

Description of four new species of *Eadya* (Hymenoptera, Braconidae), parasitoids of the *Eucalyptus* Tortoise Beetle (*Paropsis charybdis*) and other *Eucalyptus* defoliating leaf beetles

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Abstract

Eucalyptus L'Héritier, 1789 (Myrtales: Myrtaceae) plantations are a global economic resource with a wide array of uses. As this forestry crop grows in popularity around the world, the exotic introduction of pests such as the leaf beetles belonging to the genera *Paropsis* Oliver, 1807 and *Paropsisterna* Motschulsky, 1860 increases in frequency. These pest introductions have spurred a need to understand the natural enemies of these pests for use in classical biological control programs. One such enemy, *Eadya paropsidis* Huddleston & Short, 1978 (Hymenoptera: Braconidae), has shown potential as a biological control agent against *Paropsis charybdis*, an exotic pest of New Zealand *Eucalyptus* plantations. However, observations made by biocontrol researchers have raised concerns that *E. paropsidis* is a complex of cryptic species. A comprehensive large-scale phylogenetic study utilizing both host and molecular data (Peixoto et al. 2018), as well as a morphological multivariate ratio analysis, was utilized to ensure accurate delimitation of the species of *Eadya*. Here we formally describe the three new species (*Eadya annleckieae* Ridenbaugh, 2018, **sp. n.**, *Eadya daenerys* Ridenbaugh, 2018, **sp. n.**, *Eadya spitzer* Ridenbaugh, 2018, **sp. n.**), and one additional new species discovered in the Australian National Insect Collection (*Eadya duncan* Ridenbaugh, 2018, **sp. n.**). All distributions and host associations for *Eadya* are listed as well as a redescription of the originally described *E. paropsidis* and *E. falcata*. An illustrated key to all known species is included to assist biological control researchers. The value of citizen science observations is discussed, along with the need for a further understanding of mainland *Eadya* populations given the recent spread of paropsine pests. Finally, we discuss the subfamilial placement of *Eadya*, and suggest it belongs within Euphorinae based on morphological characters.

Keywords

Parasitoid wasps, Taxonomy, DNA Barcoding, Morphometrics, Biological Control, Tasmania, New South Wales, Victoria, Euphorinae, Eucalyptus

Introduction

Although native to Australia, the cultivation of production and trade of goods derived from *Eucalyptus* L'Héritier, 1789 (Myrtales: Myrtaceae) is a massive global industry. The largest subdivision of this industry is the *Eucalyptus* oil market (Coppen 2003). *Eucalyptus* oil is a coveted aromatic/medicinal product with major producers in Australia, Brazil, Chile, China, India, Portugal, Spain, and South Africa (Coppen 2003). Between 1991 and 2000, China alone exported 32,244 tons of *Eucalyptus* oil, valued at \$108 million USD (Coppen 2003). *Eucalyptus* is also one of the most important sources of commercial cellulose fiber for Asia, the Mediterranean, southern Africa, and South America (Paine et al. 2011). In North America, *Eucalyptus* is most often cultivated for use as ornamental plants (Paine et al. 2011), but has also been evaluated in the southern United States as a potential source of energy (Gonzalez et al. 2011).

Species of *Paropsis* Oliver, 1807 and *Paropsisterna* Motschulsky, 1860 are endemic Australian leaf-beetles (Coleoptera: Chrysomelidae: Chrysomelinae) that feed upon the leaves and shoots of *Eucalyptus*. These beetles have been known to cause serious damage to *Eucalyptus* plantations both within (de Little 1989; Nahrung 2004) and outside (Millar et al. 2009; Lin et al. 2017) of their native Australian range. Invasive paropsine beetles have recently become established in New Zealand (Rogan 2016), Ireland (Reid and de Little 2013), California (von Ellenrieder 2003), and South Carolina (Clemson University Extension 2012). Continued global expansion of the *Eucalyptus* industry will likely result in further incursions of invasive paropsine beetles, necessitating an understanding of their native natural enemies that could be utilized in classical biological control. The suite of predators and parasitoids that attack paropsine beetles in Australia is not well known. Additionally, the taxonomy of the beetles themselves has been in flux (Peixoto et al. 2018), with the most recent revision based solely on morphological characters (Reid 2006). Further revisions are needed using molecular characters to understand the identity and origin of the beetles themselves.

Larval endoparasitic wasps in the genus *Eadya* Huddleston & Short, 1978 (Hymenoptera: Braconidae) have great potential as biocontrol agents for invasive paropsines. Classical biological control studies have begun for *Eadya* from Tasmania to control the Eucalyptus Tortoise Beetle, *Paropsis charybdis* Stål, 1860 (Withers et al. 2012; Withers et al. 2013; Peixoto et al. 2018), a defoliating pest of *Eucalyptus nitens* (Deane & Maiden, 1899) plantations. The presence of possible cryptic species of *Eadya* spurred a large-scale molecular phylogenetic study on Tasmanian species of *Eadya* (Peixoto et al. 2018). This comprehensive study, a collaboration between biocontrol researchers and taxonomists, utilized a combination of molecular and host data taken from multiple locations over six years to reveal three new species of *Eadya* (*Eadya annleckieae* Ridenbaugh, sp. n.,

Eadya daenerys Ridenbaugh, sp. n., *Eadya spitzer* Ridenbaugh, sp. n.). *Eadya daenerys* sp. n. (referred to as *Eadya* sp.3 in Peixoto et al. 2018), is now the focus for importation into New Zealand to control *P. charybdis*.

In this paper, we formally describe these three new species discovered from Peixoto et al. (2018) using all available data, including newly collected morphological data. *Eadya paropsidis* and *E. daenerys* sp. n. are the two cryptic species that spurred the molecular phylogenetic paper of Peixoto et al. (2018). We redescribe *E. paropsidis* and use a multivariate ratio analysis to ensure these species can be accurately diagnosed. A fourth new species, *E. duncan* sp. n. was discovered from the Australian National Insect Collection (ANIC) and is also described using morphology. All known host records for all species of *Eadya* are listed so these records are available in the event of further paropsine introductions around the world. Furthermore, a well-illustrated key to *E. paropsidis* and all new and known species is provided to facilitate identification by applied researchers along with a discussion of the potential for species of *Eadya* as biological control agents. Finally, based on morphology, we suggest that *Eadya* belongs within Euphorinae, as originally placed by Huddleston and Short (1978) and not Helconinae as recovered in a one gene molecular analysis (Belshaw and Quicke 2002).

Methods

We utilized material collected from Peixoto et al. (2018), and additional museum specimens. Type specimens were deposited in the following institutions: the Australian National Insect Collection (ANIC), the American Entomological Institute (A.E.I.), and the University of Central Florida Collection of Arthropods (UCFC). All material examined and locations of deposition are listed in Suppl. material 1. Depositions of holotypes and paratypes are also listed in the descriptions, in brackets, under Type material. Terminology for morphology follows that of Sharkey and Wharton (1997) and the Hymenoptera Anatomy Ontology project (Yoder et al. 2010), while terminology for sculpture follows that of Harris (1979).

A molecular diagnostic key was created using the barcoding region (Hebert et al. 2003) of *Cytochrome c oxidase subunit 1* (*COI*) sequences obtained from Peixoto et al. (2018) under GenBank accession numbers KX99052–KX990220, and MH107809–MH107817. Sequences were translated and hand aligned in Bioedit v.7.1.3 (Hall 1999). As there were no indels in the sequence, alignment was achieved using the reading frame as a guide. Diagnostic molecular characters are listed with reference to their amino acid position on the complete *COI* reference gene of *Apis mellifera mellifera* Linnaeus, 1758 (GenBank ref AHY80993.1). Positions are listed in parenthesis followed by the corresponding diagnostic molecular characters. Species that are polymorphic at these codon sites have all observed amino acids for a given position listed in brackets.

Photographs were taken using a Canon 7D Mark II with the following lenses: MP-E 65mm 1–5× Canon macro lens, and a M Plan Apo 10× Mitutoyo objective mounted onto the EF Telephoto 70–200mm Canon zoom lens. For lighting, the MT-24EX Macro Twin Lite Canon Flash was used in conjunction with a custom made

Table 1. Abbreviations and definitions of the 8 morphological characters used for the morphometrics analysis of *Eadya paropsidis* and *Eadya daenerys*.

Abbreviation	Character name	Definition	Magnification (<i>E. paropsidis</i>)	Magnification (<i>E. daenerys</i>)
LOL	Lateral Ocellar Line	The shortest distance between the median and lateral ocellus, dorsal view (Fig. 16B)	100×	100×
OOL	Ocular Ocellar Line	The shortest distance between the lateral ocellus and the eye, dorsal view (Fig. 16B)	100×	100×
POL	Posterior Ocellar Line	The shortest distance between the lateral ocelli, dorsal view (Fig. 16B)	100x	100×
oci.l	Occipital Ocellar Line	The shortest distance from the posterior edge of the lateral ocellus at a 90° angle to the occipital carinae, dorsal view (Fig. 16B)	100×	100×
gsp.l	Genal Space	Length of the genal space taken midway between the dorsal and ventral margins of the eye from the posterior edge at a 90° angle to the occipital carinae, lateral view (see Fig. 2D, Zhang et al. 2017)	100×	100×
mlr.l	Malar Space	Length of the malar space taken from the posterior margin of the eye to the base of the mandible, anterior view (Fig. 16A)	100×	100×
hea.b	Head breadth	Greatest breadth of head, dorsal view (see Fig. 2B, Zhang et al. 2017)	50×	50×
mt1.b	Metasomal tergite 1 breadth	Greatest breadth of metasomal tergite 1 at the posterior margin, dorsal view (see Fig. 2F, Zhang et al. 2017)	50×	100×

diffuser. Multiple images were taken of each specimen and compiled into a single image using Zerene Stacker 1.04 (Zerene Systems LLC.). Scale bars were added using ImageJ 1.51 (Schneider et al. 2012). Images were edited using Adobe Photoshop Creative Cloud and Adobe Lightroom Creative Cloud (Adobe Systems Inc.). Figures were prepared using Adobe Illustrator Creative Cloud (Adobe Systems Inc.).

Of the four species supported by the molecular data presented in Peixoto et al. (2018), *E. paropsidis* and *E. daenerys* sp. n. were examined using a morphometric multivariate ratio analysis due to their cryptic morphology. For this study, species were grouped based on molecular operational taxonomic units (MOTUs) in accordance with the results of Peixoto et al. (2018). To test the validity of the MOTUs, a series of shape principal component analyses (PCAs) were performed to determine if variation was due to shape or allometric in nature. A shape PCA analysis was chosen to avoid bias towards one group or another, as an assignment to species was not required (László et al. 2013). A series of 20 female specimens, eight *E. paropsidis* and 16 *E. daenerys* sp. n. were selected based upon the number of female specimens available and the condition of those specimens (see Suppl. material 1). Female specimens were used exclusively as most type specimens are female, and to eliminate any variation that may be attributed to sexual dimorphism.

The characters evaluated in this study were as follows: Lateral ocellar line (LOL), ocular ocellar line (OOL), posterior ocellar line (POL), occipital ocellar line (oci.l), genal

space (gsp.l), malar space (mlr.l), head breadth (hea.b), and metasomal tergite 1 breadth (mt1.b). The definition of these characters and how they were measured can be found in Table 1 and are depicted in Fig. 16. For application of the PCA ratio spectrum, characters furthest from each other show the most variation and are ideal for diagnosing species, whereas those closest together account for very little variation and should be avoided (Baur and Leuenberger 2011; László et al. 2013). The allometry ratio spectrum can be applied in a similar manner, with characters closer together being favored as they are less allometric (Baur and Leuenberger 2011; László et al. 2013). Character measurements were recorded as the average of three measurements taken using a Nikon SNZ18 stereomicroscope with an ocular micrometer. The morphometrical analysis (Baur and Leuenberger 2011) was applied in R (R Core Team 2016) as outlined in Baur et al. (2014) using code modified by Zhang et al. (2017). The data and R script files for this analysis can be obtained from figshare (<https://figshare.com>, DOI: 10.6084/m9.figshare.6022259).

The host, *Paropsisterna variicollis** (Chapuis, 1877) is listed with an asterisk within descriptions due to the uncertainty surrounding the taxonomic validity of this species with respect to *Pst. obovata* (Chapuis, 1877) and *Pst. cloelia* (Stål, 1860). For a detailed discussion on the taxonomic uncertainty of this species, see Peixoto et al. (2018).

Results

Morphometrics analysis

Separating most species of *Eadya* was relatively straightforward using morphological characters (see Key to Species of *Eadya* below). However, *E. paropsidis* and *E. daenerys* sp. n. presented only size differences morphologically, with the latter species being smaller, even though they were well supported phylogenetic species based on molecular data (Peixoto et al. 2018). To examine if there were any usable morphological characters to discriminate these species, we performed a multivariate ratio analysis. The first and second shape PC were the only ones that were informative, accounting for 83.9% of the variation observed (Fig. 1A). From these two shape PCAs separation of the species was recovered from the first principal component, but not the second. Isometric size, defined by Baur and Leuenberger (2011) as the geometric mean of all body measurements, was plotted against the first principal component (Fig. 1B). A correlation between shape and size was observed, indicating that the differences in measured ratios between the two species are due to size and not shape (Fig. 1B).

A PCA and allometry ratio spectrum were generated to determine which characters were the best for delimiting the two cryptic species. The most discerning ratios according to the first principal component were *LOL:mlr.l*, *LOL:mt1.b*, and *LOL:gsp.l* (Fig. 1C). According to the allometry ratio spectrum, the ratios *LOL:gsp.l*, *LOL:mlr.l*, and *LOL:mt1.b* were the most allometric between the two groups (Fig. 1D). As the characters corresponding to the separation of these species were also the characters displaying the greatest degree of allometric variation, the variation between these species is due primarily to differences in size and not shape (László et al. 2013).

