into account to estimate the number of healthy third instar larvae that had left the plants. At high larval infestation levels, a maximum of two larvae can develop inside the same pod, feeding on the seeds separated by an internal wall. Thus, two third instar larvae can exit the plant using the same hole, which might have led to a slight under-estimation of larval numbers.
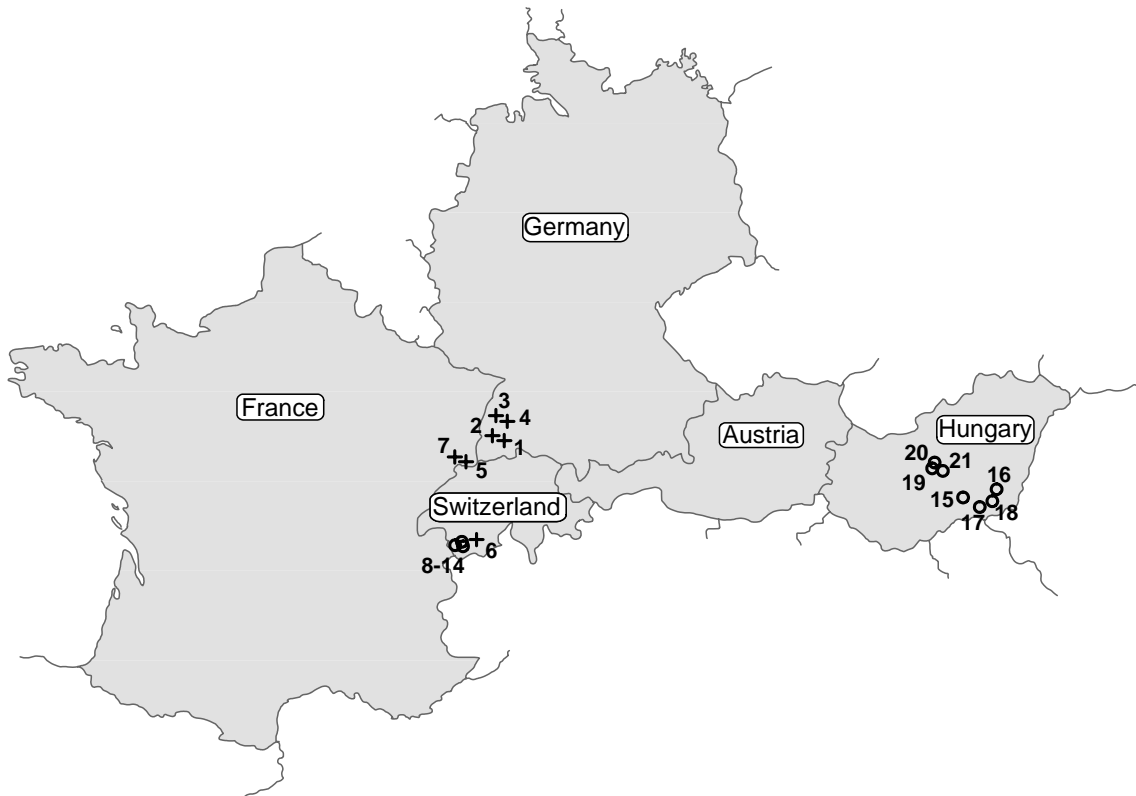
Host plant / Ceutorhynchinae species	Country	Region / Town	Site Code	GPS Coordinates Lat. N	GPS Coordinates Long. E	Elevation (m.)
Capsella bursa pastoris /	Germany	Southern Rhine Valley / Neuenburg	1	47°47'42.70"	07°34'48.80"	227
Ceutorhynchus typhae		Southern Rhine Valley / Neuenburg	2	47°50'53.00"	07°35'34.00"	218
		Southern Rhine Valley / Buggingen	3	47°51'30.93"	07°36'59.91"	216
		Southern Rhine Valley / Zienken	4	47°50'28.91"	07°35'47.45"	220
	Switzerland	Jura / Alle	5	47°26'11.79"	07°08'31.07"	497
		Valais / Aproz	6	46°12'46.48"	07°19'07.72"	481
	France	Alsace / Boron	7	47°33'19.21"	07°00'22.26"	385
Lepidium draba /	Switzerland	Valais / Chamoson	8	46°10'58.56"	07°13'51.44"	485
Ceutorhynchus turbatus		Valais / Econe	9	46°10'28.87"	07°12'54.19"	468
		Valais / Saxon	10	46°08'40.03"	07°09'51.67"	465
		Valais / Aproz	11	46°12'46.48"	07°19'07.72"	481
		Valais / Martigny	12	46°06'36.49"	07°06'12.82"	456
		Valais / Martigny	13	46°06'47.04"	07°06'54.24"	457
		Valais / Sion	14	46°13'11.80"	07°20'43.09"	483
	Hungary	Csongrad / Hodmezovasarhely	15	46°24'50.80"	20°00'03.24"	85
		Csongrad / Hodmezovasarhely	16	46°24'45.24"	20°17'21.66"	74
		Csongrad / Hodmezovasarhely	17	46°15'41.62"	20°12'24.52"	78
		Bekes / Kardoskut (1)	18	46°16'41.41"	20°19'34.25"	74
		Pest / Sari - Bugyi (2)	19	47°12'09.12"	19°13'50.22"	92
		Pest / Gyal	20	47°11'48.19"	19°06'36.40"	92
		Pest / Sari - Bugyi (1)	21	47°12'30.24"	19°13'06.06"	92

Figure 1. Location of field collection sites of *Capsella bursa-pastoris* (L.) Medicus surveyed for the occurrence of *Ceutorhynchus typhae* (Herbst) [o] and *Lepidium draba* L. for *Ceutorhynchus turbatus* Schultze [+] in 2003 and 2004, respectively.

Parasitism by ectoparasitoids

For each plant dissected, ectoparasitoid eggs and larvae found on host larvae as well as ectoparasitoid pupae found near the remains of a consumed host body were recorded to estimate the parasitism level on each collection date for every field site studied. Mortality due to host feeding by ectoparasitoid adults was considered as an indirect effect of parasitism and recorded independently. Parasitism was calculated for each site as follows:

$$\%Parasitism = \frac{\sum_1^n paras.}{\sum_1^n avail.hosts} \times 100 = \frac{\sum_{larv}^{par} + \sum_{larv}^{\dagger} + \sum_{par}^{alone}}{\sum_{larv}^{healthy} + \sum_{larv}^{par} + \sum_{larv}^{\dagger} + \sum_{holes} + \sum_{par}^{alone}} \times 100$$

Where $\sum_1^n paras.$ is the total number of parasitoids found in the plant; $\sum_1^n avail.hosts$ is the total number of hosts available for parasitism (second and third instar larvae) found in the plant; Σ_{larv}^{par} is the number of larvae found parasitized by an ectoparasitoid; $\Sigma_{larv}^{\dagger}$ is the number of larvae found dead with traces of host feeding like brownish punctures on the tegumen; Σ_{par}^{alone} is the number of parasitoids found alone near the remains of a consumed host, either as a pupa, or as a newly emerged adult which had not yet exited the plant; $\Sigma_{larv}^{healthy}$ is the number of healthy host larvae; Σ_{hole} is the number of exit holes (made by healthy third instar larvae that have left the plant), found on the shoots or pods during dissections.

