

First confirmed parasitism of pleasing fungus beetles (Coleoptera, Erotylidae) by a tropical rhyssine ichneumonid, and first record for *Cyrtorhyssa moellerii* Bingham (Hymenoptera, Ichneumonidae) from Thailand

Kittipum Chansri^{1,2}, Kanoktip Somsiri³, Donald L. J. Quicke^{2*}, Buntika A. Butcher^{2*}

1 Program in Zoology, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand **2** Integrative Insect Ecology Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand **3** Sakaerat Environmental Research Station, Nakhon Ratchasima 30370, Thailand

Corresponding author: Buntika A. Butcher (buntika.a@chula.ac.th)

Academic editor: T. Spasojevic | Received 30 May 2023 | Accepted 1 October 2023 | Published 11 October 2023

<https://zoobank.org/F47B0A1E-DB24-46D2-85A9-C6663A053622>

Citation: Chansri K, Somsiri K, Quicke DLJ, Butcher BA (2023) First confirmed parasitism of pleasing fungus beetles (Coleoptera, Erotylidae) by a tropical rhyssine ichneumonid, and first record for *Cyrtorhyssa moellerii* Bingham (Hymenoptera, Ichneumonidae) from Thailand. Journal of Hymenoptera Research 96: 783–804. <https://doi.org/10.3897/jhr.96.107196>

Abstract

The first record of the Darwin wasp, *Cyrtorhyssa moellerii* Bingham, 1898 (Hymenoptera, Ichneumonoidea, Rhyssinae) from Thailand is presented. Members of both sexes are fully described and illustrated. The biology of *C. moellerii*, a parasitoid of the pleasing fungus beetle *Encaustes opaca* Crotch, 1876 (Coleoptera, Erotylidae), is reported for the first time. Hosts were associated with standing deadwood of *Anthoshorea henryana* (Pierre ex Laness.) P. S. Ashton & J. Heck (Dipterocarpaceae) in dry evergreen forest, Nakhon Ratchasima province, northeastern Thailand. DNA barcodes (cytochrome c oxidase subunit 1 sequence (COI)) were generated for both host and parasitoid and phylogenetic trees constructed for these and other members of the same family and subfamily respectively. A key is provided to separate the three known species of *Cyrtorhyssa*. This is the first confirmed host record for a tropical species of Rhyssinae as well as the first from Erotylidae.

Keywords

Coleoptera, dead wood, *Encaustes*, host record, Rhyssinae

* These authors contributed equally to this work.

Introduction

Rhyssine Darwin wasps (Ichneumonidae) have mostly been recorded as parasitoids of various woodwasps belonging to the families Siricidae, Xiphydriidae and the monotypic Anaxyelidae from northwest America (Hanson 1939; Couturier 1949; Quicke 2015). In India and Pakistan, *Rhyssa persuasoria himalayensis* Wilkinson, 1927 is reported to attack many species of Siricidae (Kamath and Gupta 1972). In Costa Rica, *Epirhyssa mexicana* Cresson, 1874 have been recorded probing dead wood with cerambycid larvae inside (Gauld 1991), and Porter (1978) also suggested that Neotropical *Epirhyssa* Cresson, 1865 species were parasitoids of xylophagous beetles but without any definitive records. In China although most hosts records involve siricid woodwasps (Sheng and Sun 2010), there are also several reports of long horn beetles (Cerambycidae) acting as hosts. *Moechotypa diphysis* (Pascoe, 1871) is reported as being attacked by *E. lurida* Sheng & Sun, 2010, *Rhyssella approximata* (Fabricius, 1793) and *Triancyra galloisi* (Uchida, 1928); *Anoplophora glabripennis* (Motschulsky, 1854) and *Cerambyx cerdo* Linnaeus, 1758 are reported to be hosts of *Megarhyssa praecegens* (Tosquinet, 1889), *M. jezoensis* (Matsumura, 1912) and *R. approximata* (Sheng and Sun 2010). *Massicus raddei* (Blessig & Solsky, 1872) has been recorded as the host of *M. praecegens* (Cao et al. 2020).

Cyrtorhyssa Baltazar, 1961 is an endemic Asian genus known only from the Indo-Chinese region. It comprises three species: *C. moellerii* Bingham, 1898 from India (Sikkim) and Myanmar (Tenasserim), *C. mesopyrrha* Mocsary, 1905 from Indonesia (Sumatra, Java, Kalimantan), Malaysia (Sarawak) and the Philippines (Kamath and Gupta 1972) and *C. xishuangensis* Wang, 1982 from China (Wang 1982). Up until now, no host records have been reported for any members of the genus.