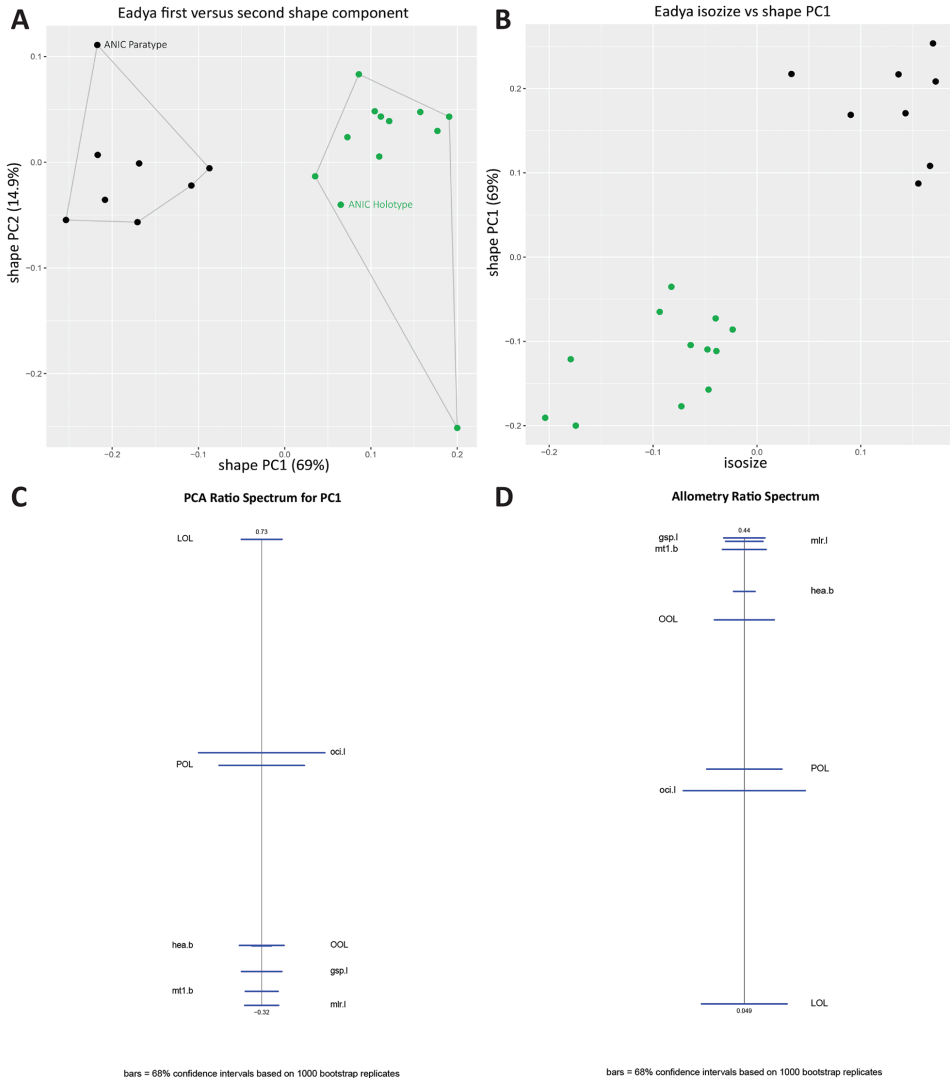


Figure 1. Multivariate morphometric ratio analysis of female specimens of *Eadya paropsidis*, and *Eadya daenerys* Ridenbaugh, sp. n. **A** Scatterplot of the first shape principal component plotted against the second shape principal component. Black - *Eadya paropsidis*, Green - *Eadya daenerys* sp. n. **B** Scatterplot of isosize plotted against the first shape principal component. Black - *Eadya paropsidis*, Green - *Eadya daenerys* sp. n. **C** Ratio spectrum for the first principal component with horizontal bars representing 68% confidence based on 1000 bootstrap replicates **D** Allometry ratio spectrum with horizontal bars representing 68% confidence based on 1000 bootstrap replicates.

When applied to *E. paropsidis* and *E. daenerys* sp. n., the morphometrical analysis only supported one species, contrasting with the results of Peixoto et al. (2018). These results indicate that the two species are truly cryptic, as the molecular and ecological data strongly supported the separation of these two species (Peixoto et al. 2018). With

this in mind, the four new species of *Eadya* are formally described using morphological and molecular characters, while purposely avoiding ratios to account for the allometric variation observed between *E. paropsidis* and *E. daenerys* sp. n.

Taxonomic descriptions

Eadya can be recognized from other braconid genera by the following combination of characteristics: head large, subcubic and as wide as thorax, clypeus flat, labrum flat, interantennal carina present; forewing with r-m crossvein present, 3RSb curved and meeting R1a before apex of wing, and 2cu-a absent; metasoma petiolate.

Eadya annleckieae Ridenbaugh, sp. n.

<http://zoobank.org/150ABAF3-37F2-405C-A86B-5768BEF6D68A>

Figs 2A–C; 3A–E

Diagnosis. *Eadya annleckieae* sp. n. can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged across ventral margin, without medial tubercles (Fig. 3A); frons with weak inter-antennal carinae and lateral carina with a faint elevated ridge wrapping around the antennal socket (Fig. 3A, B); occipital carina simple (Fig. 3B); occiput normal; notaulus wide and rugulose (Fig. 3C); scutellar sulcus divided into two distinct foveae with rugulose sculpturing along the posterior margins (Fig. 3C); sternaulus rugulose (Fig. 3D); propodeum rounded in appearance from lateral angle, without transverse carinae (Fig. 3E), and not creating a distinct posterior face when viewed laterally; propodeal spiracle circular; head black except for mandible orange with base black and apex ferruginous, maxillary and labial palp orange (Figs 2A; 3A), antenna dark brown (Figs 3C); pronotum black (Figs 2B; 3B); propleuron black (Fig. 3D); hindwing hyaline with dark brown veins (Fig. 2C); legs orange except for hind tibia dark orange with apex black (Fig. 2A); amino acid sequence (112–118) LRRLTNI (Fig. 15).

Description. Female. Body length 6.46mm. Ovipositor length 1.72mm.

Color. Head black except for mandible orange with base black and apex ferruginous, maxillary and labial palp orange, and antenna dark brown (Figs 2A; 3A, C); prothorax black (Fig. 2A); mesoscutum black (Fig. 2B); mesopleuron black with the dorsal posterior margin orange (Fig. 3D); scutellum black except for the posterior margin directly behind the scutellar sulcus orange (Fig. 2B); sternum black; metathorax orange (Fig. 2A); forewing and hindwing hyaline with dark brown veins (Fig. 2C); legs orange except for hind tibia dark orange with apex black (Fig. 2A, B); abdomen orange except for ovipositor sheath brown (Fig. 2A, B).

Head. Clypeus simple, punctate and pubescent, flanged across ventral margin, without medial tubercles (Fig. 3A); mandibles overlapping, dorsal and ventral teeth of equal length (Fig. 3A); face densely punctate, pubescent (Fig. 3A); frons rugulose, with

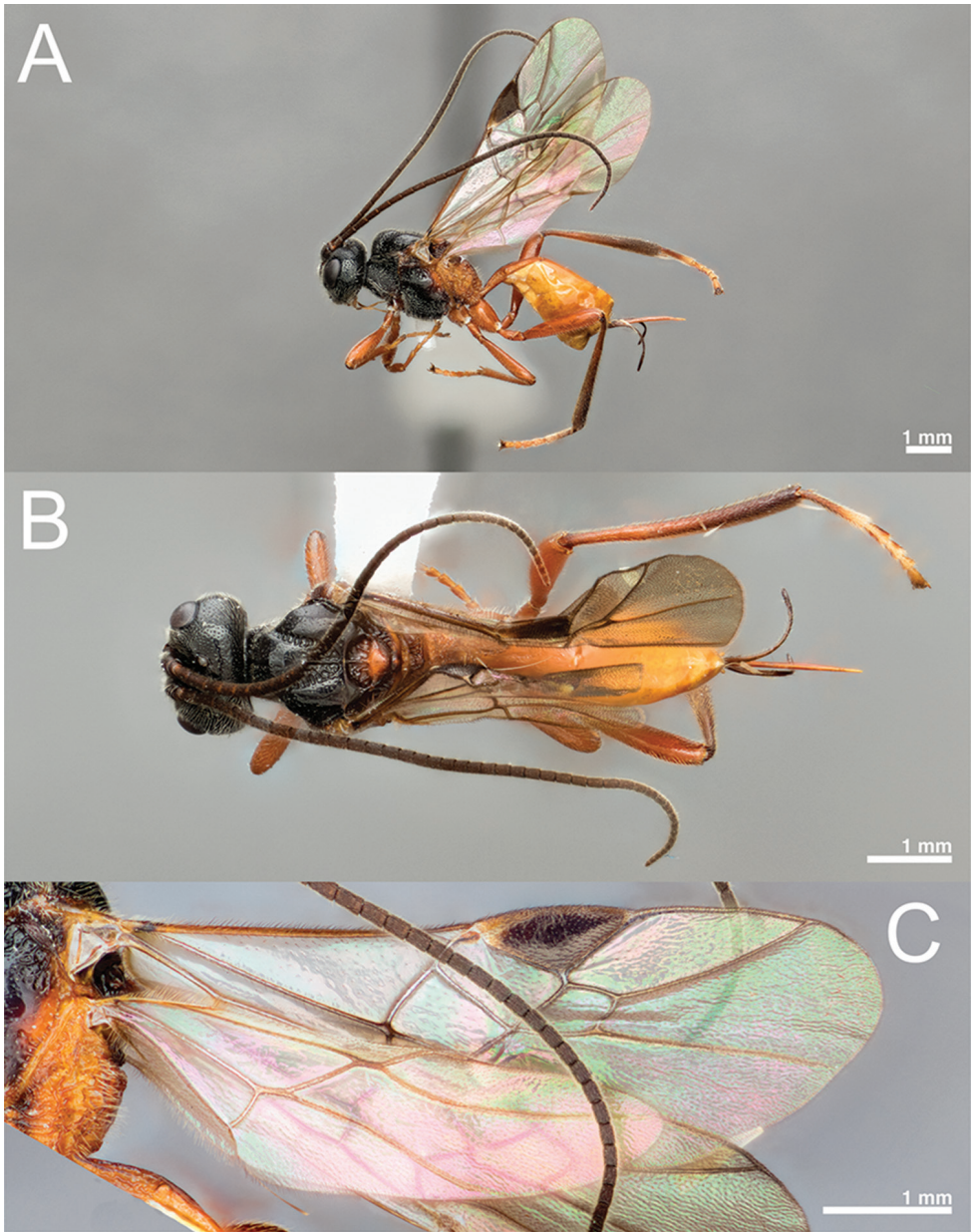


Figure 2. *Eadya annleckieae* Ridenbaugh, sp. n. holotype. **A** Lateral habitus **B** Dorsal habitus **C** Fore and hindwing. All scale bars are 1 mm in length.

a weak inter-antennal carinae and with lateral carinae with a faint elevated ridge wrapping around the antennal socket (Fig. 3A, B); vertex punctate and pubescent (Fig. 3B); occipital carina simple (Fig. 3B), reaching the hypostomal carina; hypostomal carina simple, not strongly flanged, meeting the mandible at the mandibular condyle; occiput smooth, normal.

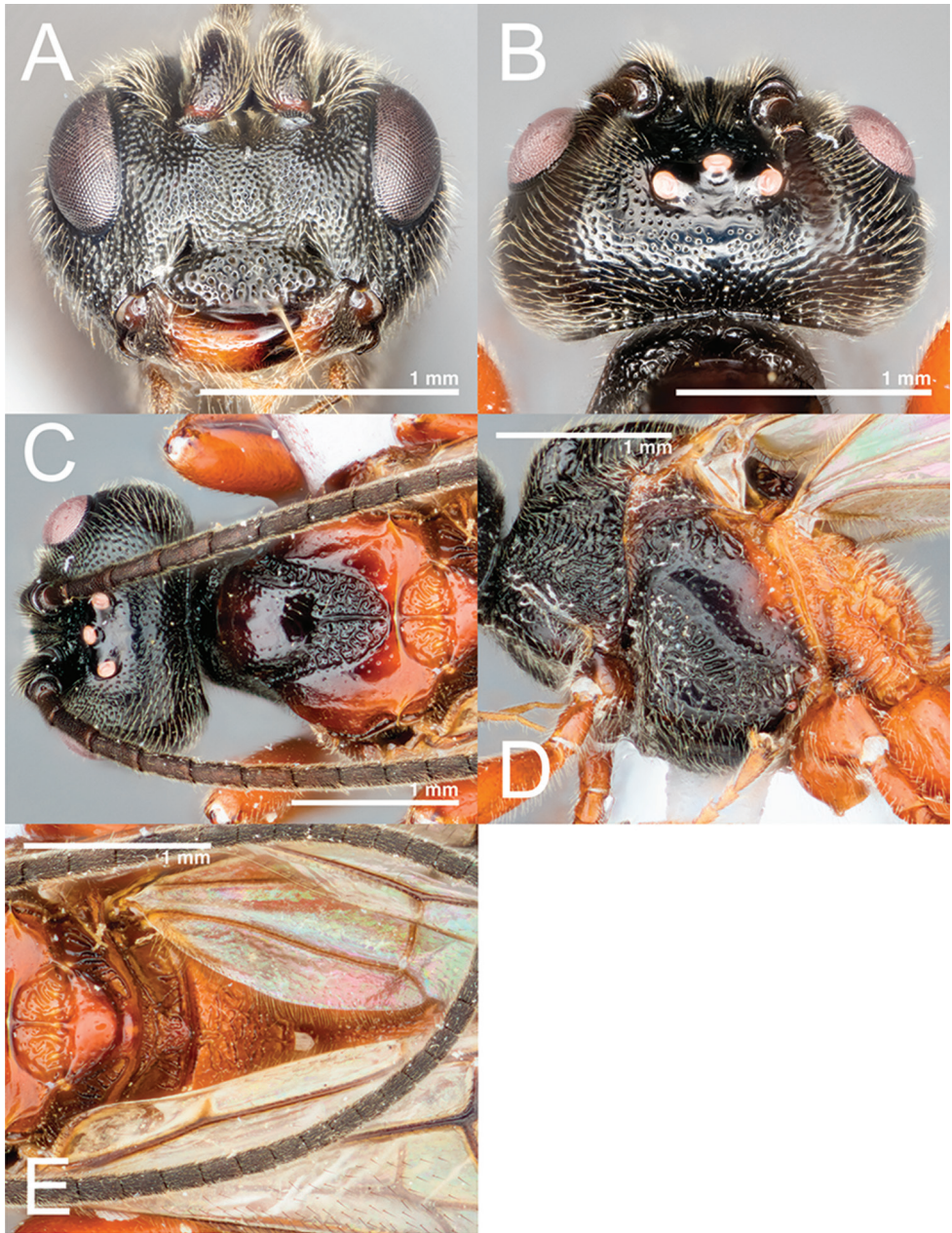


Figure 3. *Eadya annleckieae* Ridenbaugh, sp. n. holotype. **A** Head, frontal view **B** Head, dorsal view **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view. All scale bars are 1 mm in length.

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronobe present, covered in rugulose sculpturing (Fig. 3C, D); mesoscutum with posterior half of median mesonotal lobe rugulose, a distinct longitudinal carinae extending from the posterior margin to about the middle of the lobe (Figs 2B; 3C); notaulus wide and rugulose

(Figs 2B; 3C); scutellar sulcus divided into two distinct foveae with rugulose sculpturing along the posterior margins (Figs 2B; 3E); sternaulus rugulose (Fig. 3D); propodeum rugose, covered in setae but not pubescent, rounded in appearance from lateral angle, without transverse carina and not creating a distinct posterior face when viewed laterally (Figs 2A; 3D, E); propodeal spiracle circular; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent (Fig. 2A, B); tarsal claws simple.

Forewing. r-m sinuous (Fig. 2C).

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal surface smooth, lateral surface punctate with associated setae; ovipositor straight (Fig. 2A).

Male. Same as female.

