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Two new Australian species of *Stethynium* (Hymenoptera: Mymaridae), larval parasitoids of *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) on *Eucalyptus*

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Abstract
Two new species of Mymaridae, *Stethynium ophelimi* Huber and *S. breviovipositor* Huber, are described from *Ophelimus maskelli*, a gall-inducing pest of *Eucalyptus camaldulensis* accidentally introduced from Australia into the Mediterranean region and Africa. This is the first record of a species of Mymaridae reared as a larval parasitoid of a holometabolous insect. One or both of the *Stethynium* species are being considered for introduction into Israel for biological control of this pest. Galls containing late second or third instar larvae are suitable for successful development of *Stethynium*. Mean survival time in a mixed colony of adults fed with honey and water solution was 1–2 days. *Stethynium perlatipenne* Girault, syn. nov., is synonymized under *S. flavinotae* Girault.

Introduction

Members of *Stethynium* are a commonly collected component of the Australian fauna of Mymaridae, but their biology is poorly known. Existing host records indicate that they are parasitoids in eggs of Cicadellidae or Membracidae (Huber 1987; Noyes 2002) and three species, *S. flavinotae* Girault, *S. perlatipenne* Girault, and *S. notatum* Girault, were reared from galls (Girault 1915). Dahms (1984) quoted an unpublished manuscript by Girault that *S. immaculatum* Girault was reared from a gall on *Eucalyptus*, and one slide of *S. latipenne* bears a label stating that this species was "bred from small galls on surface of leaves" though the original description does not mention this. Despite these records no firm evidence that anything other than egg parasitism existed for *Stethynium*, so it was assumed that the actual hosts for species associated with galls were eggs of other insects laid in or around gall tissue. Indeed, egg parasitism was thought to be the only type of parasitism for all Mymaridae, despite various records from scale insects, e.g. *S. peccavum* Girault from *Eriococcus* on *Eucalyptus* (Girault 1938), and one from an aphid fundatrix (Lampel 1959). The senior author considers

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the scale insect records to be doubtful or, at least, accidental. Lampel's record is an error; it refers probably to the immature stage of an aphidiine braconid parasitoid, not a mymarid (Noyes and Valentine 1989, p 9). The recent interest in *Stethynium* species as potential biological control agents of newly introduced pests in the Mediterranean region and Africa has shown beyond doubt that the species described below are larval parasitoids. Observations by two of the authors (Z.M., J.L.) indicate that *Stethynium* species are commonly reared from a variety of galls, and it may be that the radiation of Australian species is due to this association with galls on eucalypts. According to present observations, they seem to be restricted to hosts that induce small, round or blister-type galls (Figures 18, 19), which are not dissimilar in size and shape from many eggs.

Stethynium in Australia contains 45 nominal species and two nominal subspecies (Lin et al. in press), which represent most of the described world fauna of this genus (Huber 1987). All of the Australian species were described by Girault, usually on the basis of only one slide-mounted specimen in very poor condition. These need to be carefully revised, together with freshly collected material properly prepared on both card (for study of colour and habitus) and slide mounts to determine their status. Two recently reared Australian species are potentially useful as biological control agents of a serious gall-forming pest, *Ophelimus maskelli* (Ashmead) (Eulophidae), on *Eucalyptus camaldulensis* leaves in the Mediterranaean region (Viggiani and Nicotina 2001; Pujade-Villar and Riba-Flinch 2004 (as *O. eucalypti*); Protasov et al. in press). Because one or both of these *Stethynium* species may eventually be released in Israel or elsewhere for biological control it is important to name them now, despite the need for a comprehensive revision of the genus to ascertain that they have not been previously described.

Materials and methods

Taxonomy

The material of both species studied was reared in quarantine in Israel, from specimens originally obtained in Australia. Except for colour, the species descriptions are kept short, with emphasis on the features that differentiate the two species from each other and, where possible, from other species whose types we examined. Morphological terms are according to Gibson (1997). The abbreviation fl_n refers to the funicle segments of females or flagellomeres of males. Measurements are in micrometres, usually with the mean followed, in parentheses, by range and number of specimens measured. Body length was measured from critical point-dried specimens; all other measurements are from slide-mounted specimens, with the holotype measurement being included in the range and calculation of means. Acronyms used to indicate depositories of material studied are: ANIC, Australian National Insect Collection, CSIRO, Canberra, Australia; BMNH, The Natural History Museum, London, UK; CNC, Canadian National Collection of Insects, Ottawa, Canada; USNM, United States National Museum of Natural History, Washington, DC, USA.

Biology

Suitable gall development stages for successful parasitism by *Stethynium* were determined using leaves with galls of the same age growing on 5-month-old *Eucalyptus camaldulensis* saplings. The saplings were exposed to *Ophelimus maskelli* for 4 days in Plexiglas cages maintained in a greenhouse in Volcani Center, Israel. Four gall age groups were tested: group A, 30–34 days after *O. maskelli* oviposition, with first visible signs of gall development

Table I. Effect (mean ± SD) of *Ophelimus maskelli* development stage on offspring production and development time of *Stethynium* spp.