A representative parasitism level was estimated for each site, that date on which the maximum number of healthy larvae still available for parasitism were present in the plant, i.e. just before exit holes appeared.

Host density was determined by considering the maximum number of hosts parasitized or available for parasitism during the season. Thus, only second and third instar larvae were used for calculation. Exit holes were recorded as equivalent to a single third instar larvae that had exited the plant, although as explained previously two larvae can exit from the same hole.

Parasitoid assemblages

Parasitoid eggs and larvae were reared on the host larvae they had been parasitizing and pupae were individually reared on a disc of filter paper in 5.5cm diameter Petri dishes. Insects were maintained in a climate chamber at 20 ± 2°C, 70% ±10 RH. and 16h L: 8h D. The insects were checked daily until emergence of the adult parasitoid. Some groups emerged the same summer season that they were collected. Mortality was rather high over rearing periods.

Identification was possible only for adults which, directly after they had emerged, were killed using Ethyl Acetate. Every specimen was card-mounted, labelled with individual information, and stored for further identification purposes. All taxonomic identifications for the family Pteromalidae were made in collaboration with Hannes Baur at the Natural History Museum of Bern (NMBE), Switzerland and Dr. Gary Gibson, Canadian National Collection of Insects, Ottawa, Canada. Eurytomidae specimens were identified by Michael Gates, USDA-ARS, National Museum of Natural History, Washington DC, United States. Where possible, Pteromalidae and Braconidae specimens were identified to species while other families were identified to genus.

Available information on life cycle, primary host association, hyperparasitism records, if existing, and plant host – weevil – parasitoid associations were obtained from the literature. Relevant references were obtained from the Taxapad programme (Yu *et al.*, 2005) and the Universal Chalcidoidea Database (Noyes, 2006). Hannes Baur, NMBE, Switzerland, and Garry Gibson, CNC, Ottawa, Canada were consulted for providing additional information on Chalcidoidea.

Results

Ceutorhynchus typhae on shepherd's purse

<u>Phenology of hosts</u>

Sites surveyed in Germany, France and Switzerland yielded similar results in both 2003 and 2004 (Figure 2). *Ceutorhynchus typhae* laid eggs during the entire sampling period. First instar larvae were present in pods of Shepherd's purse from mid-may onwards. The number of second and third instar larvae available to parasitoids increased beginning the second week of May and in Germany, Southern Rhine Valley, reached maximum densities of between 0.2±0.1 (Site 4) and 3.1±0.4 (Site 1) per plant (Table 1) on the second and third weeks of June. At all sites surveyed, third instar larvae appeared beginning the first week of May and mature larvae started to leave the plants, beginning the second week of June.

Phenology of ectoparasitoids

Ectoparasitoids were present beginning the first week of June and for a period of about two weeks (Figure 3) Overall parasitism was highest at Site 2 near Neuenburg, southern Rhine Valley, Germany. Parasitism reached a maximum of 59.5% at Site 5 near Alle, in the Swiss Jura on the 28 June 2004. Parasitism levels were between 10 and 15% in Germany (2003) and between 15 and 59.5% in Switzerland and France (2004).

Table 1. Mean number (±SE) of second and third instar larvae of *Ceutorhynchus typhae* (Herbst) available for parasitism, and percent parasitism observed in Germany, Switzerland and France in 2003 and 2004.

	Location	*Site Code*	*Mean # (±SE) of Hosts (L2/L3) per plant*			*% parasitisim*
Germany	Southern Rhine Valley / Neuenburg	*1*	1.58	±	0.45	10.0
	Southern Rhine Valley / Neuenburg	*2*	3.05	±	0.44	11.5
	Southern Rhine Valley / Buggingen	*3*	0.00	±		0.0
	Southern Rhine Valley / Zienken	*4*	0.15	±	0.08	0.0
Switzerland	Jura / Alle	*5*	4.60	±	0.29	59.5
	Valais / Aproz	*6*	1.85	±	0.33	15.0
France	Alsace / Boron	*7*	1.85	±	0.15	16.7

The relationship between host density and parasitism was significant (ANOVA, F = 18.45; df 1, 6; P = 0.028; y = 11.22x – 4.8645 ; R^2 = 0.7868) showing the number of *C. typhae* larvae available to parasitism in the plants was positively related to the level of parasitism by ectoparasitoids (Figure 2).
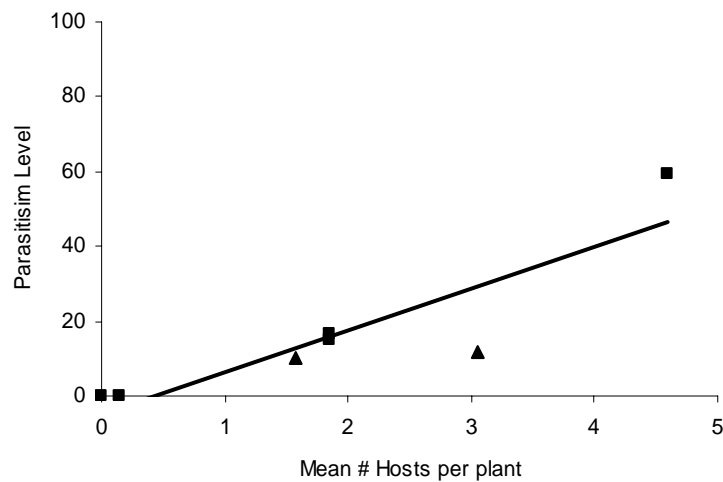