Host. *Paropsisterna nobilitata* (Erichson, 1842), *Paropsisterna variicollis**, *Paropsisterna selmani* Reid & de Little, 2013, *Paropsis charybdis*.

Variations. Paratype with propleuron black except for lateral posterior margin orange; mesoscutum orange except for the median mesonotal lobe black with the anterior margin and lateral mesonotal lobes ferruginous (Fig. 3C); mesopleuron orange except for the sternaulus and ventral margins black; scutellum orange (Fig. 3C, E); legs orange except for apex of hind tibia black and hind tarsus with tarsomere 1 yellow and white at apex, tarsomeres 2–4 white, and tarsomere 5 yellow; abdomen orange except for lateral margins of metasomal sternites 3–6 brown, the second and third to last metasomal tergites with two light brown spots near the anterior margin. Some of this variation may be the result of the DNA extraction process.

Diagnostic molecular characters. Amino acid positions (22–27) MWAGIL; (32–34) SII; (41–46) SRGSLI; (54) R; (67–73) MVMPVIM; (81) I; (90) I; (95–98) MNNM; (104–109) LPSLFI; (112–118) LRRLTNI; (126) I; (133–139) GGRHSGV; (143–144) VA; (150) I; (157) [I or K]; (167–169) FNM; (172–191) NGIAVDRVTL-FRWSVKITAF (Fig. 13).

Distribution. Tasmania.

Etymology. This species is named in honor of the science fiction author, Ann Leckie by the second author (EB).

Remarks. This species is referred to as *Eadya* sp.1 in Peixoto et al. (2018). The UCFC paratype is in poor shape due to the DNA extraction process. The flange of the inter-antennal carinae is difficult to see in the images (Fig. 3A, B), but is clear when viewing the specimens, provided the antennae are separated enough.

Type material. Holotype, Female (ANIC), “Ellendale, TAS, Female, 21a, 10 Dec 2014, D. Satchell”. Paratype, Female (ANIC), “Moina, TAS, S41°29.5' E152°04.7', *Paropsis charybdis* sentinel, Emerged 2 Jan 2013, G.R. Allen, E127”, “DNA voucher BJS196”, GenBank accession numbers KX031361, KX99032, and KX990052. Paratype, Male (UCFC), “The Lea, TAS, #12, *Eadya paropsidis* cocoon (brown). Emerged from *Pst. variicollis**, 4 Dec 2014, UCFC 0 567 827”, “DNA voucher BJS501”, GenBank accession number KX990216.

***Eadya daenerys* Ridenbaugh, 2018, sp. n.**

<http://zoobank.org/38860F10-4E44-4C6A-A396-51364FB71F09>

Figs 4A–C; 5A–F

Diagnosis. *Eadya daenerys* sp. n. can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged along ventral margin, with two medial tubercles projecting outward (Fig. 5A); frons with inter-antennal and lateral carinae flanged (Fig. 5B); occipital carina simple (Fig. 5B); occiput normal; notaulus crenulate (Fig. 5C); scutellar sulcus divided into many deep pits by longitudinal carinae (Fig. 5C); sternaulus crenulate (Fig. 5D); propodeum rounded in appearance from lateral angle (Figs 4A; 5D), without transverse carina (Fig. 5E, F), and not creating a distinct posterior face when viewed laterally; propodeal spiracle circular; head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 5A, B); pronotum black except for anterior dorsal margin orange (Figs 4A, 5B); propleuron orange; hindwing infusate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 4C); legs black (Fig. 4A, B); amino acid sequence (112–118) IRNFIGA (Fig. 15).

Description. Female. Body Length 5.77mm. Ovipositor Length 0.82mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black (Figs 4A, B; 5A, B); pronotum black except for anterior dorsal margin orange (Figs 4A, 5B); propleuron orange; mesothorax black (Figs 4A, B; 5C, D); metathorax black (Figs 4A, B; 5E, F); forewing infusate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 4C); hindwing infusate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 4C); legs black (Figs 4A, B); abdomen black except ovipositor orange (Fig. 4A).

Head. Clypeus simple, smooth with scattered setae, flanged at ventral margin, with two medial tubercles projecting outward (Fig. 5A); mandibles overlapping, dorsal tooth longer than ventral (Fig. 5A); face finely punctate with associated setae (Fig. 5A); frons rugose, inter-antennal and lateral carinae flanged, starting at the toruli and reaching the ocellar triangle (Fig. 5A, B); vertex smooth with scattered setae (Fig. 5B); occipital carina simple (See arrow, Fig. 5B), reaching the hypostomal carina; hypostomal carina strongly flanged, reaching the mandible and bending around to the mandibular condyle; occiput smooth, normal (Fig. 5B).

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronope absent, smooth except for a crenulate line extending laterally and rugulose sculpturing along the lateral posterior margin (Fig. 5B); mesoscutum with median mesonotal lobe smooth (Fig. 5C); notaulus crenulate (Fig. 5C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 5C); sternaulus crenulate (Fig. 5D); propodeum rugose and pubescent, rounded in appearance from lateral angle, without transverse carina and not creating a distinct posterior face when viewed laterally (Figs 4A; 5D, E, F); propodeal spiracle circular; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent (Fig. 4A, B); tarsal claws simple.

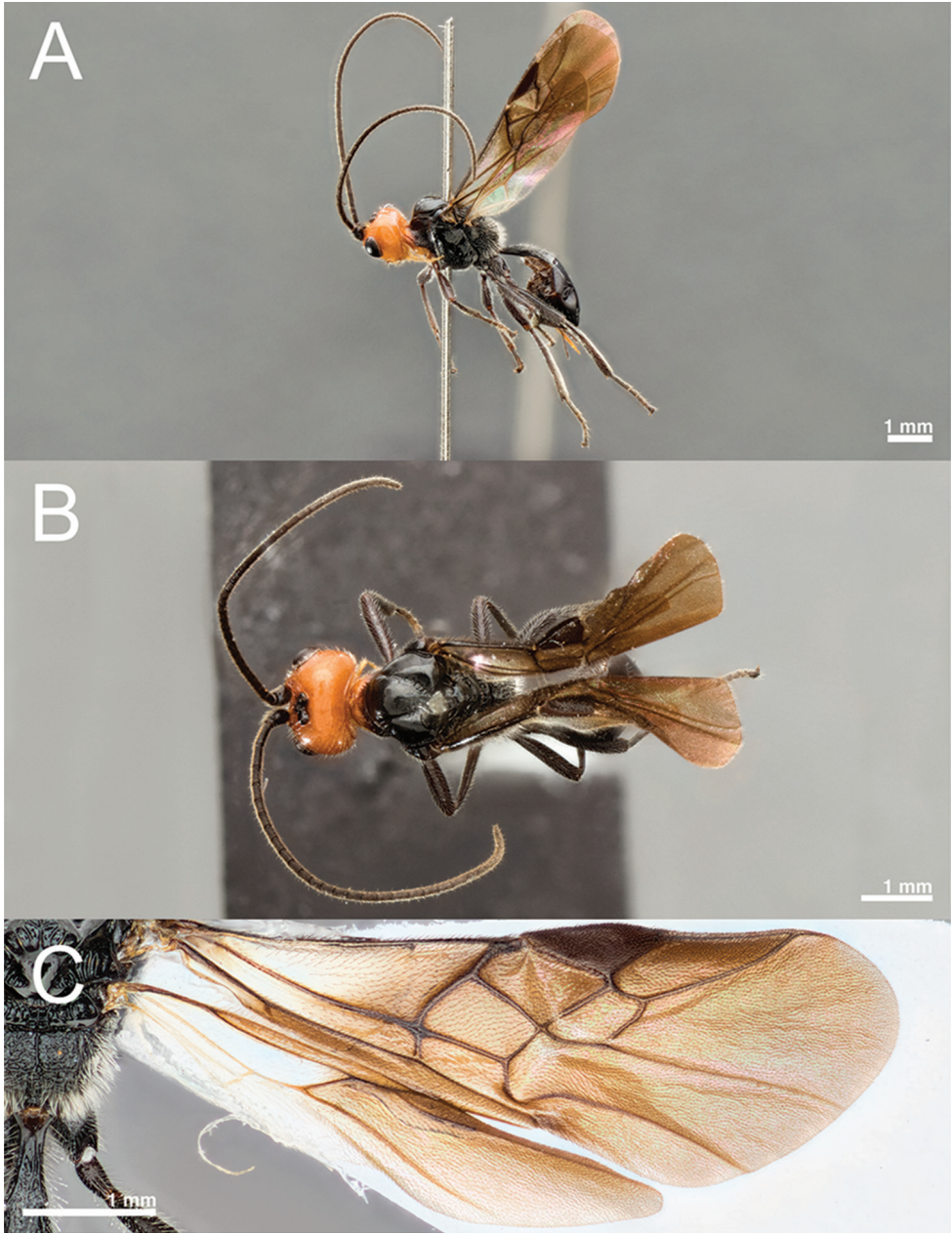


Figure 4. *Eadya daenerys* Ridenbaugh, sp. n. **A** Lateral habitus, holotype **B** Dorsal habitus, holotype **C** Fore and hindwing, paratype. All scale bars are 1mm in length.

Forewing. r-m curved slightly towards stigma before reaching the junction of 3RSa and 3RSb (Fig. 4C).

Hindwing. R1a with three hamuli.

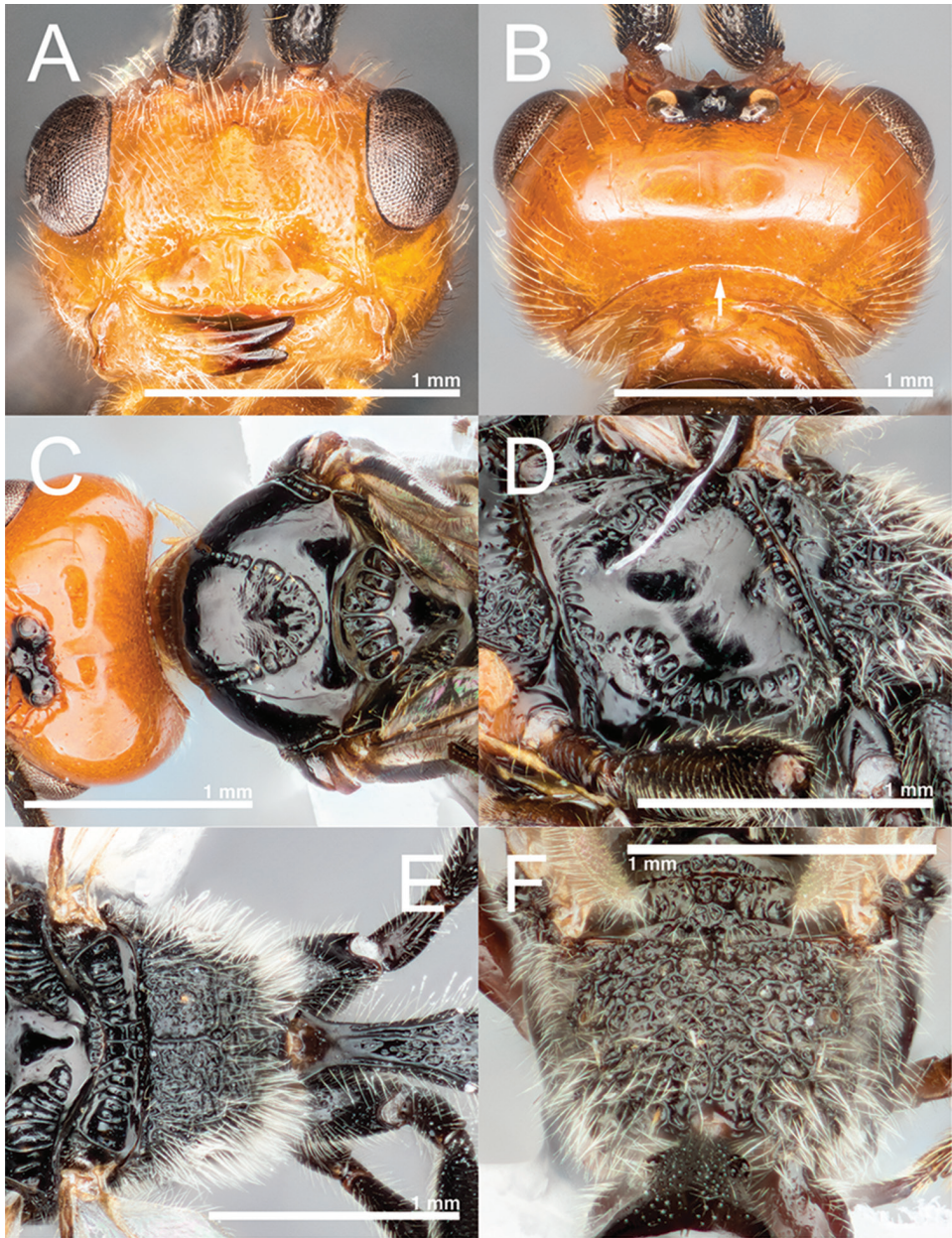


Figure 5. *Eadya daenerys* Ridenbaugh, sp. n. paratype. **A** Head, frontal view **B** Head, dorsal view, arrow indicating simple occipital carinae **C** Head and mesoscutum, dorsal view, paratype **D** Mesopleuron, lateral view, paratype **E** Propodeum, dorsal view **F** Propodeum, postero-dorsal view. All scale bars are 1 mm in length.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae (Fig. 5E); ovipositor straight (Fig. 4A).

Male. Same as female.

Host. *Paropsisterna agricola* (Chapuis, 1877), *Paropsisterna bimaculata* (Olivier, 1807), *Paropsisterna nobilitata*, *Paropsis charybdis*.

Diagnostic molecular characters. Amino acid positions (22–27) [M or R] WSGII; (32–34) RVL; (41–46) ILGRLL; (54) S; (67–73) IVIPIII; (81) I; (90) I; (95–98) INNI; (104–109) PPSL[I or V]L; (112–118) IRNFIGA; (126) I; (133–139) NLSHRGV; (143–144) [V or I]S; (150) L; (157) I; (167–169) INI; (172–191) LGL-SYDNISLLVWSVNITAI (Fig. 15).

Distribution. Australian Capital Territory, New South Wales, Tasmania.

Etymology. This species is named for Daenerys Stormborn of House Targaryen, the First of Her Name, Queen of the Andals and the First Men, Protector of the Seven Kingdoms, the Mother of Dragons, Khaleesi of the Great Grass Sea, the Unburnt, the Breaker of Chains, from the literary series *A Song of Ice and Fire* by George R.R. Martin, as well as the television series *Game of Thrones* on Home Box Office (HBO). This is a noun in apposition to the generic name in order to retain integrity of the fictional character name Daenerys.

Remarks. This species is referred to as *Eadya* sp.3 in Peixoto et al. (2018).