Gall stage at the time of parasitoid exposure (days after *O. maskelli* oviposition)[a]	Number of leaves per sapling		Number of galls per sapling	Number of parasitoids emerged		Development time (days)
	Total	Galled		Males	Females	
A (30–34)	15.0 ± 1.6	8.7 ± 1.7	477.3 ± 131.5	0.0	0.0	–
B (46–50)	10.7 ± 0.9	6.3 ± 0.9	228.0 ± 55.1	52.0 ± 8.5	55.0 ± 4.8	40.0 ± 0.8
C (58–62)	11.0 ± 1.4	7.7 ± 2.4	325.3 ± 157.5	42.9 ± 2.8	26.4 ± 11.7	37.7 ± 0.5
D (80–84)	12.0 ± 1.4	8.0 ± 0.8	366.7 ± 53.1	0.0	0.0	–

[a]Each sapling replicate was exposed to between 17 and 21 male and 18 and 21 female parasitoids.

and when the first instar larvae could be detected; group B, 46–50 days after oviposition, at the time when the gall tissue is slightly swollen and the host was in its late second instar larval stage; group C, 58–62 days after oviposition, when the host was in its third instar larval stage; group D, 80–84 days after oviposition, when many of the galls began to lose their typical gloss and the host was in its pupal stage.

A single sapling with about 12 leaves, about eight of which were galled, was placed in a ventilated Plexiglas cage (30 × 30 × 40 cm). Mean number of galls per sapling was 350 (Table I). Three saplings with each of the above four gall age groups were tested. All 12 cages were placed in a semi-shaded screen house in September 2005. About 20 males and about 20 females of *Stethynium* (see Table I) were put inside each of the 12 cages with water supply alone.

The time of emergence of *Stethynium* individuals was determined by daily collection of parasitoid offspring in each cage. The mean number ± SD of male and female parasitoid offspring per cage was calculated.

Adult longevity was determined by means of several feed treatments as follows: (1) water; (2) young fresh foliage of *E. camaldulensis*; (3) honey solution (distilled water+honey, 1:1); (4) fresh *E. camaldulensis* flowers; (5) combination of treatments 2 and 3; (6) no feed; and (7) leaves with galls of group C. The wasps, males and female separately, were placed in 95 mm-diameter Petri dishes fitted with a filter-paper disk. The water or honey solution was sprayed along a narrow strip on the cover of each Petri dish, and the feed supply was renewed daily. The wasps for testing were collected on the day of emergence, removed with a fine brush and placed in the Petri dish. Each treatment comprised three replicates, each of five wasps. The wasp mortality was registered daily. The test was conducted at 25°C and 75% relative humidity. The mean survival (± SD) rate was calculated (Table II).

Table II. Mean (± SD) *Stethynium* adult longevity (days) as affected by feed treatment at 25°C and 75% relative humidity.

Feed	Males	Females
Water	1.0 ± 0.0 C	1.0 ± 0.0 B
Young fresh foliage of *Eucalyptus camaldulensis*	1.0 ± 0.0 C	1.0 ± 0.0 B
Honey solution	2.2 ± 0.2 A	2.3 ± 0.2 A
Fresh *E. camaldulensis* flowers	1.3 ± 0.3 ABC	1.3 ± 0.1 B
Young fresh foliage of *E. camaldulensis*+honey solution	2.1 ± 0.5 AB	2.0 ± 0.4 A
No food/no water	1.0 ± 0.0 C	1.0 ± 0.0 B
Leaves with galls 58–62 days after oviposition	1.1 ± 0.1 BC	1.0 ± 0.0 B

Means followed by the same letter within each column are not significantly different (Tukey–Kramer HSD, $P=0.05$).

Taxonomy

Stethynium ophelimi Huber, n. sp.
(Figures 1–8)

Type material

Holotype: female (ANIC), cleared and dissected under three coverslips on slide labelled: (1) ''Australia: NSW Wagga Wagga, ex. *Ophelimus maskelli* leaf galls on *Eucalyptus camaldulensis*''; (2) ''em. from mature larvae vii.2005 in quarantine culture, Bet Dagan Israel, Z. Mendel''; and (3) ''*Stethynium ophelimi* Huber Holotype female dorsal''. Paratypes: 12 females and two males pinned on cards, seven females and one male on slides in Canada balsam (ANIC, BMNH, CNC, USNM).