Figure 2. Comparison between mean number of hosts and percentage parasitism observed 2003 (▲) and 2004 (●) surveys. (ANOVA, F = 18.45; df1, 6; P = 0.028; y = 11.22x – 4.8645; R^2 = 0.7868).
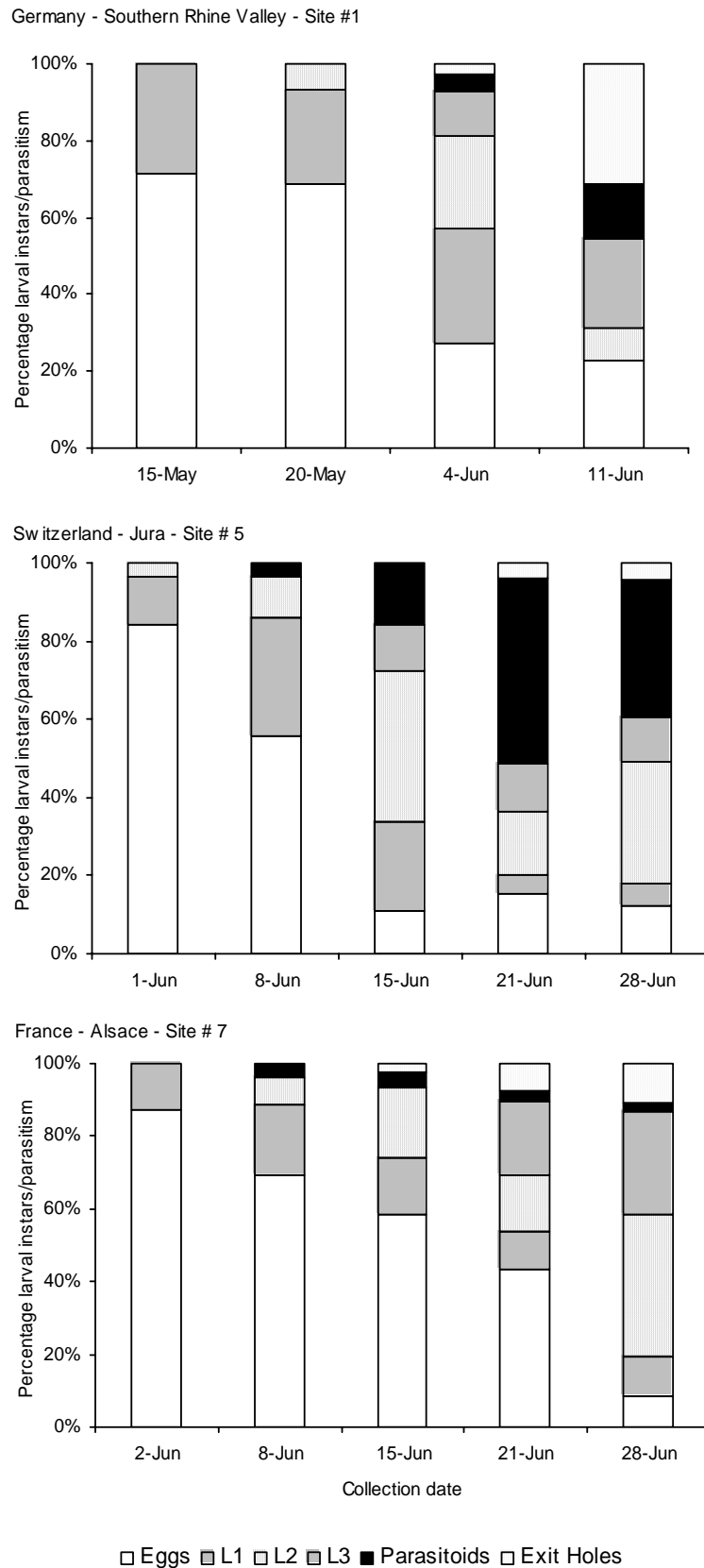
Figure 3. Proportion of eggs, larval instars 1-3 of *Ceutorhynchus typhae* (Herbst), parasitism, and exit holes in Germany/Southern Rhine Valley, Neueunburg (Site 1), Switzerland/ Jura, Alle (Site 5), and France/ Alsace, Boron (Site 7) between May and July in 2003 and 2004.

Parasitoid assemblages

Two parasitoid species associated with *C. typhae* were collected from shepherd's purse, both belonged to the family Pteromalidae (Hymenoptera). This assemblage included one new species belonging to the genus *Mesopolobus, Mesopolobus gemellus* Baur&Muller sp. nov. (Baur *et al.*, submitted; Chapter II of the thesis) of which 37 specimens were individually reared (Table 3). Only a single specimen of *S. gracilis* was found at the Site 6 near Aproz in the Swiss Valais.

Mesopolobus gemellus Baur&Muller sp. nov. (Pteromalidae):

Life cycle: Results of our study show that *Mesopolobus gemellus* females lay eggs on late instar larvae of *C. typhae* in May and June. Adults of the new generation emerge in June and July the same year, and exit the senesced pods (Baur *et al.*, submitted; Chapter II of the thesis).

Primary host association: Mesopolobus gemellus is known only from *C. typhae*. However, the species has only very recently been discovered, and since it is closely related to and might have been misidentified as *M. morys* in the past, other primary host associations may exist that will be clarified in the future.

Plant association: The species has only been collected from pods of shepherd's purse (Baur *et al.*, submitted; Chapter II of the thesis).

Stenomalina gracilis Walker (Pteromalidae):

Life cycle: *Stenomalina* spp. are solitary primary larval or pupal ectoparasitoids. Adults can be observed in the field from June to October (Graham, 1969).

Primary host association: Stenomalina gracilis is associated with weevil species in the subfamily Ceutorhynchinae, such as *C. obstrictus* (Dmoch, 1975a), *H. litura* (Vidal, 1997), *C. napi* Gyllenhal, *C. pallidactlylus* (Marsham), and *C. roberti* Gyllenhal (Kuhlmann & Mason, 2002). In this study, *S. gracilis* was associated with *C. turbatus*. Other known hosts of *S. gracilis* include Diptera (Agromyzidae, Calliphoridae, Cecidomyiidae and Chloropidae, Tephritidae), Hymenoptera (Cynipidae) and Lepidoptera (Tortricidae) (Noyes, 2006).

Plant association: *Stenomalina gracilis* has been associated with hosts developing on other Asteraceae, Brassicaceae, Cupressaceae and Fagaceae (Noyes, 2006) .

Ceutorhynchus turbatus on hoary cress

Phenology of hosts

Only eggs of *C. turbatus* were found on the first sampling dates on 20 and 26 May in 2004 (Figure 5) Eggs hatched and first instar larvae were observed beginning the last week of May or first week of June. Second instar larvae were present beginning the second week of June at all sites surveyed in Switzerland and Hungary. Infestation levels observed in Valais – Switzerland were rather similar in 2003 and 2004, with maximum densities of second and third instar larvae per plant observed at 9.3±0.2 (Site 10) near Saxon in 2003 and 11.3±0.6 near Sion (Site 14) in 2004 (Table 2). In Hungary, in 2004, densities tended to be lower on average at all sites surveyed in Hungary in 2004, with a maximum of 7.5±0.7second and third instar larvae per plant observed at the Site 17 near Hödmezövasarhely (Table 2). On average, densities of second and third instar larvae per plant determined in 2003 and 2004 were 6.8±1.7 in Switzerland, and 2.1±1.6 in Hungary.

Table 2. Mean number (±SE) of second and third instar larvae of *Ceutorhynchus turbatus* Schultze available for parasitism, and percent parasitism observed in Switerland and Hungary in 2003 and 2004.

	Location	Site Code	Mean # (±SE) of hosts (L2/L3) per plant			% Parasitisim
Switzerland	Valais / Chamoson	*8*	3.90		*	15.4
	Valais / Econe	*9*	1.88		*	80.0
	Valais / Saxon	*10*	9.15	±	0.21	39.8
	Valais / Aproz	*11*	0.75	±	0.23	18.2
	Valais / Martigny	12	10.50	±	0.72	19.9
	Valais / Martigny	13	10.10	±	1.02	32.8
	Valais / Sion	14	11.25	±	0.64	44.0
Hungary	Csongrad / Hodmezovasarhely	15	7.50	±	0.70	25.6
	Csongrad / Hodmezovasarhely	16	0.00	±	0.00	0.0
	Csongrad / Hodmezovasarhely	17	3.35	±	0.40	41.8
	Bekes / Kardoskut (1)	18	1.15	±	0.27	28.6
	Pest / Sari - Bugyi (2)	19	1.15	±	0.31	33.3
	Pest / Gyal	20	0.90	±	0.26	11.1
	Pest / Sari - Bugyi (1)	21	0.70	±	0.21	21.4