Type material. Holotype, Female (ANIC), “Frankford, TAS, Female, 3 Dec 2001, A.D. Rice, NT#5, Pin#8”. Paratype, Female (ANIC), “The Creel, Kosciusko, NSW, 8 Nov 1961, E.F. Riek, A35, Aust. Nat. Ins. Coll.”. Paratype, Female (ANIC), “Canberra, ACT, 19 Nov 1958, E.F. Riek, A34, Aust. Nat. Ins. Coll.”. Paratype, Female (ANIC), “Canberra, ACT, 26 Nov 1959, E.F. Riek, Aust. Nat. Ins. Coll.”. Paratype, Female (ANIC), “Canberra, ACT, 26 Nov 1959, E.F. Riek, Aust. Nat. Ins. Coll.”. Paratype, Male (ANIC), “Canberra, ACT, 30 Nov 1959, E.F. Riek, Aust. Nat. Ins. Coll.”. Paratype, Female (ANIC), “Canberra, ACT, 18 Nov 1960, E.F. Riek, Aust. Nat. Ins. Coll.”. Paratype, Male (ANIC), “Canberra, ACT, 24 Nov 1960, E.F. Riek, A35, Aust. Nat. Ins. Coll.”. Paratype, Female (ANIC), Black Mt., F.C.T, 10 XI 30, W. Broce, Aust. Nat. Ins. Coll.”. Paratype, Female (UCFC), “Frankford, TAS, 2 Jan 2002, Malaise Trap, A.D. Rice, MT6, UCFC 0 567 735”. Paratype, Female (UCFC), “Frankford, TAS, Female, 19 Nov 2001, A.D. Rice, NT#5, Pin #5, UCFC 0 567 736”. Paratype, Female (UCFC), “Frankford, TAS, Female, 3 Dec 2001, A.D. Rice, NT#5, Pin #9, UCFC 0 567 737”. Paratype, Female (UCFC), “Frankford, TAS, Female, 3 Dec 2001, A.D. Rice, NT#5, Pin #10, UCFC 0 567 738”. Paratype, Female (UCFC), “Frankford, TAS, Female, 19 Nov 2001, A.D. Rice NT#5, Pin #7, UCFC 0 567 741”. Paratype, Female (A.E.I.), “King William Range, I. 8-23, Tasmania, A.E.I. Sep/05”. Paratype, Male (A.E.I.), “Runnymede, TAS, 24 Nov 2015, 42 38'13.3"S 147 33'53.8"E, Malaise trap, G.R. Allen, Male, MTM2”. Paratype, Male (A.E.I.), “Ellendale, TAS, Male, 14 Dec 2015, D. Satchell, EM2”. Paratype, Male (A.E.I.), “Runnymede, TAS, 24 Nov 2015, 42 38'13.3"S 147 33'53.8"E, Malaise trap G.R. Allen, Male, MTM1”. Paratype, Female (A.E.I.), “Frankford, TAS, Female, 27 Nov 2000, A.D. Rice, Em Trap #1, Pin #3”.

Non-type material. See Suppl. material 1.

***Eadya duncan* Ridenbaugh, sp. n.**

<http://zoobank.org/10EA7B5B-E6F6-49BA-BCBD-A1388D5B5390>

Figs 6A–C, 7A–E

Diagnosis. *Eadya duncan* sp. n. can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged at ventral margin, with two medial tubercles projecting outward (Fig. 7A); frons with inter-antennal and lateral carina strongly flanged (Fig. 7B); occipital carina simple (Fig. 7B); occiput concave; notaulus narrow and impressed towards anterior margins of mesoscutum, crenulate at apex (Fig. 7C); scutellar sulcus divided into two distinct foveae with short longitudinal carinae ending before reaching anterior margin (Fig. 7C); propodeum not rounded in appearance from lateral angle (Fig. 6A), with transverse carina creating a distinct posterior face when viewed laterally; propodeal spiracle elliptical; head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 7A, B); prothorax orange (Figs 6A, 7C); hindwing infusate with dark brown veins except for anal, basal, sub-basal, and anterior half of discal cells hyaline (Fig. 6C); legs black except for fore coxa and trochanter orange, fore femur dark orange (Fig 6A).

Description. Male. Body length 6.37mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black (Figs 6A, B; 7A, B); prothorax orange (Figs 6A, B; 7B); mesothorax orange (Figs 6A, B; 7B, C); propodeum black except for medial posterior margin at the insertion of metasomal tergite 1 orange (Figs 6A, B; 7B); metapleuron black; forewing infusate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 6C); hindwing infusate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 6C); legs black except for fore coxa and trochanter orange, fore femur dark orange; abdomen black (Fig. 6A, B).

Head. Clypeus simple, smooth with scattered setae, flanged at ventral margin, with two medial tubercles projecting outward (Fig. 7A); mandibles overlapping, dorsal tooth longer than ventral (Fig. 7A); face finely punctate with associated setae (Fig. 7A); frons rugulose, inter-antennal and lateral carina strongly flanged, starting at the toruli and reaching the ocellar triangle (Fig. 7A, B); vertex smooth with scattered setae (Fig. 7B); occipital carina simple, reaching hypostomal carina (Fig. 7B); hypostomal carina strongly flanged, meeting the mandible and bending around to the mandibular condyle; occiput smooth, normal.

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronope absent, smooth except for a faint crenulate line extending laterally and rugulose sculpturing along the lateral posterior margin (Fig. 7B); mesoscutum with median mesonotal lobe smooth (Fig. 7C); notaulus impressed towards anterior margins of mesoscutum, crenulate at apex (Fig. 7C); scutellar sulcus divided into two distinct foveae with short longitudinal carinae ending before reaching anterior margin (Fig. 7C); sternaulus crenulate (Fig. 7D); propodeum rugose and pubescent, not rounded in appearance from lateral angle, with transverse carina creating a distinct posterior face (Fig. 6A); propodeal

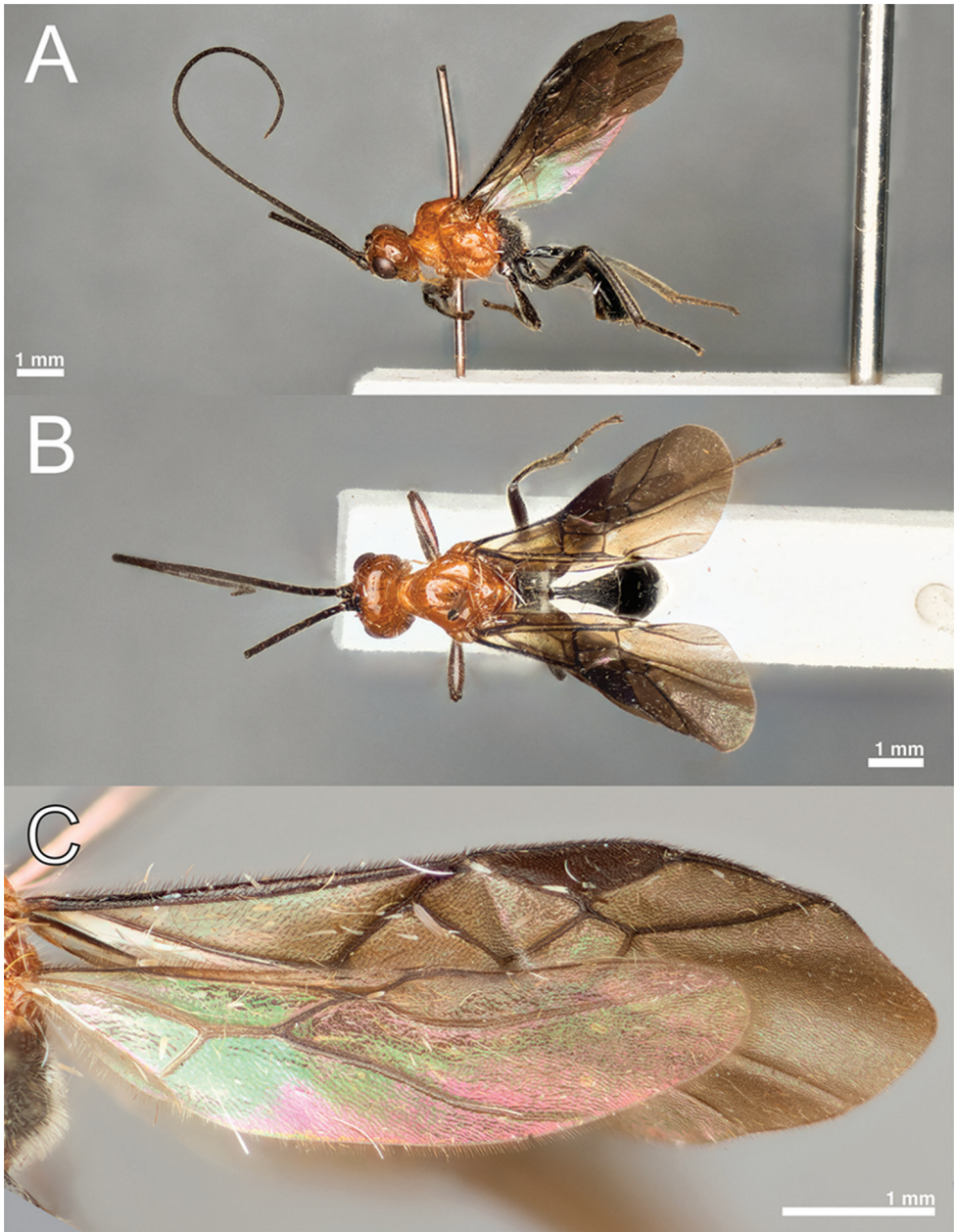


Figure 6. *Eadya duncan* Ridenbaugh, sp. n. holotype. **A** Lateral habitus **B** Dorsal habitus **C** Fore and hind wing. All scale bars are 1 mm in length.

spiracle elliptical; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent (Fig. 6A, B); tarsal claws simple.

Forewing. r-m curved slightly towards stigma before reaching the junction of 3RSa and 3RSb (Fig. 6C).

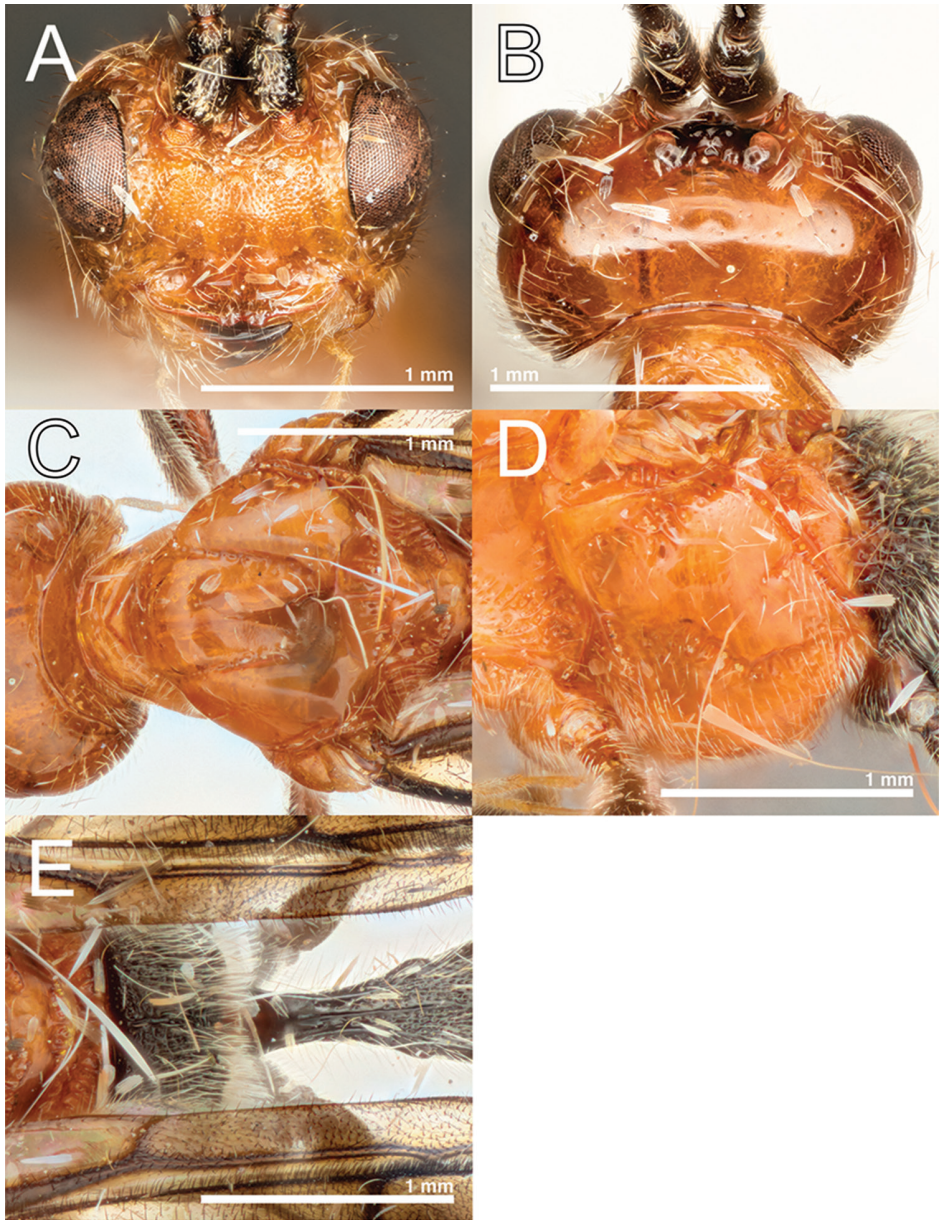


Figure 7. *Eadya duncan* Ridenbaugh, sp. n. holotype. **A** Head, frontal view **B** Head, dorsal view **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view. All scale bars are 1mm in length.

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae (Fig. 7E); ovipositor straight.

Female. Unknown.

Host. Unknown.

Distribution. New South Wales, Victoria (see discussion).

Etymology. This epithet is named in honor of the senior author's (BJS) sister in law, Julie Brant nee Duncan, who is an Australian-born beauty. This is a noun in apposition to the generic name in order to retain integrity of the surname Duncan.

Remarks. The holotype for this species was identified as a species of *Eadya* by Huddleston in 1977 and deposited at ANIC, but was not listed as material examined in the original description of *Eadya*. The flange of the inter-antennal carinae is difficult to see in the images (Fig. 7A, B), but is clear when viewing the specimen, provided the antennae are separated enough.

Type material. Holotype, Male (ANIC), "Upper Kangaroo Valley, NSW, 24 Nov 1960, E.F. Riek, A44, Aust. Nat. Ins. Coll."