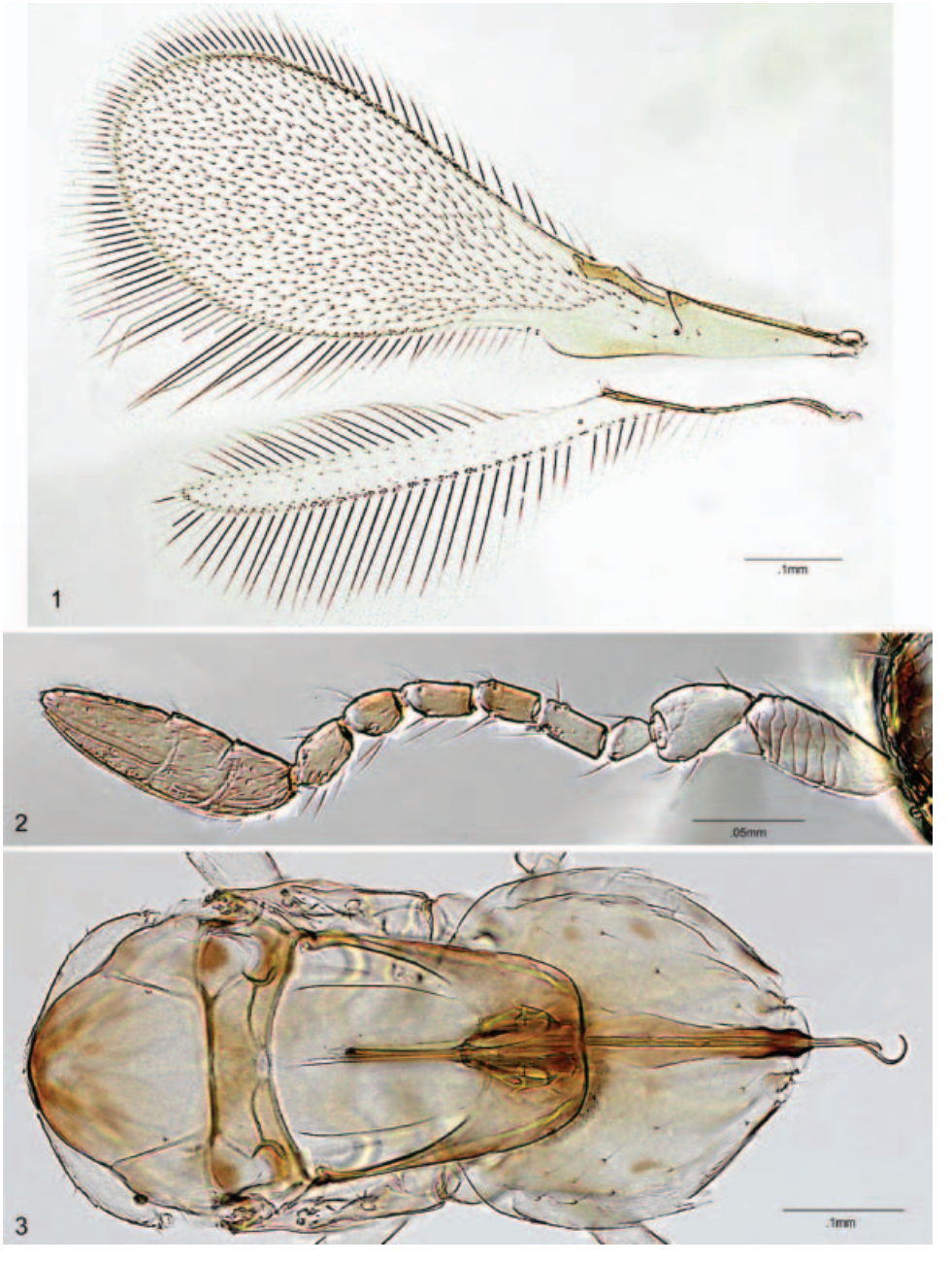
Diagnosis

Ovipositor sheaths distinctly thickened apically and black-tipped (Figure 4); ovipositor distinctly hooked at apex, and produced forward in a large loop at base of metasoma and forward inside mesosoma at least to base of mesocoxa or, in dorsal view, to at least midpoint of posterior scutellum (Figures 3, 4); fore wing with lobe of posterior margin behind venation evenly rounded (Figure 1); anterior scutellum with narrow longitudinal median strip (=area between placoid sensilla, visible on slide-mounted specimens) not contrasting strongly with slightly darker lateral areas, and the lateral areas contrasting very weakly with light-coloured posterior scutellum.

The types of eight species of Australian *Stethynium* (*cuvieri* Girault, *daltoni* Girault, *flavinotae*, *latipenne* Girault, *lavoisieri* Girault, *notatum* Girault, *perlatipenne* Girault, and *vesalii* Girault) were compared directly with *ophelimi*. None of them is conspecific with *ophelimi*. *Stethynium cuvieri*, *lavoisieri*, *flavinotae*, and *vesalii* have black-tipped ovipositor sheaths (*S. latipenne* is known only from males so the ovipositor sheath colour is unknown). *Stethynium cuvieri* has a much narrower fore wing and shorter funicle segments, *S. lavoisieri* has a straight ovipositor (its antennae are mostly missing except for the clava and in such poor condition they cannot be compared with *S. ophelimi*), *S. flavinotae* has distinctly wider hind wings, and *S. vesalii* has shorter funicle segments, with three of them wider than long.