* only one sampling date

Phenology of ectoparasitoids

At all sites, parasitoids were present earlier in the season in Hungary than in Switzerland at all sites surveyed. First evidence of parasitism occurred the second week of June in Hungary, as

soon as second instar larvae were observed, whereas in the Swiss Valais, parasitism was evident later, beginning the last week of June (Figure 5). All healthy larvae had left the plants on the 24 June in Hungary at the Site 20 near Gyal, whereas at the same period all larval instars were observed in the plants dissected from sites surveyed in Switzerland (Figure 5). Parasitism levels in Switzerland ranged between 15.4 to 80% in 2003, and 18.2 to 44% in 2004. Similar parasitism levels (11.1 to 41.8%) were observed in Hungary, except for Site 18 near Hödmezövasarhely where no parasitoids were found. The relationship between number of *C. turbatus* larvae available for parasitism in the plants and level of parasitism was not significant (ANOVA, F = 0,5359; df 1, 6; P = 0.09; y = 0.9274x + 25.296; R^2 = 0.0427) (Figure 4).
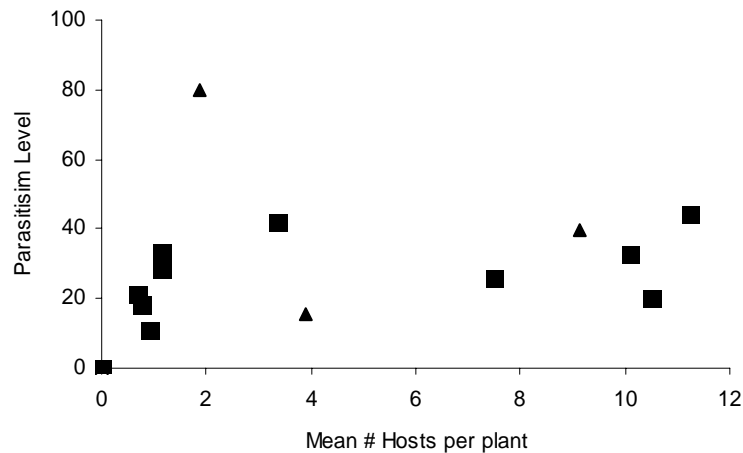

Figure 4. Comparison between mean number of hosts and percentage parasitism observed 2003 (▲) and 2004 (●) surveys. (ANOVA, F = 0.5359; df 1, 6; P = 0.09; y = 0.9274x + 25.296; R^2 = 0.0427)

Parasitoid assemblage

The ten parasitoid species associated with *C. turbatus* from hoary cress belonged to the hymenopteran families: Pteromalidae, Eulophidae, Eurytomidae, and Eupelmidae (Table 3). Of the species found some are poorly known and taxonomic expertise is not available. Available information such as life cycle, primary host association and hyperparasitism, and plant association are provided for all members of the parasitoid assemblage of *C. turbatus* in alphabetic order:

Baryscapus sp. (Eulophidae):

Life cycle: Baryscapus sp. belong to the Tetrastichinae, which are usually primary endoparasitoids of the eggs, larvae or pupae of gallicolous Diptera, Hymenoptera or Lepidoptera (Noyes, 2006). A number of species develop as ectoparasitoids or as facultative or obligate hyperparasitoids. Others are predators on the eggs of various insects. Although little is known of the biology of *Baryscapus* species, many different life styles seem to occur among the genus: some species are primary ectoparasitoids of Aranea eggs (Noyes, 2006),
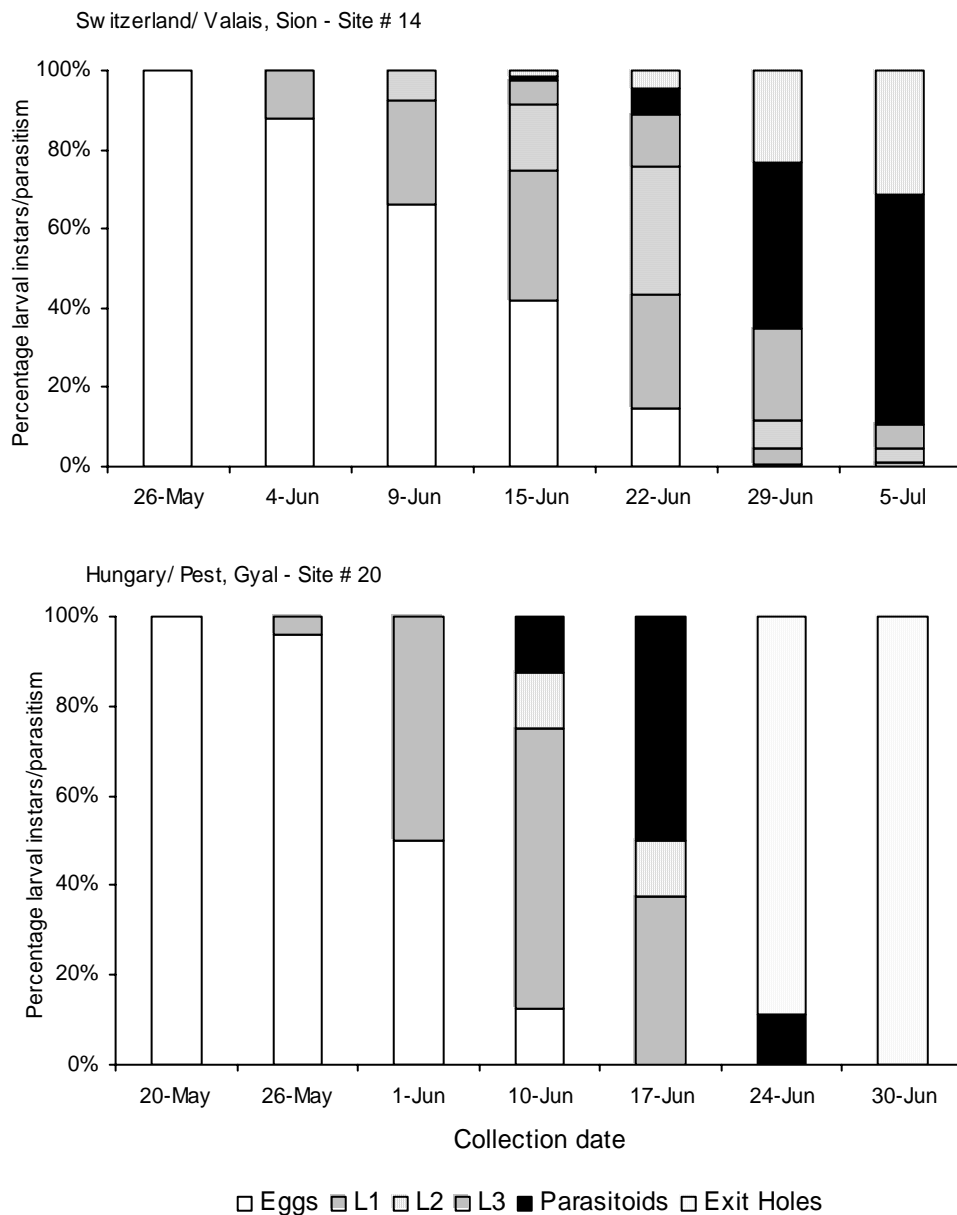
Figure 5. Proportion of eggs, larval instars 1-3 of *Ceutorhynchus turbatus* Schultze, parasitism, and exit holes in Switzerland/ Valais, Sion (Site 14) and Hungary/ Pest, Gyal (Site 20) between May and July in 2004.

others are pupal endoparasitoids and overwinter in the coccons of their hosts (Mey, 1993). In our study specimens were found that were larval ectoparasitoids of *C. turbatus* in pods of shepherd's purse.