Only one species of rhyssine wasp has previously been recorded from Thailand, viz *Myllenyx kuchingensis* Kamath & Gupta, 1972 (Kamath and Gupta 1972). The record is more than 50 years old and there is no associated biological information. Thus, this discovery of *C. moellerii* in 2021 is the second officially reported rhyssine wasp from Thailand and the last published information on *C. moellerii* dates from over a century ago. Here we establish the first host record of *C. moellerii* which was reared from a particularly large-bodied, wood-boring erotylid beetle (*E. opaca*). Moreover, this is also the first confirmed case of an erotylid as a host for rhyssine ichneumonids.

Methods

Observation were made in the dry evergreen forest at Sakaerat Environmental Research Station, Nakhon Ratchasima Province, northeastern, Thailand (Fig. 1A). The parasitoid wasps and their host were collected from a standing dead *Anthoshorea henryana* (Pierre ex Laness.) P. S. Ashton & J. Heck (Dipterocarpaceae) tree (Fig. 1B) during January and February 2021. Adults male and female *C. moellerii* (Fig. 2A, B) were collected on and around the tree, approximately 0.5–2.0 m above the ground (Fig. 1C, D), and preserved in 95% ethanol. Hosts and their remains were dissected from



Figure 1. Dry evergreen forest, Sakaerat Environmental Research Station, Thailand (**A**), dead wood of *A. henryana* (**B**), teneral stage of *Encaustes opaca* inside the deadwood (**C**), pupa of *C. moellerii* inside the deadwood (**D**).

the wood using a hammer and chisel. Living immature beetles and wasps were also collected and reared in a clear plastic container at room temperature. Morphological terminology follows Broad et al. (2018) except for wing venation which follows Sharkey and Wharton (1997); see also fig. 2.2 in Quicke (2015). We provide the alternative nomenclature in parentheses in the relevant places below. Specimens were imaged using a Leica M205C, Leica DMC5400, Digital Camera, and LAS X software.

DNA barcodes (cytochrome c oxidase subunit 1 sequence, COI) were generated from the wasps and the beetle host legs by the Center for Biodiversity Genomics, University of Guelph, using standard methods (Hebert et al. 2003). Host and parasitoid barcodes, as well as sequence from all available relatives, and close outgroups from GenBank database (National Center for Biotechnology Information [NCBI] (2023)) were assembled for phylogenetic analysis. Details of the sequences used in the analyses are presented in Tables 1, 2, respectively, together with specimen provenances and Genbank Accession Number. For the Ichneumonidae analysis, DNA sequences were available for an additional 27 species of Rhyssinae representing seven genera. In addition, four spe-