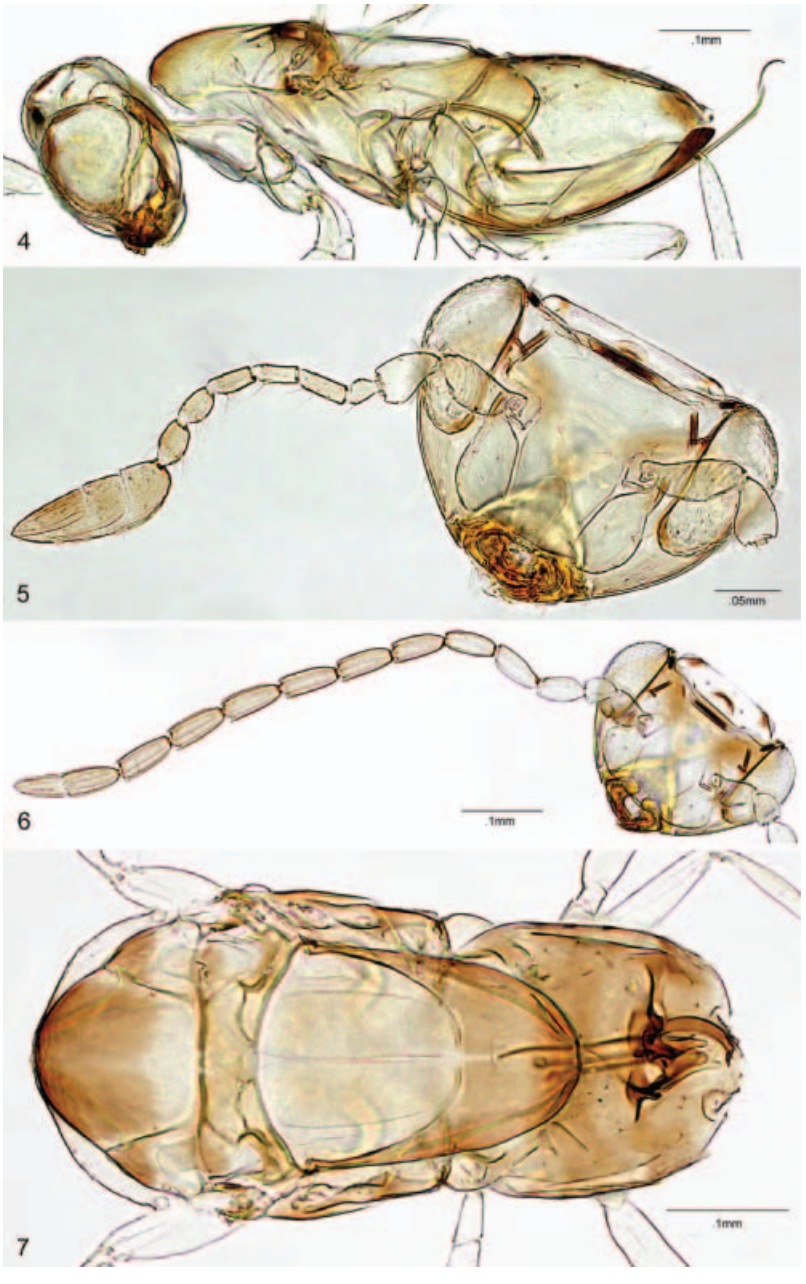
Description

Female. Body pale yellow to cream coloured, with lower face often having a pink tinge. Eyes and ocelli grey, sometimes with a pink tinge. Teeth of mandible, trabeculae, a diffuse streak on occiput between eye and foramen magnum, a small dot on anterior margin of axilla, and base of ovipositor dark brown. Anterior two-thirds of mesoscutum, anterior scutellum except for narrow longitudinal strip medially (Figure 3), and propodeum medially darker yellow to very light brown. A vaguely triangular area medially on anterior half of gaster varying from light to dark brown and sometimes, very small, usually faint, light brown areas elsewhere on terga dorsally and laterally. Two minute dots on anterolateral corner of axilla and posterior scutellum, respectively, and apex of ovipositor sheath black. Funicle and clava dusky yellow to light brown. Wings uniformly hyaline, and venation light brown. Legs pale yellow, except for dark brown apical tarsomere of each leg.



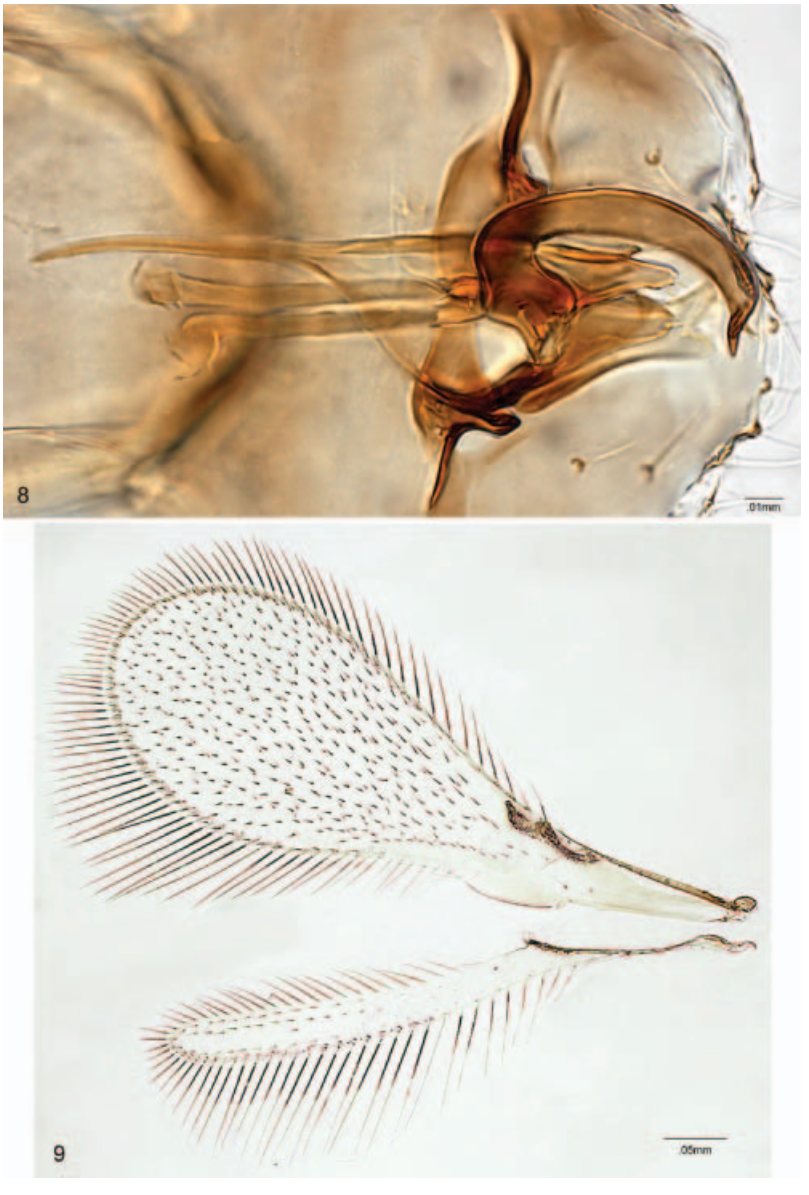
Figures 1–3. *Stethynium ophelimi* sp. n., female. (1) Wings. (2) Antenna. (3) Holotype, mesosoma+metasoma, dorsal.