Eadya falcata Huddleston & Short, 1978

Figs 8A–C; 9A–E

Diagnosis. *Eadya falcata* can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged at ventral margin, with two medial tubercles projecting outward (Fig. 9A); frons with inter-antennal and lateral carina flanged (Fig. 9A, B); occipital carinae simple (Fig. 9B); occiput normal (Fig. 9B); notaulus impressed towards anterior margin of mesoscutum, crenulate at apex (Fig. 9C); scutellar sulcus divided into two distinct foveae with short longitudinal carina ending before reaching anterior margin (Fig. 9C, E); sternaulus crenulate (Fig. 9D); propodeum rounded in appearance from lateral angle, without transverse carina, and not creating a distinct posterior face when viewed laterally (Fig. 8A); propodeal spiracle elliptical; head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 9A, B); pronotum orange except for lateral posterior margins black (Figs 8A; 9B, C); propleuron orange; hindwing infusate with dark brown veins except for anal, basal, discal, and subbasal cells hyaline (Fig. 8C); legs black except for foreleg orange with femur, tibia, and tarsus black (Fig. 8A, B).

Description. Female. Body Length 5.26mm. Ovipositor Length 1.80mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black; pronotum orange except for lateral posterior margins black (Fig. 9A, B); propleuron orange; mesothorax black (Figs 8A, B; 9C, D); metathorax black (Figs 8A, B; 9D, E); forewing infusate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 8C); hindwing infusate with dark brown veins except for anal, basal, discal, and subbasal cells hyaline (Fig. 8C); legs black except for foreleg orange with femur, tibia, and tarsus black (Fig. 8A, B); abdomen black except for ovipositor orange (Fig. 8A).

Head. Clypeus simple, smooth with scattered setae, flanged at ventral margin, with two medial tubercles projecting outward (Fig. 9A); mandibles overlapping, dorsal tooth longer than ventral (Fig. 9A); face finely punctate with associated setae (Fig. 9A); frons smooth, inter-antennal and lateral carina flanged, starting at the toruli and reaching

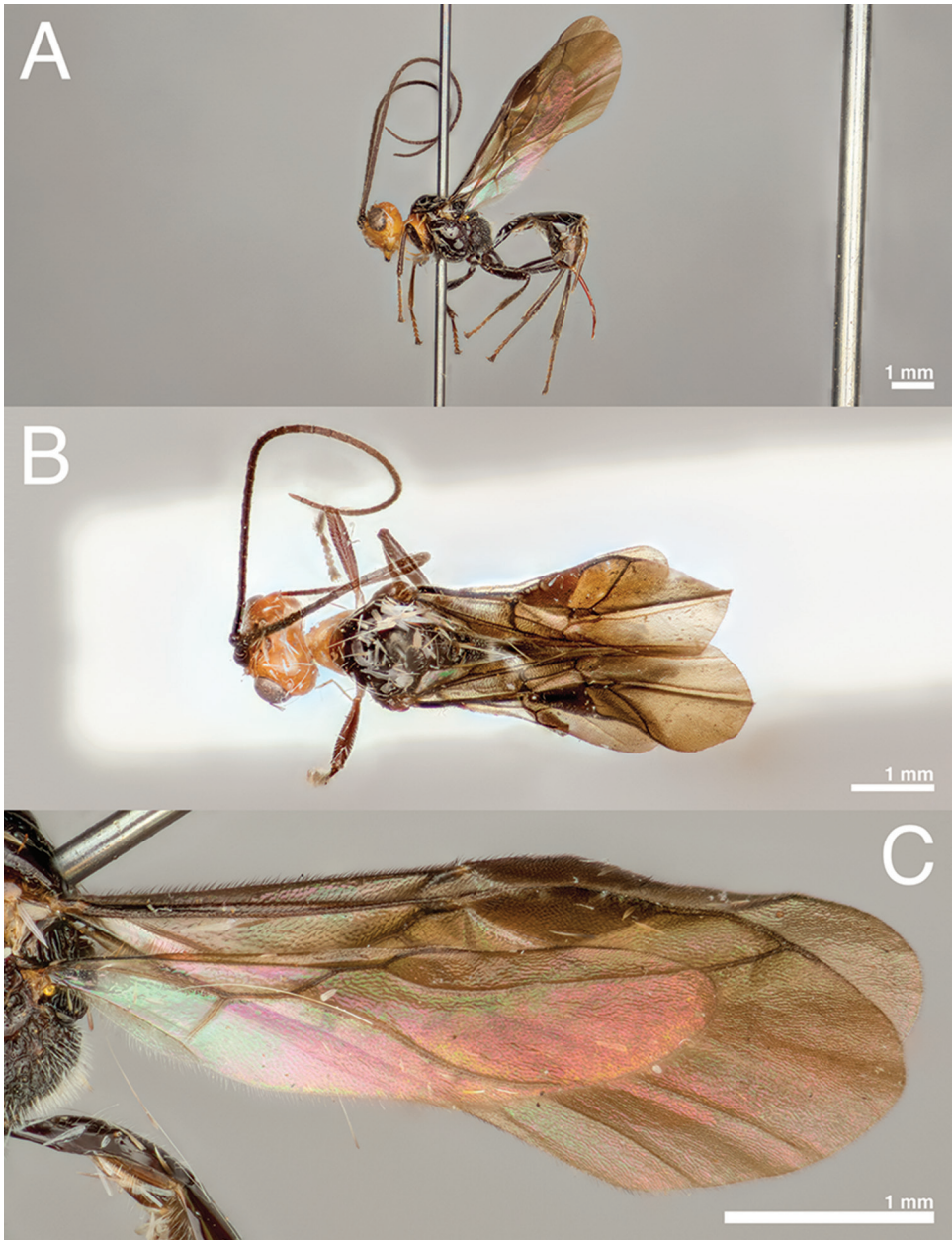


Figure 8. *Eadya falcata* holotype. **A** Lateral habitus **B** Dorsal habitus **C** Fore and hindwing. All scale bars are 1mm in length.

the ocellar triangle (Fig. 9A, B); vertex smooth with scattered setae (Fig. 9B); occipital carina simple, reaching hypostomal carina (Fig. 9B); hypostomal carina strongly flanged, meeting the mandible and bending around to the mandibular condyle; occiput smooth, normal.

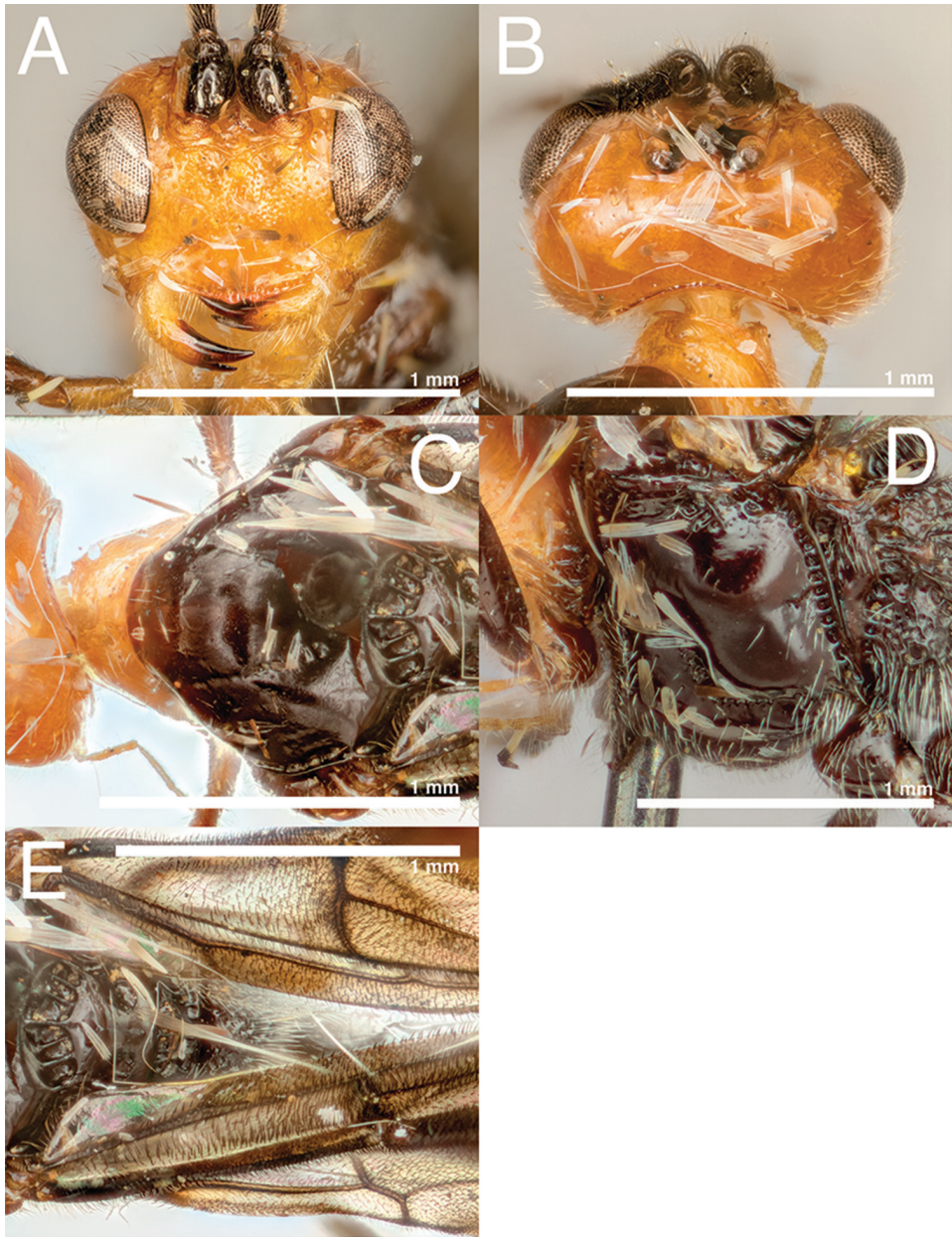


Figure 9. *Eadya falcata* holotype. **A** Head, frontal view **B** Head, dorsal view, arrow pointing to emarginate occipital carinae **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view.

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronope absent, smooth (Fig. 9B); mesoscutum with median mesonotal lobe smooth (Fig. 9C); notaulus impressed towards anterior margin of mesoscutum, crenulate at apex (Fig. 9C);

scutellar sulcus divided into two distinct foveae with short longitudinal carinae ending before reaching anterior margin (Fig. 9C, E); sternaulus crenulate (Fig. 9D); propodeum rugose and pubescent (Fig. 9E), rounded in appearance from lateral angle (Fig. 8A), without transverse carinae and not creating a distinct posterior face when viewed laterally; propodeal spiracle elliptical; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent; tarsal claws simple (Fig. 8A, B).

Forewing. r-m curved slightly towards stigma before reaching the junction of 3R_{sa} and 3R_{sb}.

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae; ovipositor curved downward (Fig. 8A).

Male. Same as female.

Host. Unknown.

Variations. Paratype with foreleg coxa orange and trochanter, trochantellus, femur, tibia, and tarsus black.

Distribution. Western Australia.

Remarks. The crenulation at the apex of the notaulus is difficult to see in the holotype due to damage caused by pinning (Fig. 9C). However, this character is much better preserved in the paratype.

Type material examined. Holotype, Female (ANIC), "18 miles W. of Mogumber, WA. 13 April 1968, I.F.B Common & M.S. Upton, 039, *Eadya falcata*, Female, Holotype, det. T.Huddleston, 1977, Aust. Nat. Ins. Coll.". Paratype, Male (ANIC), "18 miles W. of Mogumber, WA. 13 April 1968, I.F.B Common & M.S. Upton, *Eadya falcata*, Male, Paratype, det. T.Huddleston, 1977, Aust. Nat. Ins. Coll.".

***Eadya paropsidis* Huddleston & Short, 1978**

Figs 10A–C; 11A–F

Diagnosis. *Eadya paropsidis* can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged at ventral margin, with two medial tubercles projecting outward (Fig. 11A); frons with inter-antennal and lateral carina strongly flanged (Fig. 11B); occipital carina emarginate (Fig. 11B); occiput strongly concave; notaulus crenulate (Fig. 11C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 11C); sternaulus crenulate (Fig. 11D); propodeum not rounded in appearance from lateral angle (Fig. 10A), with transverse carina creating a distinct posterior face (Fig. 11E, F) when viewed laterally; propodeal spiracle circular; head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 11A, B); pronotum orange except for lateral posterior margins black (Figs 10A, 11B); propleuron orange; hindwing infusate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 10C); legs black except for foreleg orange with tibia dark orange medially

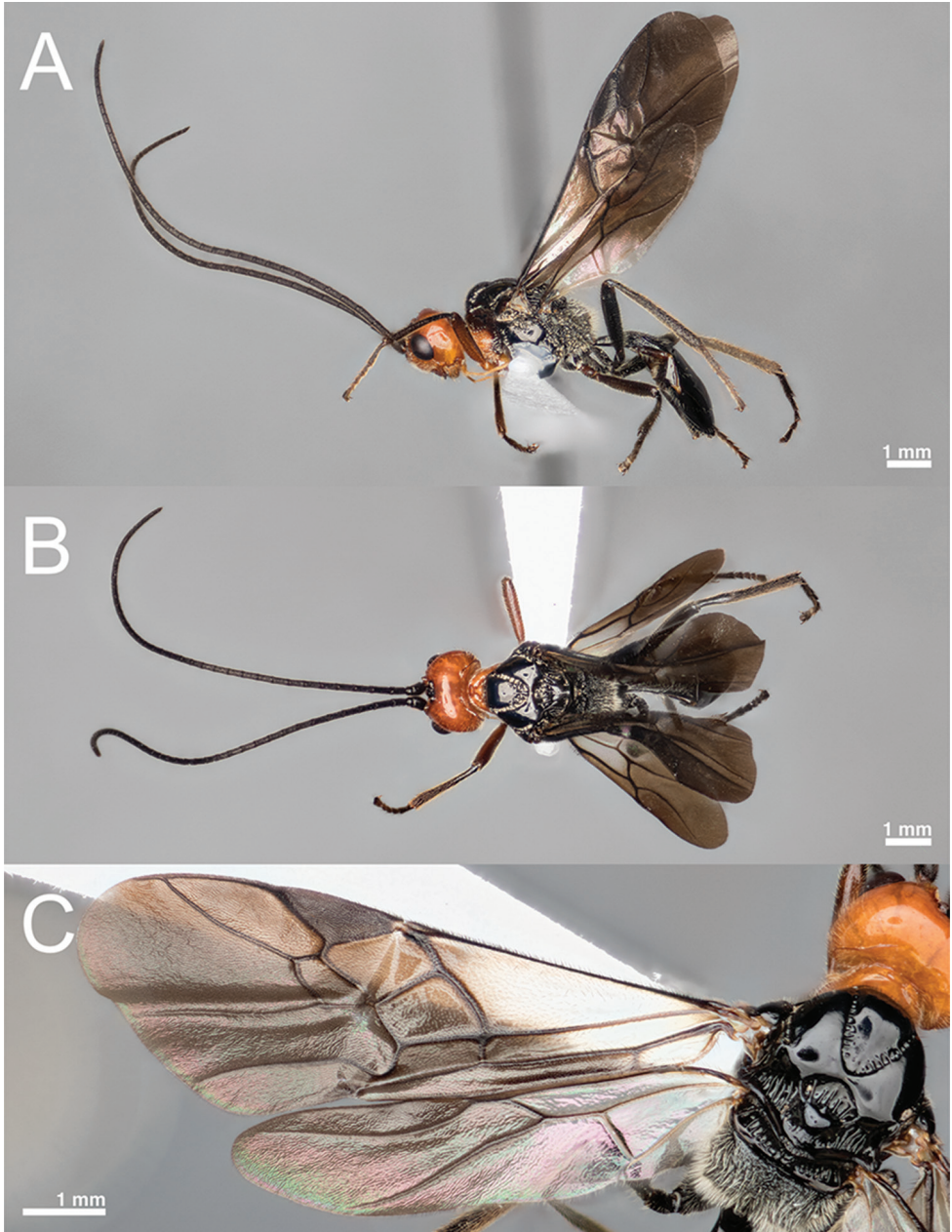


Figure 10. *Eadya paropsidis*. **A** Lateral habitus **B** Dorsal habitus **C** Fore and hindwing. All scale bars are 1mm in length.

and anterior and posterior apices brown, tarsi black (Fig. 10A); amino acid sequence (112–118) TRNFIGI (Fig. 15).