Primary host association: Species of *Baryscapus* are associated with hosts among Coleoptera, Diptera (Agromyzidae), Lepidoptera (Gracillariidae, Lyonetiidae, Notodontidae, Tischeriidae, Tortricidae and Yponomeutidae), Neuroptera (Chrysopidae), and Aranea (Tomisidae) (Noyes, 2006).

Plant association: *Baryscapus* sp. hosts develop in plant species of Brassicaceae, Fagaceae, Hippocastanaceae, Pinaceae, Rutaceae, Salicaceae, Verbenaceae (Noyes, 2006).

Closterocerus spp. (Eulophidae):

Species of the genus *Closterocerus* have a broad host range (Hansson, 1990; Hansson, 1994; Gumovski, 2001; Yu *et al.*, 2005). Some species are known to be primary parasitoids of leaf-miners and gall-formers, and others are also reported from Symphyta eggs (Argidae, Diprionidae), armored scales (Daspididae), and psyllids (Psyllidae) (Schauff, 1991; Hansson, 1996; Yu *et al.*, 2005).

Eupelmus sp. (Eupelmidae):

The majority of Eupelmidae species are parasitoids and facultative hyperparasitoids on the immature stages of Lepidoptera, Homoptera, Hymenoptera, Coleoptera, Neuroptera and Orthoptera (Clausen, 1940; Noyes, 2006). A small number of species are predators on the eggs or larvae of various insects, or on the eggs of spiders and a few are solitary, primary endoparasitoids of eggs of Lepidoptera, Orthoptera and Hemiptera (Yu *et al.*, 2005). Most eupelmids are solitary, but some species are gregarious (Clausen, 1940). Most are ectoparasitoids, including some that develop gregariously on dipterous pupae within puparia, and a few species are solitary endoparasitoids of Coccoidea (Noyes, 2006).

Eurytoma curculionum Mayr (Eurytomidae):

Life cycle: *Eurytoma curculionum* is a larval ectoparasitoid of concealed Coleoptera (Fischer, 1965). Less frequently it is a hyperparasitoid of late instar larvae of chalcid wasps (Fischer, 1965). *Eurytoma curculionum* is bivoltine, adults emerge in late May and June, a partial second generation occurs in August (Fischer, 1965).

Primary host association: Eurytoma curculionum is associated with Coleoptera such as Apionidae (Fischer, 1965; Vidal, 1997), Buprestidae (Boucek, 1977) and Curculionidae

(Dmoch, 1975a; Boucek, 1977). In this study, *E. curculionum* was for the first time associated with *C. turbatus.*

Plant association: Records can be found of *E. curculionum* associated with hosts developing in *Brassicaceae, Polygonaceae, Rubiaceae* and *Scrophulariaceae* (Yu *et al.*, 2005).

Mesopolobus morys Walker (Pteromalidae):

Life cycle: Although some aspects of its biology, such as overwintering, still remain relatively obscure, the biology of the species has been well documented. *M. morys* is a solitary larval ectoparasitoid of concealed hosts (Bonnemaison, 1957; Dmoch, 1975b; Dmoch, 1975a; Buechi, 1993; Murchie & Williams, 1998). Females lay their eggs in spring and adults of the new generation emerge during the same season (Dmoch & Klimek, 1975). The new generation adults overwinter and are active again the following spring. The species completes a single generation per year, however, a partial second generation may occur when conditions are optimal (Muller *et al.*, 2003; Muller *et al.*, 2004).

Primary host association: The species has a host range that includes Coleoptera (Apionidae, Curculionidae) and Diptera (Cecidomyiidae) (Graham, 1969; Noyes, 2006).

Plant association: Insect hosts that allow complete development of the species are associated with plants of the families Brassicaceae, Fabaceae and Tamaricaceae (Noyes, 2006).

Pteromalus sequester Walker (Pteromalidae):

Life cycle: Female *P. sequester* lay eggs externally on the larvae or pupae of their hosts, adults of the new generation generally overwinter in their host's food plants (Parnell, 1964).

Primary host association and hyperparasitism: Most of the known hosts of *P. sequester* are concealed and include Coleoptera (Apionidae, Bruchidae, Curculionidae), Diptera (Cecidomyiidae), Hymenoptera (Eurytomidae) and Lepidoptera (Pyralidae). A few records documented *P. sequester* as a hyperparasitoid of Hymenoptera such as species of Pergidae (Noyes, 2006).

Plant association: *Pteromalus sequester* is associated with insect hosts that develop in a wide range of plant species of the Asteraceae, Caryophyllaceae, Chenopodiaceae, Convolvulaceae, Cupressaceae, Euphorbiaceae, Fabaceae, Malvaceae, Plantaginaceae, Scrophulariaceae, Tamaricaceae and Zygophyllaceae (Noyes, 2006).

Pteromalus sp. (Pteromalidae):

Life cycle: *Pteromalus* species have a wide range of life styles, being either solitary or gregarious, larval or pupal, ecto- or endoparasitoid (Noyes, 2006). Some species such as *Pteromalus puparum* (Linnaeus) overwinter as mature larvae within their host's pupae (Takagi, 1985).

Primary host association: Species of *Pteromalus* are associated with hosts in a wide range of families among Coleoptera, Dictyoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera (Noyes, 2006). In addition, Noyes (2006) listed several species that are known to develop as hyperparastioids of Diptera (Tachinidae), Hymenoptera (Braconidae, Chalcididae, Encyrtidae, Eulophidae, Ichneumonidae). In our study, individuals of *Baryscapus* sp. were found as ectoparasitoids of larval *C. turbatus*.

Plant association: As a consequence of the wide range of known hosts, the species of *Pteromalus* develop in association with a broad variety of plants among Anacardiaceae, Asteraceae, Betulaceae, Boraginaceae, Brassicaceae, Cyperaceae, Ericaceae, Fabaceae, Fagaceae, Hippocastanaceae, Malvaceae, Oleaceae, Onagraceae, Pedaliaceae, Pinaceae, Poaceae, Rosaceae, Rutaceae, Salicaceae, Scrophulariaceae, Tamaricaceae, Tyhpaceae, Ulmaceae, Urticaceae (Noyes, 2006).

Syntomopus sp. (Pteromalidae):

Life cycle: *Syntomopus* spp. are primary pupal ectoparasitoids (Graham, 1969). Results obtained in this study indicate that *Syntomopus* sp. is a primary larval ectoparasitoid. Adults appear in the field in May and July-August, and there are probably two generations per year (Graham, 1969).

Primary host association: Hosts of *Syntomopus* sp. include Diptera (Agromyzidae) and Lepidoptera (Pterophoridae) (Yu *et al.*, 2005). The records from this study indicate that *Syntomopus* sp. is an ectoparasitoid of *C. turbatus.*

Plant association: Whereas other hosts of *Syntomopus* spp. are known to develop in hosts associated with Asteraceae, Apiaceae, Fabaceae, Solanaceae and Urticaceae (Graham, 1969; Vidal, 1993; Andriescu & Mitroiu, 2001). Recorded here is the first association of a *Syntomopus* sp. from a host feeding on hoary cress, hoary cress.