Body length 590 (490–640, $n=10$). Head width 271 (245–301, $n=4$). Antenna (Figure 5) with inner surface of scape transversely striate; funicle with all segments at least slightly longer than wide and without longitudinal sensilla; clava with six longitudinal sensilla. Length/width ratios ($n=5$): scape 2.77–2.86, pedicel 1.41–1.57, fl_1 1.52–2.00, fl_2 2.18–2.88, fl_3 1.96–2.67, fl_4 1.79–2.36, fl_5 1.31–1.85, fl_6 1.48–1.63, clava 2.79–3.15.



Figures 4–7. *Stethynium ophelimi*. (4) Female body, lateral. (5) Holotype, head, anterior, and antenna. (6) Male head and antenna. (7) Male mesosoma and metasoma, dorsal.

Measurements length (width) (n=5 or 6): scape 69–91 (29–32), pedicel 46–55 (32–35), fl_1 24–29 (13–18), fl_2 30–44 (13–17), fl_3 28–39 (11–16), fl_4 31–37 (14–17), fl_5 27–32 (14–22), fl_6 28–35 (20–23), clava 115–134 (39–46). Mesosoma with adnotaular seta midway between anterior and posterior margin or slightly closer to posterior margin (Figure 3). Posterior scutellum length/width=0.80–0.92. Mesophragma posteriorly truncate



Figures 8, 9. *Stethynium* spp. (8) *S. ophelimi*, male genitalia, dorsal. (9) *S. breviovipositor* sp. n., wings.

(Figure 3). Fore wing length (including humeral plate) 773 (707–823, $n=3$), width 253 (219–282), length/width 3.07 (2.92–3.03, $n=3$), with discal microtrichia dense (including behind stigmal vein), with lobe of posterior margin behind venation evenly rounded (Figure 1), and with longest marginal cilia 143 (123–161), just over half as long as wing width. Hind wing length 685 (632–735, $n=3$), width 50 (46–53), and longest marginal cilia 138 (134–145), about 2.75 times hind wing width. Metasoma with lateral margins of gaster converging in apical half to rather narrow apex (Figure 3). Ovipositor sheaths distinctly thickened apically and black-tipped (Figure 4), evenly curved along entire length, and not exserted; ovipositor evenly curved along its entire length, distinctly hooked at apex,

exserted slightly beyond apex of gaster (in slide-mounted specimens), and produced forward in a large loop at base of metasoma and inside mesosoma at least to base of mesocoxa or, in dorsal view, at least to midpoint of posterior scutellum (Figures 3, 4).

Male. Yellow, but with much more extensive dark areas than in female, especially on mesoscutum and gaster. Dark brown areas are: a very narrow ring around each ocellus and two small spots beside upper orbit opposite lateral ocelli, a diffuse oblique streak between posterior eye margin and foramen magnum, most of midlobe of mesonotum except for posterior one-fifth to one-quarter, especially medially, a spot anteriorly on lateral lobe of mesoscutum, this joined to brown of midlobe by a lighter brown area, a large spot anteriorly and smaller one posteriorly on axilla, a small spot on mesopleuron below wing base, a slightly larger one laterally on propodeum at junction with metapleuron, and entire dorsal surface of propodeum; most of gaster dorsally and laterally (with scattered lighter brown to yellow areas), and apical gastral sterna. Anterior scutellum brown-yellow, contrasting slightly with bright yellow of posterior scutellum. Pedicel and fl_1 dusky yellow, remaining flagellomeres light grey brown. Transverse trabecula, teeth of mandible, minute spot at tegula, and apical tarsomere of each leg coloured as in female.

Body length 666 (640–691, $n=2$, critical point-dried specimens). Antennal measurements (length only, $n=1$): scape (not measurable), pedicel 40, fl_1 59, fl_2 67, fl_3 61, fl_4 65, fl_5 71, fl_6 71, fl_7 71, fl_8 72, fl_9 73, fl_{10} 67, fl_{11} 63. Length/width ratio of fl_6 2.49. Each flagellomere apparently with eight longitudinal sensilla. Genitalia with aedeagus, in lateral view, evenly rounded dorsally and parameres near aedeagal apex (Figures 7, 8).