Description. Female. Body Length 6.29mm. Ovipositor Length 1.08mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black (Figs 10A, B; 11A, B); pronotum orange except for lateral posterior margins black (Figs 10A, B; 11A, B); propleuron orange; mesothorax black (Figs 10A, B; 11C, D); metathorax black (Figs 10A, B; 11D, E, F); forewing infuscate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 10C); hindwing infuscate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 10C); legs black except for foreleg orange with tibia dark orange medially and anterior and posterior apices brown, tarsi black (Fig. 10A, B); abdomen black except for ovipositor orange (Fig. 10A, B).

Head. Clypeus simple, smooth with scattered setae, flanged at ventral margin, with two medial tubercles projecting outward (Fig. 11A); mandibles overlapping, dorsal and ventral teeth of equal length (Fig. 11A); face finely punctate with associated setae (Fig. 11A); frons rugulose, inter-antennal and lateral carina strongly flanged, starting at the toruli and reaching the ocellar triangle (Fig. 11A, B); vertex smooth with scattered setae (Fig. 11B); occipital carinae emarginate (See arrow, Fig. 11B), reaching hypostomal carina; hypostomal carina strongly flanged, meeting the mandible and bending around to the mandibular condyle; occiput smooth, strongly concave (Fig. 11B, see arrow).

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronope absent, smooth except for a faint crenulate line extending laterally and rugulose sculpturing along the lateral posterior margin (Fig. 11B, C); mesoscutum with rugulose sculpturing along the posterior margin of median mesonotal lobe (Fig. 11C); notaulus crenulate (Fig. 11C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 11C); sternaulus crenulate (Fig. 11D); propodeum rugose and pubescent, not rounded in appearance from lateral angle, with transverse carina (see arrows, Fig. 11F) creating a distinct posterior face when viewed laterally (Figs 10A; 11E, F); propodeal spiracle circular; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent (Fig. 10A, B); tarsal claws simple.

Forewing. r-m curved slightly towards stigma before reaching the junction of 3RSa and 3RSb (Fig. 10C).

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae (Fig. 9E); ovipositor straight.

Male. Same as female.

Host. *Paropsis atomaria* Olivier, 1807, *Paropsis tasmanica* Baly, 1866, *Paropsis charybdis*.

Diagnostic molecular characters. (22–27) MWSGII; (32–34) SVL; (41–46) ILGRLI; (54) S; (67–73) IVIPIII; (81) V; (90) M; (95–98) INNI; (104–109) PPSLIL; (112–118) TRNFIGI; (126) I; (133–139) NLRHRGI; (143–144) IS; (150) L; (157) M; (167–169) INI; (172–191) LGLNYDNISLLVWSVNITAI (Fig. 15).

Distribution. Australian Capital Territory, Victoria, New South Wales, Tasmania.

Type material examined. Holotype, Female (ANIC), “Canberra, A.C.T., Em. 1. 1. 58 cx, host larva coll. 4. 1. 57. Parasite of *Paropsis reticulata*. C.I.E. COLL. NO. 18079. *Eadya paropsisidis* Holotype det. T. Huddleston, 1977. ANIC Database No. 32

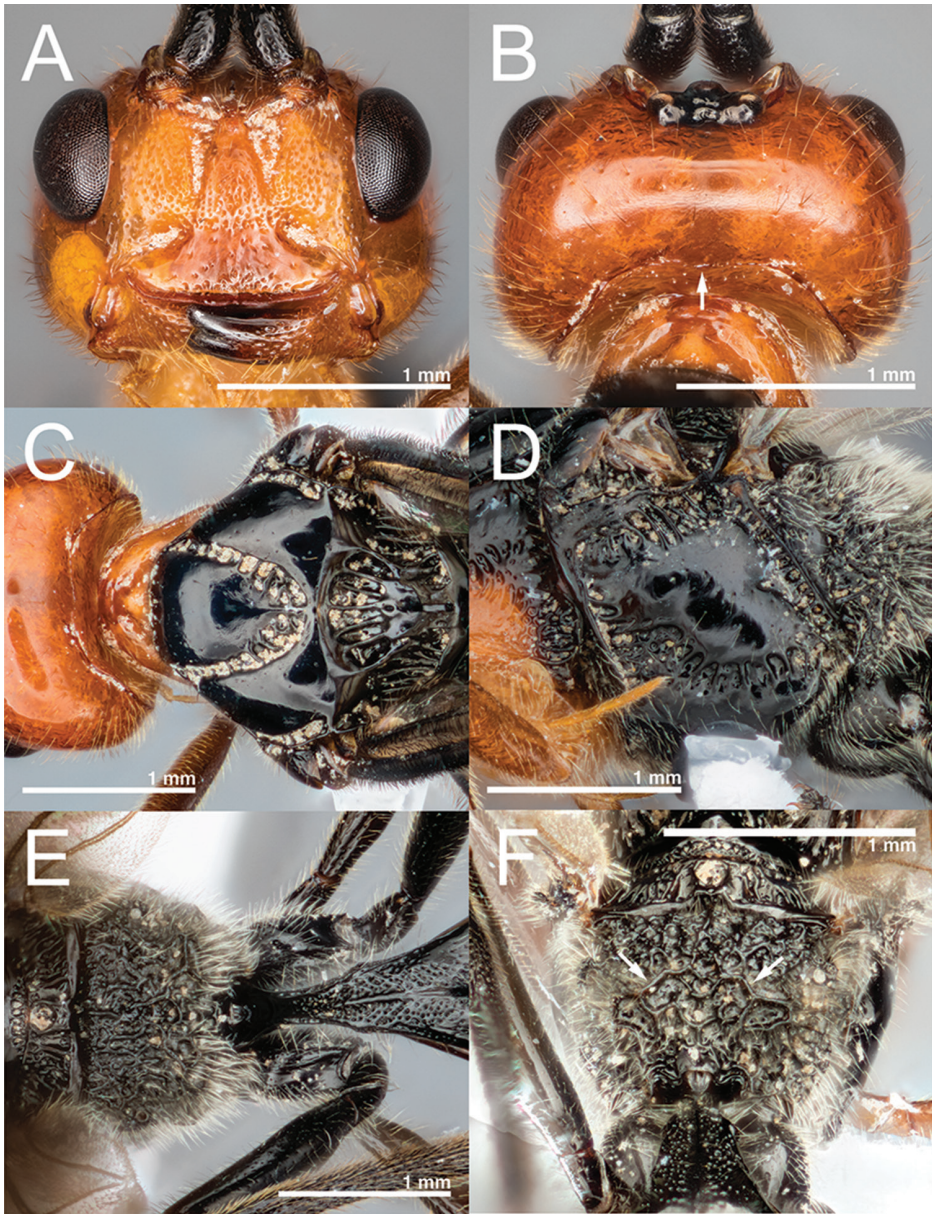


Figure 11. *Eadya paropsidis*. **A** Head, frontal view **B** Head, dorsal view, arrow pointing to emarginate occipital carinae **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view **F** Propodeum, dorsal view, with arrows indicating transverse carinae. All scale bars are 1 mm in length.

111891". Paratype, Female (ANIC), "Canberra, A.C.T., 1. 10. 1957, Dissected from cocoon, Parasite of *Paropsis reticulata*, CIE COLL No 18079. *Eadya paropsidis* Paratype Female det T. Huddleston, 1977, Aust. Nat. Ins. Coll."

Non-type material examined. See Suppl. material 1.

***Eadya spitzer* Ridenbaugh, sp. n.**

<http://zoobank.org/68DF4AF7-FA6A-48A4-9305-CC8D6D4EECF7>

Figs 12A–C; 13A–C; 14A–E

Diagnosis. *Eadya spitzer* sp. n. can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged at ventral margin, with two medial tubercles projecting outward (Fig. 14A); frons with inter-antennal and lateral carina flanged (Fig. 14B); occipital carina simple (Fig. 14B); occiput simple; notaulus impressed towards anterior margin of mesoscutum, foveate at apex (Fig. 14C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 14C); sternaulus crenulate (Fig. 14D); propodeum rounded in appearance from lateral angle (Fig. 13A), without transverse carinae (Fig. 14E), and not creating a distinct posterior face when viewed laterally; propodeal spiracle circular; head orange except for antenna, apex of mandible, and ocellar triangle black, median of clypeus brown (Figs 14A, B); prothorax orange (Figs 12A, 13A, 14B); hindwing infusate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 13C); legs black except for fore coxa and trochanter orange (Fig. 13A); amino acid sequence (112–118) IRNFIGM (Fig. 15).

Description. Female. Body length without abdomen 3.30mm. Abdomen 2.86mm. Ovipositor 1.17mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black (Figs 12A, B; 13A, B; 14A, B), median of clypeus brown; prothorax orange (Figs 12A, B; 13A, B; 14A, B, C); mesoscutum orange (Figs 12A, B; 13A, B; 14A, B); mesopleuron black except for anterior dorsal margin orange (Figs 13A; 14D); metathorax black (Figs 12B; 13A, B; 14D, E); forewing infusate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 13C); hindwing infusate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 13C); legs black except for fore coxa and trochanter orange (Figs 12A; 13A, B); abdomen black except for ovipositor orange (Figs 12C; 13A).

Head. Clypeus simple, smooth with scattered setae, flanged as ventral margin, with two medial tubercles projecting outward (Fig. 14A); mandibles overlapping, dorsal tooth longer than ventral (Fig. 14A); face finely punctate with associated setae (Fig. 14A); frons rugose, inter-antennal and lateral carina flanged, starting at the toruli and reaching the ocellar triangle (Fig. 14A, B); vertex smooth with scattered setae (Fig. 14B); occipital carina simple (Fig. 14B), reaching the hypostomal carina; hypostomal carina strongly flanged, reaching the mandible and bending around to the mandibular condyle; occiput smooth, normal (Fig. 14B).

Mesosoma. Pronotum exposed in dorsal view (Fig. 14B, C); pronope absent, subpronope absent, smooth except for a faint crenulate line extending laterally and rugulose sculpturing along the lateral posterior margin (Fig. 14B); mesoscutum with median mesonotal lobe smooth (Fig. 14C); notaulus impressed towards anterior margin of mesoscutum, foveate at apex (Fig. 14C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 14C); sternaulus crenulate (Fig. 14D); propo-

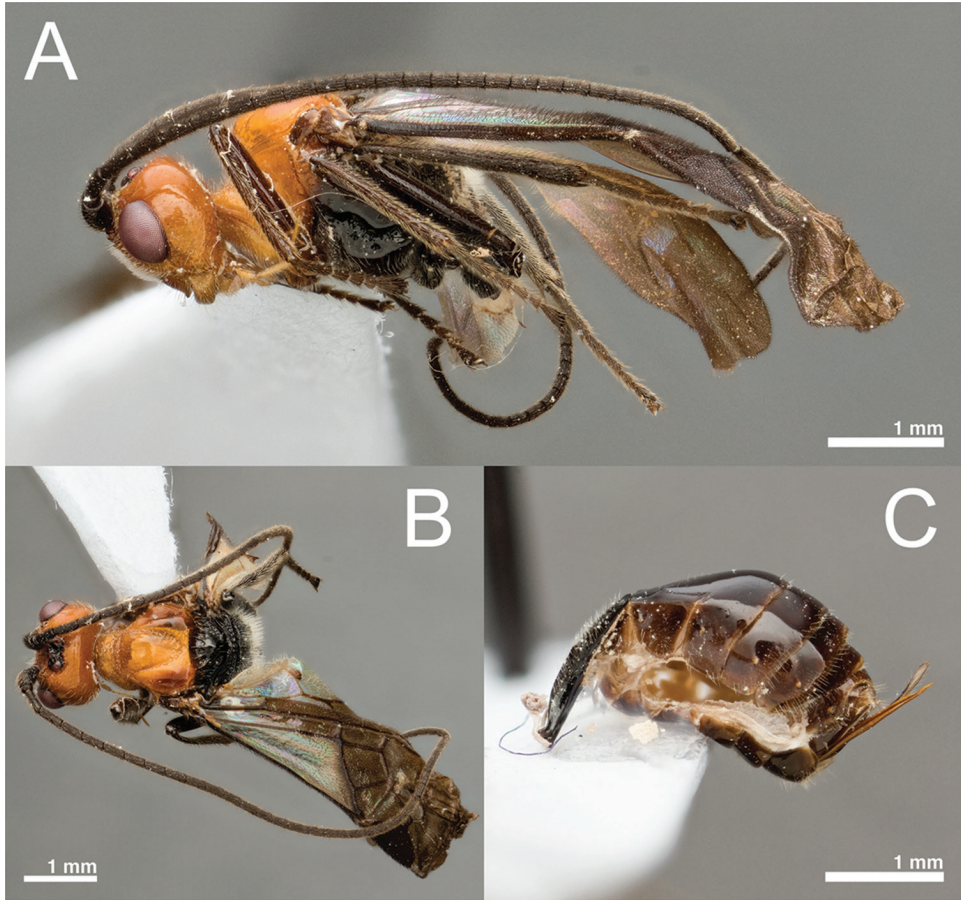


Figure 12. *Eadya spitzer* Ridenbaugh, sp. n. holotype. **A** Lateral habitus **B** Dorsal habitus **C** Metasoma, lateral view. All scale bars are 1 mm in length.

deum rugose and pubescent, rounded in appearance from lateral angle, without transverse carinae and not creating a distinct posterior face when viewed laterally (Figs 13A; 14E); propodeal spiracle circular; coxa, trochanter, trochantellus, and femur covered in setae; tibia and tarsus pubescent; tarsal claws simple (Figs 12A; 13A, B).

Forewing. r-m curved slightly towards stigma before reaching the junction of 3RSa and 3RSb (Fig. 13C).

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae (Fig. 14E); ovipositor straight (Figs 12C; 13A).

Male. Unknown.

Host. *Paropsis charybdis*, *Paropsis aegrota elliotti* Selman, 1983.

Variations. Paratype with clypeus orange (Fig. 14A). This variation may be the result of the DNA extraction process of the Holotype.