Trichomalus cf. *perfectus* Walker (Pteromalidae):

Note: Identifications of *T.* cf *perfectus* was difficult to confirm because only two specimens were collected and a series of each species is needed to study variations in several morphological characters that differentiate the species.

Life cycle: The related species, *Trichomalus perfectus*, is a solitary larval ectoparasitoid (Bonnemaison, 1957), in spring adults fly away from their overwintering sites in forest habitats (Szczepanski, 1972; Dmoch & Klimek, 1975) and are active from May until August (Graham, 1969). *Trichomalus perfectus*, completes only one generation per year (Dmoch, 1975a; Dmoch, 1975b; Dmoch & Klimek, 1975), however, a possible partial second generation may occur if acceptable hosts (e.g., *C. obstrictus* on summer canola) are present later in the season (Graham, 1969).

Primary host association: Trichomalus perfectus, is restricted to species of Ceutorhynchinae (Coleoptera) (Noyes, 2006). However, there are a few records of *T. perfectus* being reared from Diptera (Cecidomyiidae) (Vidal, 1993) and Hymenoptera (Torymidae) (Mitroiu, 2001).

Plant association: *Trichomalus perfectus* is associated with hosts that develop in Asteraceae, Brassicaceae, Cupressaceae, Poaceae and Resedaceae (Dmoch, 1975a; Vidal, 1997; Kuhlmann & Mason, 2002).

Trichomalus sp. (Pteromalidae):

Life cycle: *Trichomalus* species are all thought to be larval ectoparasitoids (Graham, 1969). Except for certain species involved in biological control programmes such as *T. perfectus* (see above), the biology of the genus is mostly unknown. Adults can be observed flying from May to September. In most species females overwinter in dry places such as amongst the foliage of coniferous trees (Graham, 1969).

Primary host association and hyperparasitism: *Trichomalus* spp. are associated with primary hosts of Coleoptera (Apionidae, Buprestidae, Chrysomelidae, Curculionidae), Diptera (Cecidomyiidae, Ephydridae), Hymenoptera (Cynipidae Diprionidae Tenthredinidae Torymidae) and Lepidoptera (Lyonetiidae, Yponomeutidae) (Graham, 1969; Noyes, 2006). In addition, records have been made of species of *Trichomalus* associated with parasitoid hosts among Diptera (Tachinidae) and Hymenoptera (Braconidae, Ichneumonidae) (Noyes, 2006).

Plant association: Species of the genus *Trichomalus* are known to be associated with hosts that develop in plants among the families Asteraceae, Brassicaceae, Cupressaceae, Poaceae, Resedaceae and Rosaceae (Graham, 1969; Dmoch, 1975a; Vidal, 1997; Kuhlmann & Mason, 2002; Noyes, 2006).

Table 3. Species and their proportion of the parasitoid assemblage of parasitoids associated with *Ceutorhynchus turbatus* (Schultze) for each sample site in 2003 and 2004.

Year	Site	Parasitoid Species	# Individuals	%
2003	Switzerland/ Valais - Site 10	*Mesopolobus morys* Walker[1]	34	100.0
	Switzerland/ Valais - Site 9	*Mesopolobus morys* Walker[1]	2	66.7
		Pteromalus sequester Walker gr.[1]	1	33.3
	Switzerland/ Valais - Site 14	*Mesopolobus morys* Walker[1]	4	57.1
		Pteromalus sequester Walker gr.[1]	3	42.9
2004	Switzerland/Valais - Site 11	*Pteromalus sequester* Walker gr.[1]	2	100.0
	Switzerland/Valais - Site 12	*Mesopolobus morys* Walker[1]	13	86.7
		Pteromalus sequester Walker gr.[1]	2	13.3
	Switzerland/Valais - Site 13	*Pteromalus sequester* Walker gr.[1]	20	52.6
		Mesopolobus morys Walker[1]	18	47.4
	Switzerland/Valais - Site 14	*Pteromalus sequester* Walker gr.[1]	27	55.1
		Mesopolobus morys Walker[1]	21	42.9
		Eupelmus sp. [4]	1	2.0
	Hungary/Csongrad - Site 15	*Mesopolobus morys* Walker[1]	14	50.0
		Pteromalus sequester Walker gr.[1]	11	39.2
		Baryscapus sp. [2]	2	7.2
		Eupelmus sp. [4]	1	3.6
	Hungary/Csongrad - Site 16	*Baryscapus* sp. [2]	1	50.0
		Pteromalus sequester Walker gr.[1]	1	50.0
	Hungary/Csongrad - Site 17	*Pteromalus sequester* Walker gr.[1]	20	29.4
		Closterocerus sp. [2]	13	19.1
		Baryscapus sp. [2]	10	14.7
		Eurytoma curculionum Mayr[3]	8	11.8
		Mesopolobus morys Walker[1]	8	11,8
		Eupelmus sp. [4]	2	2,9
		Trichomalus cf. perfectus Walker[1]	2	2,9
		Trichomalus sp. [1]	2	2,9
		Eurytoma curculionum Mayr[3]	1	1,5
		Pteromalus sp. [1]	1	1,5
		Syntomopus sp. [1]	1	1,5
	Hungary/Bekes - Site 18	*Pteromalus sequester* Walker gr.[1]	11	61,1
		Closterocerus sp. [2]	4	22,2
		Baryscapus sp. [2]	1	5,6
		Eurytoma curculionum Mayr[3]	1	5,6
		Eupelmus sp. [4]	1	5,6
	Hungary/ Sari - Site 19	*Mesopolobus morys* Walker[1]	2	50,0
		Pteromalus sequester Walker gr.[1]	2	50,0
	Hungary/Gyal - Site 20	*Pteromalus sequester* Walker gr.[1]	2	50.0
		Eupelmus sp. [4]	1	25.0
		Mesopolobus morys Walker[1]	1	25.0
	Hungary Sari - Site 21	*Mesopolobus morys* Walker[1]	2	40.0
		Closterocerus sp. [2]	1	20.0
		Eurytoma curculionum Mayr[3]	1	20.0
		Pteromalus sequester Walker gr.[1]	1	20.0

Chalcidoidea : 1Pteromalidae, 2 Eulophidae, 3Eurytomidae, 4Eupelmidae

Discussion

The two most common parasitoids of *C. obstrictus*, *T. perfectus* and *M. morys*, together with the less common *S. gracilis* account for up to 90% of *C. obstrictus* parasitism in Europe (Williams, 2003). It has recently been shown by Gibson *et al.* (2005) that neither *T. perfectus* nor *M. morys* are present in North America. *Stenomalina gracilis* however, occurs on *C. obstrictus* in low numbers in British Columbia (Gibson *et al.*, in press). Prior to introduction

of *T. perfectus* and *M. morys* for classical biological control of *C. obstrictus* in Canada, and before considering *S. gracilis* for augmentative biological control, it is essential to document their host ranges for the identification and exclusion of polyphagous agents to ensure potential non-target effects are avoided. Based on the principles proposed by Kuhlmann *et al.* (2006a), the phenological and geographical overlap of target (*C. obstrictus*) and non-target species (*C. turbatus* and *C. typhae*), added to the fact that the target and non-target species share the same feeding niche, clearly points towards a higher risk that parasitoids of the target species could attack the two non-target species. Following these principles, results of our studies should facilitate accurate prediction of potential non-target host impacts in the area of introduction.