Stethynium breviovipositor Huber, n. sp.
(Figures 9–17)

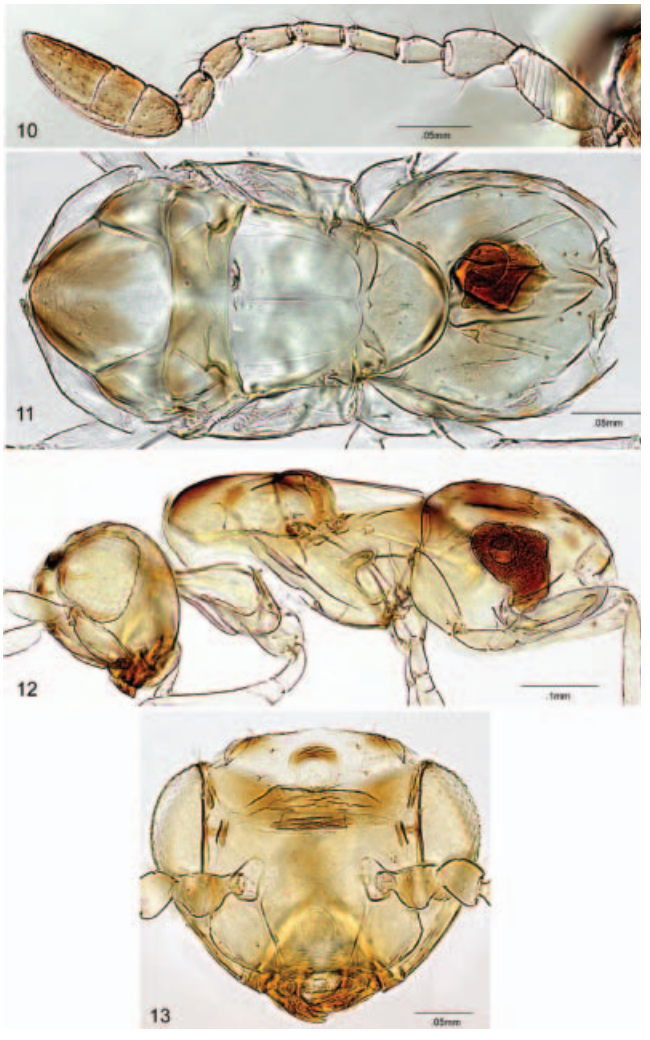
Type material

Holotype: female (ANIC), cleared and dissected under three coverslips on slide labelled: (1) ''Australia: NSW Wagga Wagga, ex. *Ophelimus maskelli* leaf galls on *Eucalyptus camaldulensis*''; (2) ''em. from mature larvae vii.2005 in quarantine culture, Bet Dagan Israel, Z. Mendel''; and (3) ''*Stethynium breviovipositor* Huber Holotype female dorsal''. Paratypes: 14 females and seven males pinned on cards, six females and two males on slides in Canada balsam (ANIC, BMNH, CNC, USNM).

Diagnosis

Ovipositor and sheaths short, arising midway along gaster at about level of apex of mesophragma (Figures 11, 12); gaster with large orange inclusion internally near base of ovipositor; fore wing with lobe of posterior margin behind venation somewhat flattened (Figure 1); anterior scutellum with narrow longitudinal median strip (=area between placoid sensilla on slide-mounted specimens) contrasting strongly with darker lateral areas, and the lateral areas contrasting distinctly with light-coloured posterior scutellum.

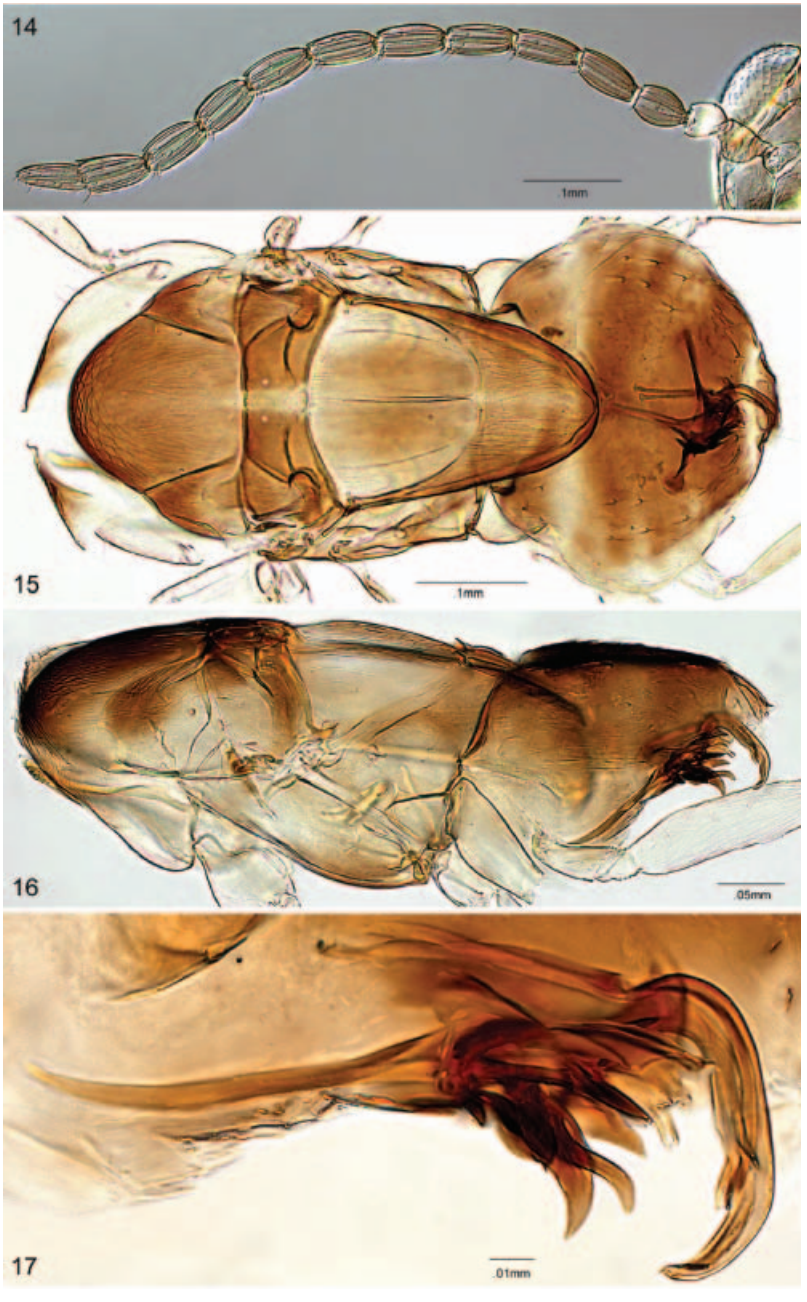
Among the species examined, none are similar to *S. breviovipositor*. All have either distinctly wider or narrower fore wings except *S. flavinotae*, which differs by its long, black-tipped ovipositor. No species has the large orange inclusion inside the gaster characteristic of *S. breviovipositor*. *Stethynium ophelimi* (described above) differs most distinctly from *S. breviovipositor* by its much longer, black-tipped ovipositor sheaths.