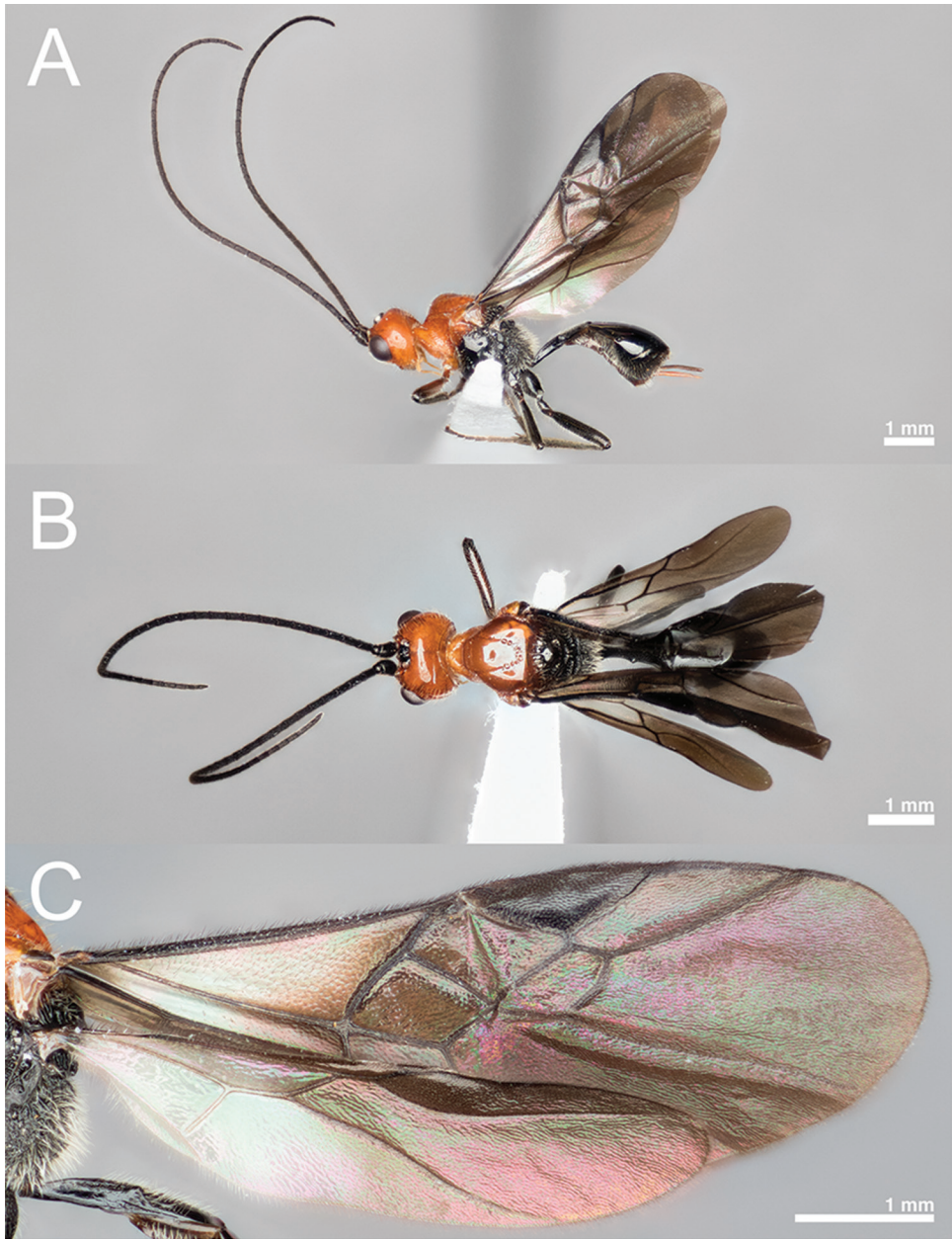


Figure 13. *Eadya spitzer* Ridenbaugh, sp. n. paratype. **A** Lateral habitus **B** Dorsal habitus **C** Metasoma, lateral view. All scale bars are 1mm in length.

Diagnostic molecular characters. (22–27) IWSGII; (32–34) SVL; (41–46) [M or K]LGRLL; (54) S; (67–73) IVIPIII; (81) I; (90) MM; (95–98) INNI; (104–109) PPSLIL; (112–118) IRNFIGM; (126) M; (133–139) NLRHRGI; (143–144) MS; (150) L; (157) I; (167–169) INI; (172–191) LGLNYDNISLLVWSVNITAI (Fig. 15).

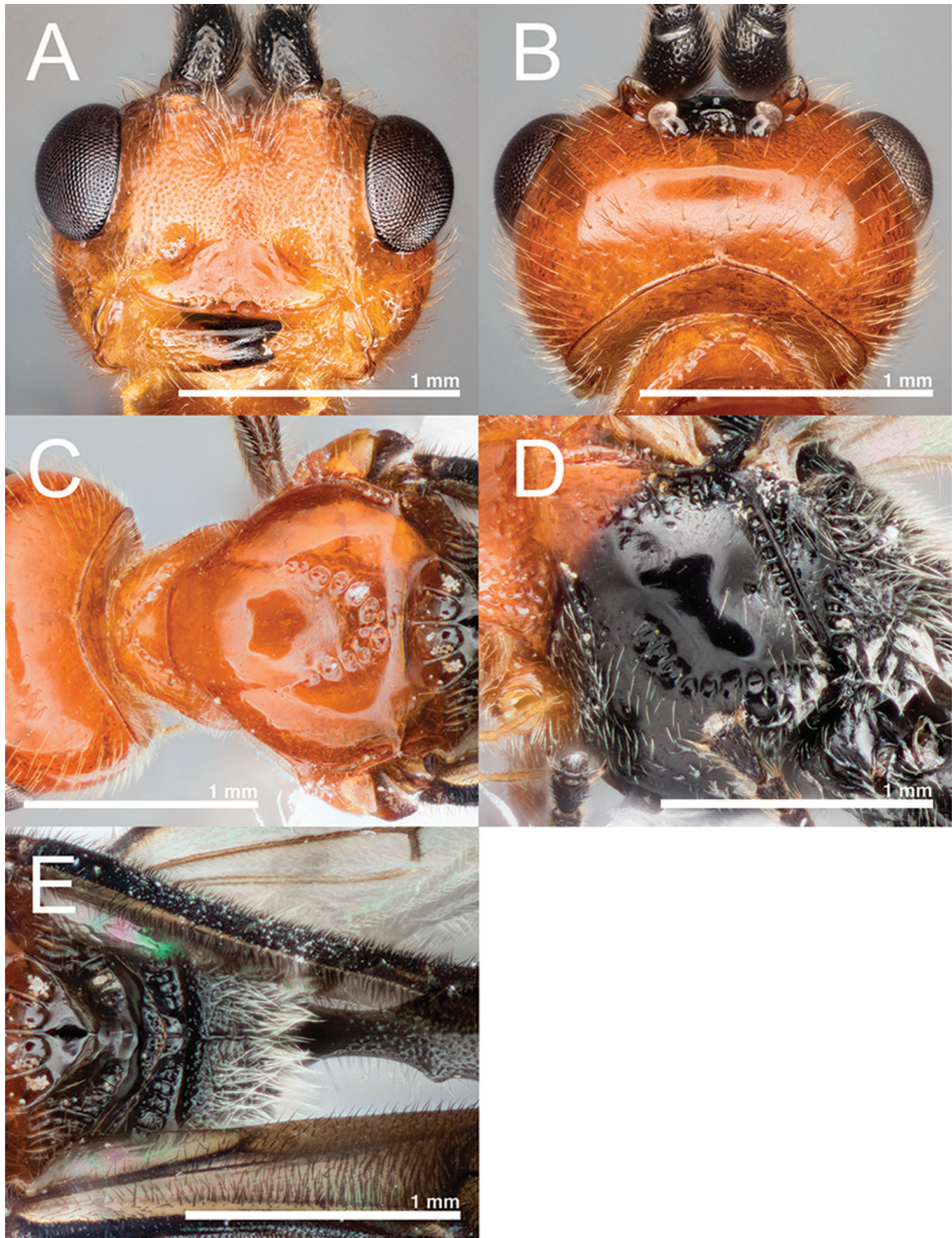


Figure 14. *Eadya spitzer* Ridenbaugh, sp. n. paratype. **A** Head, frontal view **B** Head, dorsal view **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view.

Distribution. Tasmania.

Etymology. This species is named in honor of Edwin Spitzer, the first author's (RDR) late grandfather. This is a noun in apposition to the generic name in order to retain integrity of the surname Spitzer.

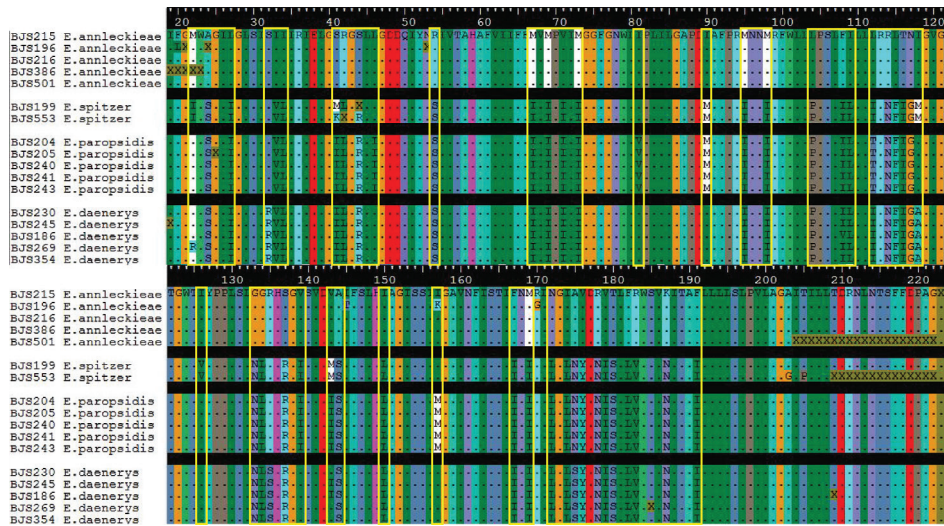


Figure 15. Cytochrome c oxidase subunit 1 amino acid sequences from Peixoto et al. (2018). Boxes indicate diagnostic molecular characters. For each sequence a unique corresponding DNA voucher code is listed as BJS followed by a number.

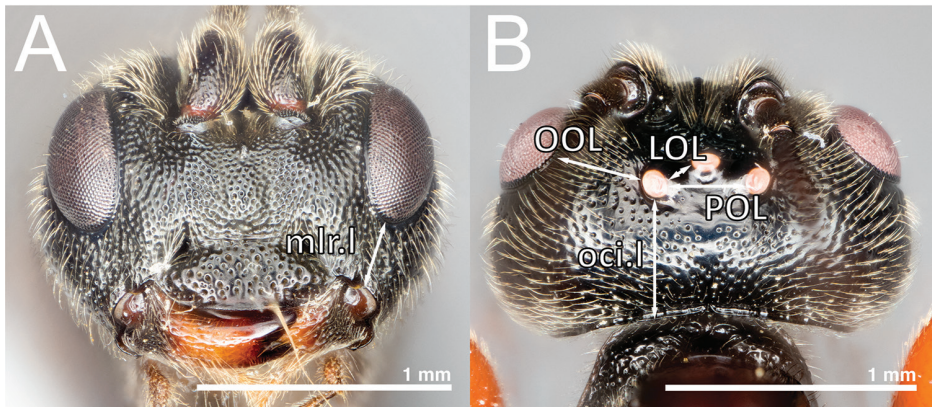


Figure 16. Characters used in the morphometric analysis. **A** Frontal view of the head illustrating the morphometric character malar space (mlr.l), *Eadya annleckieae* Ridenbaugh, sp. n. paratype **B** Dorsal view of the head illustrating the morphometric characters lateral ocellar line (LOL), ocular ocellar line (OOL), posterior ocellar line (POL), and occipital ocellar line (oci.l), *Eadya annleckieae* Ridenbaugh, sp. n. paratype. All scale bars are 1mm in length.

Remarks. The paratype is for this series is badly damaged, missing both antennae, all six legs, and the abdomen excluding metasomal tergite 1. However, the specimen was photographed before destruction and can be seen in Figures 13A–C and 14A–E. This species is referred to as *Eadya* sp.2 in Peixoto et al. (2018).

Type material. Holotype, Female (ANIC), “The Lea, TAS, 11 Dec 2012, Emerged 26 Dec 2012, G.R. Allen, Field collected in *P. charybdis*, E135”, “BJS 199”, GenBank

accession numbers KX989902, and MH107810. Paratype, Female (ANIC), “Runnymede Site #1, TAS, 13 Dec 2015, 42°38'11.1"S, 147°33'54.7"E, Flying adult, D. Satchell, Female”.

Key to the species of *Eadya*

- 1 Propodeum with transverse carinae (See arrows, Fig. 11F) creating a distinct posterior face when viewed from the lateral angle (Fig. 10A)..... **2**
- Propodeum without transverse carinae (Fig. 5F), rounded in appearance when viewed from the lateral angle (Fig. 4A) **3**
- 2 Occipital carinae simple (See arrow, Fig. 5B); propodeal spiracles elliptical; mesothorax orange (Fig. 7C, D) ***E. duncan* Ridenbaugh, sp. n.**
- Occipital carinae emarginate (See arrow, Fig. 11B); propodeal spiracles circular; mesothorax black (Fig. 11C, D)..... ***E. paropsidis* Huddleston & Short, 1978**
- 3 Notaulus impressed towards anterior margin of mesoscutum, crenulate at apex (Fig. 9C); propodeal spiracles elliptical; hindwing infusate except for anal, basal, discal, and subbasal cells hyaline (Fig. 8C); ovipositor downcurved (Fig. 8A); Distribution: Western Australia ... ***E. falcata* Huddleston & Short, 1978**
- Notaulus rugulose (Fig. 3C), crenulate (Fig. 5C), or impressed towards anterior margin of mesoscutum and foveate at apex (Fig. 14A); propodeal spiracles circular; hindwing either completely hyaline (Fig. 2C) or infusate except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 13C); ovipositor straight (Fig. 13A); Distribution: Australian Capital Territory, New South Wales, Tasmania **4**
- 4 Head black (Fig. 3A, B); sternaulus rugulose (Fig. 3D); scutellar sulcus divided into two distinct foveae with rugulose sculpturing along the posterior margins (Fig. 3C)..... ***E. annleckieae* Ridenbaugh, sp. n.**
- Head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 14A, B); sternaulus crenulate (Fig. 14D); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 14C)..... **5**
- 5 Pronotum orange (Fig. 12A); mesoscutum orange (Fig. 14C); legs black except for fore coxa and trochanter orange; notaulus impressed towards anterior margins of mesoscutum, foveate at apex (Fig. 14C) ***E. spitzer* Ridenbaugh, sp. n.**
- Pronotum black except for anterior dorsal margin orange (Figs 4A, 5B) mesoscutum black (Fig. 5C); legs black; notaulus crenulate (Fig. 5C) ***E. daenerys* Ridenbaugh, sp. n.**

Discussion

With the description of the four new species described here, the distribution of *Eadya* has expanded to include Tasmania, the Australian Capital Territory, New South Wales,

Victoria, and Western Australia. As Peixoto et al.'s (2018) study was limited to Tasmania, much is still unknown about mainland populations of *Eadya*. Of the six species of *Eadya* now known, two (*E. annleckieae* sp. n. and *E. spitzer* sp. n.) are known solely from Tasmania. This may not be an accurate distribution given our limited knowledge of mainland *Eadya* and because both *E. paropsidis* and *E. daenerys* sp. n. have been recorded from both Tasmania and mainland Australia.