The phenology of these ectoparasitoids is closely linked with those of their hosts. All ectoparasitoids encountered during our surveys laid eggs on late instar larvae of *C. turbatus* and *C. typhae*. The parasitoid larvae fed on their hosts for two to three weeks and pupated inside the pods. Pupation took approximately one to two weeks and adults of the new generation emerged through the openings of the senescent pods. Our studies on the phenology of *C. turbatus* support the results obtained by Cripps *et al.* (in press), Schwarzländer *et al.* (2002) and Hinz *et al.* (2003; 2004). Little was known regarding the phenology of *C. typhae* prior to our studies. Females of both species lay eggs in pods of their host plants between May and June. Larvae develop through three instars until they are mature and chew a hole in the pod wall to exit and pupate in the soil. Weevils of the new generation emerge between June and July of the same year. Both species overwinter in the adult stage, and the weevils become active again the following spring. Larvae of *C. obstrictus* also develop during May and July (Bonnemaison, 1957) confirming that its phenology overlaps with those of *C. turbatus and C. typhae* in the field. Furthermore, several studies showed that the geographical distribution of these three species also overlaps in Europe (Bonnemaison, 1957; Hinz *et al.*, 2003; Hinz *et al.*, 2004; Cripps *et al.*, in press). Our result on the target and non-target host phenology comparison indicate that phenology overlap conditions are met, which could lead to a higher risk of non-target effects, according to Kuhlmann *et al.* (2006a), in the case of classical biological control of *C. obstrictus* in Canada. The remaining question of our study aimed at answering whether these three Ceutorhynchionae host species share some of the parasitoid species among their parasitoid assemblages, with particular interest on the candidate biological control agents of *C. obstrictus*, *M. morys* and/or *T. perfectus*.

Several new host-parasitoid associations were identified in our study. Concerning the parasitoid assemblage associated with *C. typhae*, our observations led to the discovery of two species, one of them, *Mesopolobus gemellus*, is new to science. This species was responsible

for 98% of the parasitism at all sites studied. The second species associated with *C. typhae* was the pteromalid *S. gracilis,* with only a single parasitism record on *C. typhae*. Overall parasitism reached on average 25% of all *C. typhae* hosts available to parasitoids at all sites surveyed.

In contrast to *C. typhae* on shepherd's purse, *C. turbatus* on hoary cress had a more species rich parasitoid assemblage with 10 species involved. Interestingly, *M. morys*, the candidate agent for *C. obstrictus*, was the most common parasitoid of *C. turbatus* and reached up to 40% of total parasitism. In addition, this species was present at almost all sites where *C. turbatus* was collected on hoary cress in Switzerland and Hungary. The second most important ectoparasitoid species parasitizing *C. turbatus* was *P. sequester*, which represents 37% of the parasitoid assemblage and was also present at almost all sites studied. The eurytomid wasp *E. curculionum* had previously been associated with *C. obstrictus* by Dmoch (1975a). Our study provides the first record of its association with *C. turbatus* on hoary cress. Interestingly, while this parasitoid is known to occur in France (Thompson, 1955; Herting, 1973) and Germany (Freese, 1995; Vidal, 1997), we only observed occurrences of *E. curculionum* on *C. turbatus* in Hungary. Several other parasitoid species such as *Closterocerus* sp., *Baryscapus* sp., *Trichomalus* sp., *Pteromalus* sp. and *Syntomopus* sp. were also encountered only in Hungary during our surveys, and their association with *C. turbatus* is new to science. Two specimens of a *Trichomalus* species near *T. perfectus* were collected, together representing less than 1% of all the parasitoid specimens obtained during our surveys.

Kruess & Tscharntke (2000) demonstrated that fragmentation of habitats negatively affects species diversity and species abundance, and that species diversity can be dramatically reduced by both area-loss and increasing habitat isolation. Our results indicate that parasitoid assemblages associated with *C. turbatus* were more species-rich in Hungary (4.4±1.2) than in Switzerland (1.9±0.3). Furthermore, Tscharntke & Brandl (2004) defined that species richness and abundance of natural enemies are often closely correlated, so that the "single species" impact of a species-poor, but individual-rich community of beneficials cannot easily be separated from the "multiple-species" impact of a species-rich community with only few individuals per species. During our studies, we found no significant difference in average parasitism levels between Switzerland (36.4±6.6%) and Hungary (27.0±4.3%), which tends to confirm the conclusions drawn by Tscharntke & Brandl (2004). In addition, the parasitoid assemblage of *T. typhae* is on average less species-rich (1.1±0.1) than that of *T. turbatus* (3.1±0.7). However, as mentioned above, species-richness depends on a wide range of factors

and thus can vary from one sampling region to the other. As a consequence, to be able to accurately assess differences in parasitoid assemblage species-richness between species, surveys should be carried out in parallel in the same sampling regions.

Concerns regarding non-target effect issues in arthropod biological control have been rising for over ten years (Howarth, 1991; Simberloff & Stiling, 1996; Thomas & Willis, 1998; Stiling & Simberloff, 2000; Louda, 2003; Stiling, 2004). Consequently, a number of studies have dealt with this topic, and several guidelines have been drawn (Babendreier *et al.*, 2005; Simberloff, 2005; Wright *et al.*, 2005; Kuhlmann *et al.*, 2006b) to help accurately assess the risks associated with the introduction of non-native species for biological control of arthropods. Our work benefited from following these guidelines and recommendations, and our results show that accurate prediction of potential risks can be made. In addition, these results prove that, as proposed by Babendreider *et al.*(2005), field studies in the area of origin of candidate agents are very valuable and can aid in the prediction of potential risks in the area of introduction.

As an outcome of these natural enemy host range surveys in Europe, it is clear that *M. morys* was the most common species parasitizing *C. turbatus* on hoary cress in Switzerland and Hungary. These findings will have direct implications on the biological control of *C. obstrictus*. In the case of both an introduction of *M. morys* as a biological control agent against *C. obstrictus*, and the release of *C. turbatus* as a biological control agent against hoary cress, potential conflicts might occur between the two programmes. In contrast, no evidence was found that *T. perfectus* attacks either *C. turbatus* or *C. typhae,* and therefore it appears that this species would pose no risk should it be introduced for biological control of *C. obstrictus* in Canada.

Acknowledgments

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Discussion

One of the many constraints biological control practitioners very frequently encounter during the course of their research is that the taxonomy of the groups involved is often unclear (Huber *et al.*, 2002; Van Driesche & Reardon, 2004). As Bigler *et al.* (2005) emphasized, there is a need to provide an accurate identification of the candidates prior to introduction of candidate biological control agents. In addition, sufficient characterization of the agent to allow its unambiguous recognition should be provided, as well as relevant supporting taxonomic information such as general diagnostic descriptions and details of any taxonomic difficulties within the group (Bigler *et al.*, 2005).