Figures 10–13. *Stethynium breviovipositor*, female. (10) Antenna. (11) Mesosoma and metasoma, dorsal. (12) Body, lateral. (13) Head, anterior.

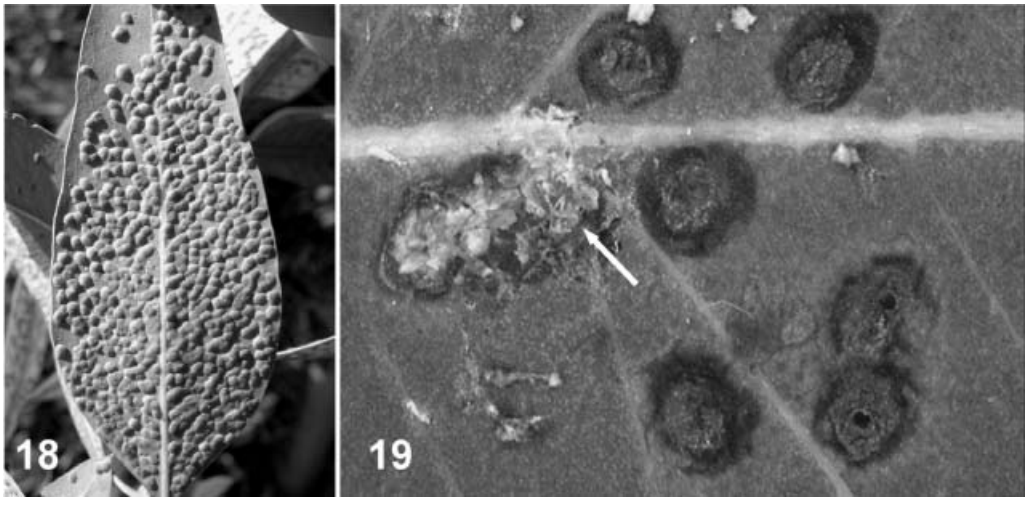
Description

Female. Body pale yellow to cream coloured. Eyes and ocelli grey, sometimes with a pink tinge. Trabeculae black. The following brown: mandible (especially the teeth), a minute area partly around each ocellus, a faint, diffuse area or, sometimes, more distinct small spot on each side of foramen magnum, anterior two-thirds of midlobe of mesoscutum, a spot medially on lateral lobe of mesoscutum, a spot on anterior margin of axilla and a paler one on posterior margin, anterior scutellum except for narrow, longitudinal median strip (Figure 11), propodeum dorsally, and gaster dorsally, except laterally and sometimes also apically. A large orange inclusion inside gaster (Figures 11, 12) often gives a greyish appearance (seen externally) to apicoventral part of gaster. Ovipositor scarcely darker than surrounding sterna. Wings uniformly hyaline, with venation light brown. Funicle and, especially, clava light brown to brown. Legs pale yellow, except for dark brown apical tarsomere of each leg.



Figures 14–17. *Stethynium breviovipositor*, male. (14) Antenna. (15) Mesosoma and metasoma, dorsal. (16) Mesosoma and metasoma, lateral. (17) Genitalia, lateral.