Interestingly, knowledge on *Eadya* distribution has grown from a citizen science observation. Citizen science initiatives are a valuable, yet underutilized, resource for biodiversity research which can survey large geographical areas over extended periods of time (Silvertown 2009; Theobald et al. 2015). In November of 2012, a series of photos taken in Melbourne depicting a wasp stinging beetle larvae and labeled “? *Eadya paropsidis*” was uploaded to ProjectNoah.org (Ridgway 2012). The photos were tagged with the following description:

“A small (7mm) wasp with an orange head, thorax and first pair of legs. The rest of the wasp was black. The larvae being parasitized were those of the eucalyptus leaf beetle (*Paropsis atomaria*), probably the 2nd instar”.

Although the image quality and detail was not sufficient to positively identify the beetle larvae, the images of the wasp coupled with the contributor's description matches that of *E. duncan* sp. n., and represents a new distribution record. With this observation, the distribution of *E. duncan* sp. n. is expanded to include Victoria, AUS in addition to New South Wales, AUS. Thus, citizen science observations can be invaluable for expanding knowledge on species and provides additional collecting localities for future research into this relatively unknown species.

Host records for *Eadya* outside of Tasmania are incomplete as well, with only *E. paropsidis* recorded from *Paropsis atomaria* (synonym *P. reticulata*) in the Australian Capital Territory and New South Wales (Huddleston and Short 1978). Again this may not represent the entire complement of possible hosts for *E. paropsidis* given the plastic nature of host usage in *Eadya* (Peixoto et al. 2018). Thus, there may be more host associations to be discovered with focused sampling and careful rearing. *Eadya daenerys* sp. n. from Tasmania has been considered as a potential biocontrol agent for *Paropsis charybdis* in New Zealand (Withers et al. 2012), and continues to be a promising candidate (Peixoto et al. 2018). With two mainland species of *Paropsisterna* (*Pst. m-fuscum* and *Pst. variicollis**) recently introduced as pests outside of Australia (von Ellenreider 2003; Paine et al. 2011; Clemson University Extension 2012; Rogan 2016), establishing accurate host records for *Eadya* could prove beneficial for future biocontrol efforts.

Much is still unknown about the species of *Eadya*, but as the popularity of *Eucalyptus* grows internationally as an ornamental landscape and forestry product (Paine et al. 2011), and with it the number of invasive pests, future biocontrol programs may look to *Eadya* for classical biological control. Although Peixoto et al. (2018) has added much to our understanding, further research into the biology of *Eadya* is required, with a particular focus on the host associations and distributions of mainland Australian populations. The sooner this research can be completed the more likely rapid

measures can be taken to control additional incursions of paropsine beetles in new countries and regions.

Finally, it is prudent to discuss the subfamily placement of *Eadya*. In the original description, Huddleston and Short (1978) placed *Eadya* within Euphorinae, but without much justification. Shaw (1985) in his analysis of Euphorinae relationships, agreed that *Eadya* belonged within Euphorinae, likely as a basal member because *Eadya* has a complete second submarginal cell (r-m cross vein present) and a long ovipositor, similar to *Meteorus* (a long suspected basal taxon of Euphorinae (Stigenberg et al. 2015)). In a subsequent molecular phylogenetic analysis, based on 28S (D2-D3) rDNA, Belshaw and Quicke (2002) recovered *Eadya* within the Helconoid complex, sister to species of Diospilini (Brachistinae - following Sharanowski et al. 2011). They erected the tribe Eadyini within Helconinae to accommodate this aberrant taxon. The presence of an inter-antennal carina is shared among *Eadya* as well as several members of Helconinae (*sensu stricto* - following Sharanowski et al. 2011) providing some morphological evidence for this placement. However, *Eadya* attacks exposed leaf-feeding beetle larvae, not concealed xylophagous beetle larvae as do species of Helconinae *s.s.* Further, the morphological characters of *Eadya* are far more consistent with placement in Euphorinae (Shaw 1985; 1997) than Helconinae, and include: forewing vein 2cu-a absent; forewing vein 3RS curved, reaching the costa and therefore creating a small marginal cell; and a petiolate metasoma. Further, *Eadya* COI sequences share the greatest similarity to other Euphorines based on BLAST searches (Peixoto et al. 2018). Thus, the presence of an inter-antennal carina is likely convergent with members of Helconinae. We suggest that *Eadya* is indeed a member of Euphorinae, and forthcoming molecular phylogenetic analyses (Stigenburg, unpublished data; Sharanowski, unpublished data) will formally test that assertion.

Conclusions

Three new species from the genus *Eadya* are described (*Eadya annleekieae* Ridenbaugh, sp. n., *Eadya daenerys* Ridenbaugh, sp. n., *Eadya spitzer* Ridenbaugh, sp. n.) based upon the results of Peixoto et al. (2018), along with a fourth new species discovered in the Australian National Insect Collection (*Eadya duncan* Ridenbaugh, sp. n.). In addition to these descriptions, the distribution of *Eadya* is expanded from the Australian Capital Territory, New South Wales, and Western Australia, to include Tasmania and Victoria. Host records for all newly described species are listed along with two new host records for *Eadya paropsidis* (*Paropsis tasmanica* Baly, 1866, and *Paropsis charybdis* Stål, 1860). Finally, based upon several morphological characters (forewing vein 2cu-a absent; forewing vein 3RS curved, reaching the costa and therefore creating a small marginal cell; and a petiolate metasoma) and COI sequences presented in Peixoto et al. (2018), we suggest the placement of *Eadya* within the subfamily Euphorinae.

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References

- Baur H, Kranz-Baltensperger Y, Cruaud A, Rasplus JY, Timokhov AV, Gokhman VE (2014) Morphometric analysis and taxonomic revision of Anisopteromalus Ruschka (Hymenoptera: Chalcidoidea: Pteromalidae)—an integrative approach. *Systematic Entomology* 39: 691–709. <https://doi.org/10.1111/syen.12081>
- Baur H, Leuenberger C (2011) Analysis of ratios in multivariate morphometry. *Systematic Biology* 60: 813–825. <https://doi.org/10.1093/sysbio/syr061>
- Belshaw R, Quicke DL (2002) Robustness of ancestral state estimates: evolution of life history strategy in ichneumonoid parasitoids. *Systematic Biology* 51: 450–477. <https://doi.org/10.1080/10635150290069896>
- Coppen JJW (2003) *Eucalyptus*: the genus *Eucalyptus*. CRC Press.
- de Little DW (1989) Paropsine chrysomelid attack on plantations of *Eucalyptus nitens* in Tasmania. *New Zealand Journal of Forestry Science* 19: 223–227.
- Clemson University Extension (2012) *Eucalyptus Leaf Beetle*. <https://www.clemson.edu/public/regulatory/plant-protection/plant-pest-regulations/state-plant-pest-information/pest-alerts/elb.html> [accessed 17 Aug 2017]
- Gonzalez R, Treasure T, Wright J, Saloni D, Phillips R, Abt R, Jameel H (2011) Exploring the potential of *Eucalyptus* for energy production in the Southern United States: Financial analysis of delivered biomass. Part I. *Biomass and Bioenergy* 35: 755–766. <https://doi.org/10.1016/j.biombioe.2010.10.011>
- Hall T (1999) BioEdit v 7.2.6.1. <http://www.mbio.ncsu.edu/BioEdit/bioedit>
- Harris RA (1979) Glossary of surface sculpturing. *Occasional Papers in Entomology* 28: 31.
- Hebert PDN, Cywinska A, Ball SL (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B: Biological Sciences* 270: 313–321. <https://doi.org/10.1098/rspb.2002.2218>
- Huddleston T, Short JRT (1978) A new genus of Euphorinae (Hymenoptera: Braconidae) from Australia, with a description of the final instar larva of one species. *Austral Entomology* 17: 317–321. <https://doi.org/10.1111/j.1440-6055.1978.tb01497>

- László Z, Baur H, Tóthmérész B (2013) Multivariate ratio analysis reveals *Trigonoderus pedicellaris* Thomson (Hymenoptera, Chalcidoidea, Pteromalidae) as a valid species. *Systematic Entomology* 38: 753–762. <https://doi.org/10.1111/syen.12026>
- Lin H, Murray TJ, Mason EG (2017) Incidence of and defoliation by a newly introduced pest, *Paropsisterna variicollis* (Coleoptera: Chrysomelidae), on eleven durable *Eucalyptus* species in Hawke's Bay, New Zealand. *New Zealand Plant Protection* 70: 45–51. <https://doi.org/10.30843/nzpp.2017.70.26>
- Millar JG, Paine TD, Bethke JA, Garrison RW, Campbell KA, Dreistadt SH (2009) *Eucalyptus Tortoise Beetles*. Pest Note UC ANR Publication 74104.
- Nahrung HF (2004) Biology of *Chrysophtharta agricola* (Coleoptera, Chrysomelidae), a pest of *Eucalyptus* plantations in south-eastern Australia. *Australian Forestry* 67: 59–66. <https://doi.org/10.1080/00049158.2004.10676207>
- Paine TD, Steinbauer MJ, Lawson SA (2011) Native and exotic pests of eucalyptus: a worldwide perspective. *Annu Rev Entomol* 56: 181–201. <https://doi.org/10.1146/annurev-ento-120709-144817>
- Peixoto L, Allen GR, Ridenbaugh RD, Quarrell SR, Withers TM, Sharanowski BJ (2018) When taxonomy and biological control researchers unite: species delimitation of *Eadya* parasitoids (Braconidae) and consequences for classical biological control of invasive paropsine pests of *Eucalyptus*. *PLoS One*, Accepted.
- Reid CAM (2006) A taxonomic revision of the Australian Chrysomelinae, with a key to the genera (Coleoptera: Chrysomelidae). *Zootaxa* 1292: 1–119.
- Reid CAM, de Little DW (2013) A new species of *Paropsisterna* Motschulsky, 1860, a significant pest of plantation eucalypts in Tasmania and Ireland (Coleoptera: Chrysomelidae: Chrysomelinae). *Zootaxa* 3681: 395–404. <https://doi.org/10.11646/zootaxa.3681.4.4>
- Ridgway L (2012) Leafbeetle larvae and parasitoid wasp. <http://www.projectnoah.org/spot-rings/16368138> [accessed 16 Aug 2017]
- Rogan BJ (2016) Biosecurity inspector reports *Paropsisterna variicollis* in Hawke's Bay. *Forest Health News*. Scion, Rotorua, New Zealand.
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature methods* 9: 671–675. <https://doi.org/10.1038/nmeth.2089>
- Sharanowski BJ, Dowling AP, Sharkey MJ (2011) Molecular phylogenetics of Braconidae (Hymenoptera: Ichneumonoidea), based on multiple nuclear genes, and implications for classification. *Systematic Entomology* 36: 549–572. <https://doi.org/10.1111/j.1365-3113.2011.00580>
- Sharkey MJ, Wharton RA (1997) Morphology and Terminology. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) *Manual of the New World genera of the family Braconidae*. The International Society of Hymenopterists, 19–38.
- Shaw SR (1985) A phylogenetic study of the subfamilies Meteorinae and Euphorinae (Hymenoptera: Braconidae). University of Maryland.
- Shaw SR (1997) Subfamily Euphorinae. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) *Manual of the New World Genera of the Family Braconidae*. The International Society of Hymenopterists, Washington, D.C., 235–254.
- Silvertown J (2009) A new dawn for citizen science. *Trends in ecology & evolution* 24: 467–471. <https://doi.org/10.1016/j.tree.2009.03.017>

- Stigenberg J, Boring CA, Ronquist F (2015) Phylogeny of the parasitic wasp subfamily Euphorinae (Braconidae) and evolution of its host preferences. *Systematic Entomology* 40: 570–591. <https://doi.org/10.1111/syen.12122>
- R Core Team (2016) R: A language and environment for statistical computing. Vienna, Austria. <http://www.R-project.org>
- Theobald EJ, Ettinger AK, Burgess HK, DeBey LB, Schmidt NR, Froehlich HE, Wagner C, HillerRisLambers J, Tewksbury J, Harsch M (2015) Global change and local solutions: Tapping the unrealized potential of citizen science for biodiversity research. *Biological Conservation* 181: 236–244. <https://doi.org/10.1016/j.biocon.2014.10.021>
- von Ellenrieder N (2003) Eucalyptus leaf beetle (*Chrysophtharta m-fuscum*). Calif Dept Food Agric Pest Sheets CDFA, Sacramento, CA.
- Withers TM, Watson M, Watt M, Nelson T, Harper L, Hurst M (2013) Laboratory bioassays of new synthetic and microbial insecticides to control Eucalyptus tortoise beetle *Paropsis charybdis*. *New Zealand Plant Protection* 66: 138–147.
- Withers TM, Allen GR, Patel VS, Satchell D, Manley G (2012) Investigating the potential of *Eadya paropsidis* (Braconidae) from Tasmania as a biocontrol agent for *Paropsis charybdis* in New Zealand. *New Zealand Plant Protection* 65.
- Yoder MJ, Miko I, Seltmann KC, Bertone MA, Deans AR (2010) A gross anatomy ontology for Hymenoptera. *PLOS One* 5: e15991. <https://doi.org/10.1371/journal.pone.0015991>
- Zerene Systems LLC (2016) Zerene Stacker v 1.04. <http://zerenesystems.com/cms>
- Zhang MY, Ridenbaugh RD, Sharanowski BJ (2017) Integrative taxonomy improves understanding of native beneficial fauna: revision of the Nearctic *Peristenus pallipes* complex (Hymenoptera: Braconidae) and implications for release of exotic biocontrol agents. *Systematic Entomology* 42: 596–608. <https://doi.org/10.1111/syen.12233>

Supplementary material I

Table S1

Authors: Ryan D. Ridenbaugh, Erin Barbeau, Barbara J. Sharanowski

Data type: species data

Explanation note: List of all materials examined along with collecting localities, its type designation and location of deposition, and associated DNA voucher number or unique identifier. *Eadya annleckieae* Ridenbaugh, sp. n. is referred to as *Eadya* sp.1, *Eadya spitzer* Ridenbaugh, sp. n. is referred to as *Eadya* sp.2, and *Eadya daenerys* Ridenbaugh, sp. n. is referred to as *Eadya* sp.3.

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