Without a doubt, the attempt at biological control of *Ceutorhynchus obstrictus* (Marsham) [=*C. assimilis* (Paykull); see Colonnelli (2004)] initiated in 1949 would have benefited from following these guidelines, and the succession of misidentifications retrospectively demonstrated by Gibson *et al.* (2005) illustrates remarkably well Bigler's (2005) statement. This first classical biological control attempt resulted in the release of three European pteromalid parasitoid species in Canada (McLeod, 1953). In their recent study, Gibson *et al.* (2005) demonstrated that the species introduced had been misidentified. In addition, the records of species subsequently reared from *Brassica* spp. seedpods pods in Canada and reported in the literature as the three originally introduced species were also shown to be based on misidentifications. Therefore, two of the three species introduced, *Trichomalus perfectus* and *Mesopolobus morys,* never established. These misidentifications could have had an important impact on the new attempt at biological control of *C. obstrictus* initiated by Agriculture and Agri-Food Canada in 2000. In fact, as both species were still thought to be present in British Columbia, Canada, a decision could have been made to redistribute them within Canada without further investigation, potentially leading to the use of misidentified species and consequently to a greater risk of associated non-target effects.

Fortunately, prior to any action, a decision was made to study in detail the taxonomy of *Trichomalus* and *Mesopolobus* in parallel in North America and in Europe and to assess the ecological host range of selected *C. obstrictus* parasitoids in Europe

First, the taxonomic status of *Mesopolobus* and *Trichomalus* species associated with the pest and other Ceutorhynchinae species in Europe was revised to prevent such failures in the

future. Consequently to this study, *Trichomalus rusticus* (Walker) is now treated as a valid species, resurrected from synonymy under *T. lucidus* (Walker), and *T. lyttus* (Walker) is transferred from synonymy under *T. lucidus* and newly placed in synonymy with *T. rusticus*. In addition, a new species of *Mesopolobus* has been described, *Mesopolobus gemellus* Baur & Muller sp. nov., and associated with *Ceutorhynchus typhae* (Herbst) [=*C. floralis* Paykull] on shepherd's purse, *Capsella bursa-pastoris* (L.) Medicus [Brassicaceae]. However, as the species has only recently been described, and is very closely related to *M. morys* and may have been misidentified as such in the past, additional host associations that might exist will be described in the future.

These results emphasize the importance of taxonomy in classical biological control programs and document the need to follow recently proposed guidelines (Bigler *et al.*, 2005; Gibson *et al.*, 2005; Wright *et al.*, 2005; Kuhlmann *et al.*, 2006a; Kuhlmann *et al.*, 2006b; Gibson *et al.*, in press; Gillespie *et al.*, submitted). Furthermore, it should be taken into consideration that the problems resulting from poor knowledge of the taxonomy of candidate agents could affect inundative biological control programs, when a species is redistributed from an area of a country where it is already present to other areas where its presence is needed.

The potential threat to the environment that might exist in case of an introduction of candidate biological control agents for the control of *C. obstrictus* in Canada was studied following recent recommendations made by Bigler *et al.* (2005), Barbendreier *et al.* (2005) and Kuhlmann *et al.* (2006b) who emphasized the fact that the potential risk for non-target effects is higher when target and non-target species 1) show an overlap in time of occurrence; 2) have overlapping geographical distributions and 3) share the same feeding niches in habitats. As a result of this approach, the very first step in every biological control project should be to collect all available information on the potential host(s) of the candidate biological control agent(s) (Babendreier *et al.*, 2005). However, documenting parasitoid host ranges is far more complicated than collecting data on host parasitoid species load because it involves rearing parasitoids to the adult stage for identification from different species of hosts rather than rearing or dissecting many individuals of a single host species (Kuhlmann *et al.*, 2006b). Nevertheless, even though they are sometimes difficult to conduct, field surveys in the country of origin are very valuable (Babendreier *et al.*, 2005). Following this principle, we carried out studies in Europe to 1) document the phenology of four non-target Ceutorhynchinae hosts with the aim of verifying whether or not the target and non-target species show an overlap in time of occurrence with the target, *C. obstrictus*; 2) assess the

parasitoid assemblages associated with these species in order to determine the potential for *T. perfectus* and *M. morys* to utilize non-target hosts ecologically related to the target host.

Results showed that in Europe, *M. morys* is the most abundant species parasitizing the seed feeder *Ceutorhynchus turbatus* (Schultze), a candidate parasitoid for biological control of the weed hoary cress, *Lepidium draba* L. (=*Cardaria draba*) [Asteraceae], in North America. In case both species would be further considered for introduction, there exists a risk that potential conflicts could occur between these arthropod biological and weed biological control programmes in the future. Decisions might have to be made on time by balancing environmental risks and potential benefits as proposed by Bigler *et al.* (2006).

Furthermore, *Stenomalina gracilis* Walker was found to be associated with *Microplontus edentulus* (Schultze), a biological control agent against the weed scentless chamomile, *Tripleurospermum perforatum* (Mérat) Laínz (=*Matricaria perforata* Mérat) [Asteraceae], in North America. Recently Gibson *et al.* (in press) documented the presence of *S. gracilis* in Canada in the same region where it had been released in 1949. A consequence of the study by Gibson *et al.* (in press) is that *S. gracilis* could be legally considered for further release or redistribution in the future. We propose that *S. gracilis* should not be further considered because our results show that its use would potentially lead to conflicting situations with weed biological control in Canada.

On the other hand, *T. perfectus* has not been found parasitizing any of the four non-target Ceutorhynchinae host species studied. Taking this into consideration, it appears from the current state of our knowledge that *T. perfectus* possesses desirable qualities for introduction into Canada (e.g. narrow host range, ability to successfully suppress pest populations in Europe).

Our results constitute a first step in understanding the risks associated with an introduction of these potential agents to North America. In their attempt at comparing results obtained in fundamental host range vs. ecological host range assessment studies, Haye *et al.* (2005) concluded that ecological host range studies in the area of origin provide a useful supplement to interpret laboratory host range testing to predict the behaviour of a biological control agent after introduction. Thus, Haye *et al.* (2005) propose that a complete host range evaluation should combine data from the literature, field collections, and laboratory experiments, the latter being the next step that future studies on the biological control of *C. obstrictus* will envisage. Indeed, eventhough the life cycles of the candidate parasitoids *T. perfectus* and *M. morys* has already been documented in the past (Bonnemaison, 1957; Dmoch, 1975b; Dmoch,

1975a; Buechi, 1993; Murchie & Williams, 1998), several characteristics of their biology (e.g. overwintering behaviour, cold hardiness, reproductive potential) that may be easier to investigate in laboratory studies remain unknown and will have to be assessed prior to any introduction.

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Curriculum Vitae

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Sept. 83 - June 88	**Primary school** in Vanves, France;
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March 02 – Apr. 06	**Research Assistant at the CABI Bioscience Switzerland Centre – Arthropod biological control section:** in charge of the cabbage seedpod weevil biological control programme. European surveys for candidate parasitoids, assessment of their ecological host range and clarification of their taxonomic status.

Erklährung

Hiermiet erkläre ich,
- dass ich diese Arbeit selbständig angefertig habe,
- dass ich wörtlich oder inhaltlich aus anderen Quellen entnommene Stellen als solche kenntlich gemacht habe,
- dass ich die Inanspruchnahme persönlicher Hilfe namenltlich aufgeführt habe,
- dass die Arbeit bisher weder ganz noch zum Teil im Rahmen eines Prüfungsverfahrens vorgelegen hat.

Fûr die mündliche Prüfung wähle ich die Form der Disputation.

Einer Zulassung von Zuhörerinnen und Zuhörern bei der mündlichen Prüfung widerspreche ich nicht.