Body length 585 (485–640, $n=10$, critical point-dried specimens). Head width 262 (224–292, $n=5$). Antenna (Figure 5) with inner surface of scape transversely striate; funicle with all segments at least slightly longer than wide and without longitudinal sensilla; clava with six longitudinal sensilla. Length/width ratios ($n=4$ or 5, except scape=2): scape 2.30–2.42, pedicel 1.43–1.59, fl_1 1.38–1.71, fl_2 2.13–2.48, fl_3 2.25–2.52, fl_4 1.85–2.35,



Figures 18, 19. (18) Leaf of *Eucalyptus camaldulensis* with heavy infestation of *Ophelimus maskelli* galls. (19) Leaf of *E. camaldulensis* showing four intact *O. maskelli* galls, two galls with emergence holes (bottom right), and two dissected galls (left), one of which contains an unemerged adult *Stethynium* sp. (arrow).

fl_5 1.41–1.89, fl_6 1.54–1.96, clava 2.81–3.11. Measurements length (width) (n=5 or 6): 82–92 (31–37), pedicel 49–55 (25–36), fl_1 26–33 (17–19), fl_2 35–44 (15–18), fl_3 35–41 (15–16), fl_4 32–40 (16–20), fl_5 21–40 (19–23), fl_6 32–44 (19–25), clava 112–134 (38–44). Mesosoma with adnotaular seta slightly nearer anterior than posterior margin (Figure 11). Posterior scutellum length/width=0.69–0.78. Mesophragma posteriorly widely rounded (Figure 11). Fore wing length (including humeral plate) 654 (587–730), width 238 (205–268), length/width 2.76 (2.68–2.87, n=3), with discal microtrichia moderately sparse (especially behind stigmal vein), with lobe of posterior margin behind venation somewhat flattened (Figure 9), and with longest marginal cilia 128 (124–136), just over half as long as wing width. Hind wing length 568 (502–654), width 44 (41–49), and longest marginal cilia 119 (108–129), about 2.7 times hind wing width. Metasoma with lateral margins of gaster more or less parallel-sided, converging to wide and blunt apex (Figure 11). Ovipositor sheaths straight, evenly narrow along entire length (Figure 12), and not exserted; ovipositor straight along its entire length and not extending beyond apices of sheaths (Figures 11, 12).

Male. Body mostly black. Yellow (sometimes pale brown): face, most of vertex except around ocelli, gena, lower occiput, pronotal shoulders, narrow longitudinal median streak on anterior scutellum, posterior scutellum (especially lateral margins), and prosternum. Mesosternum varies from almost black to moderately light brown. Flagellum light brown.

Body length 605 (485–665, n=7, critical point-dried specimens). Antennal measurements (n=1, length only): scape 71, pedicel 43, fl_1 56, fl_2 61, fl_3 62, fl_4 67, fl_5 71, fl_6 70, fl_7 74, fl_8 75, fl_9 71, fl_{10} 73, fl_{11} 71. Length/width ratio of fl_6 1.94. Each flagellomere apparently with eight longitudinal sensilla. Genitalia with aedeagus, in lateral view, flattened dorsally and parameres at some distance from aedeagal apex (Figures 16, 17).

Biology

Galls containing late second or third instar larvae were found to be suitable for successful development of *Stethynium*. A female wasp (the species could not be ascertained) inserts its

ovipositor into the gall, most probably through a stomatal pore; these are found on both the adaxial and abaxial surfaces of the galls. Perhaps, *S. ophelimi* manages to parasitize second larvae of *O. maskelli*, reaching them with its relatively long ovipositor, whereas *S. breviovipositor* may only reach mature host larvae when they fill the gall chamber and are thus easier to reach with a short ovipositor. It seems that eggs and first instar larvae are ignored by the wasp, whereas the pupa is unsuitable for development. *Stethynium* survived for a relatively short period. Male and female survival seemed to be similar. Food has a significant effect on adult survival ($F_{7,\ 16}=26.9$; $P<0.0001$, one-way ANOVA; SAS Institute 2002). Wasps fed with honey and water solution, with or without fresh young leaves present, lived for about 2 days. Wasps that were not given food or water, or that were given only water, or that were placed on leaves with galls 58–62 days after oviposition, survived for 1 day only. The low mean survival on galled foliage and *Eucalyptus* flowers suggests that neither nectar nor pollen feeding, nor host feeding occur.

Discussion

The *Stethynium* used in the experiments were not recognized as consisting of two distinct species until after the experiments had been performed and the colony had died out. Observations on oviposition were done with females from a newly established colony, obtained from Australia after the first colony had died out. Thus, the data presented here cannot be restricted to either one or the other species. Nevertheless, some useful information can be stated. The adult lifespan of both species is relatively short. When fed with honey, and with access to water, species of other genera of Mymaridae that have been studied under laboratory conditions can live longer than 1–2 days.

Girault (1915) reared *S. flavinotae* and *S. perlatipenne* from fleshy galls on gum, presumably from the same rearing because three specimens (holotype of *S. flavinotae* and two syntypes of *S. perlatipenne*) are on the same slide and have the same published type locality, given by Girault as Melbourne but with a question mark. Girault distinguished *S. perlatipenne* from *S. flavinotae* as ''more robust, both wings very much broader''. The forewing length/width ratio is 2.77 for *S. flavinotae* and 2.58 for *S. perlatipenne*. Other than this and the smaller body size of *S. flavinotae* the species are identical. The range of length to width of the forewing is similar to that of *S. breviovipositor* and represents only individual variation according to the senior author. Therefore, *S. perlatipenne*, syn. nov., is synonymized under *S. flavinotae*.

Acknowledgements

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