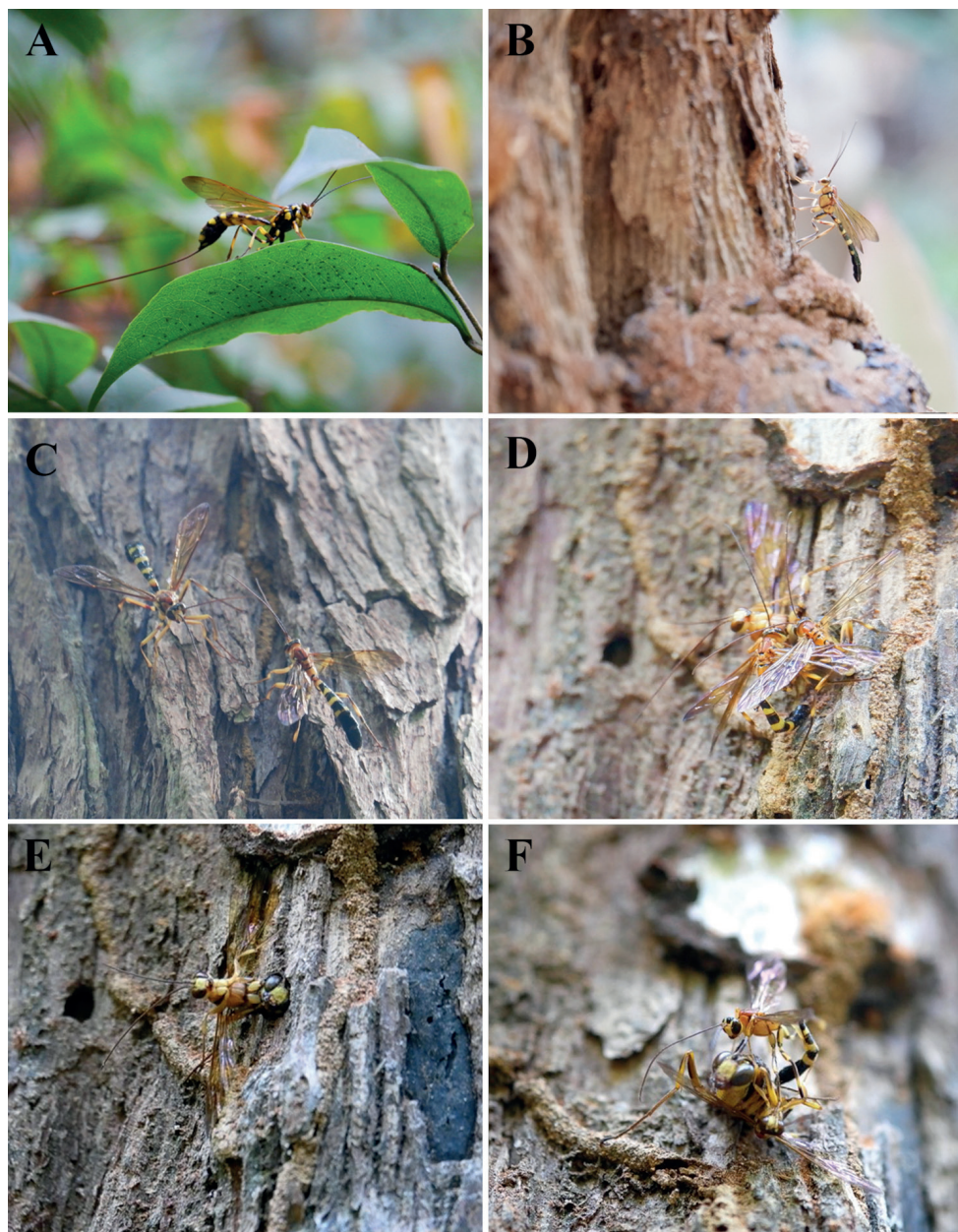


Figure 2. Adult female *C. moellerii* in the natural habitat (A), male of *C. moellerii* during tergal stroke behaviour to marking the location of closely emergence female (B), male aggregation behaviour (C), mating behaviour of female and male *C. moellerii* in natural habitat (D–F).

cies of the closely related subfamily Poemeniinae were included as outgroups (Quicke et al. 2009; Spasojevic et al. 2021). For the analysis of Erotylidae, barcodes from 25 other species were available, representing five subfamilies. Members of two genera of Languriidae were included as outgroups (Bocak et al. 2014). Maximum likelihood phy-

Table 1. List of rhyssine wasp species, including Thai *C. moellerii* and outgroups, with their provenance, GenBank Accession Number, and references.

Subfamily	Species	Provenance	GenBank accession No.	Reference
Poemeniinae	<i>Deuteroxorides elevator</i>	Germany	JF963193	Quicke et al. 2012
	<i>Neoxorides caryae</i>	USA	MK959447	Bennett et al. 2019
	<i>Poemenia albipes</i>	Canada	MG355017	Dewaard 2017 unpublished
	<i>Poemenia hectica</i>	Russia	MZ627402	Roslin et al. 2022
Rhyssinae	<i>Cyrtorhyssa moellerii</i>	Thailand	OQ272136	present study
	<i>Cyrtorhyssa moellerii</i>	Thailand	OQ272137	present study
	<i>Epirhyssa latimandibularis</i>	Thailand	OQ272138	present study
	<i>Epirhyssa corralesi</i>	Costa Rica	OQ272125	
	<i>Epirhyssa curtisi</i>	Costa Rica	OQ272126	
	<i>Epirhyssa frobergi</i>	Costa Rica	OQ272135	
	<i>Epirhyssa mexicana</i>	Costa Rica	OQ272133	
	<i>Epirhyssa oranensis</i>	Costa Rica	OQ272132	
	<i>Epirhyssa porteri</i>	Costa Rica	OQ272131	
	<i>Epirhyssa praecincta</i>	Costa Rica	OQ272134	
	<i>Epirhyssa prolasia</i>	Costa Rica	OQ272129	
	<i>Epirhyssa sapporensis</i>	South Korea	KU753248	Suk and Won 2016 unpublished
	<i>Epirhyssa theloides</i>	Costa Rica	OQ272130	
	<i>Megarhyssa atrata</i>	Canada	OQ272127	
	<i>Megarhyssa greeniei</i>	USA	HM422919	iBOL 2010 unpublished
	<i>Megarhyssa nortoni</i>	USA	KU496775	Sikes et al. 2017
	<i>Megarhyssa n. nortoni</i>	Canada	KR787310	Hebert et al. 2016
	<i>Megarhyssa macrura</i>	Canada	KR929825	Hebert et al. 2016
	<i>Myllenyxis</i> sp.	Malaysia	JF963636	Quicke et al. 2012
	<i>Rhyssa amoena</i>	Germany	JF963813	Quicke et al. 2012
	<i>Rhyssa crevieri</i>	Canada	KR799965	Hebert et al. 2016
	<i>Rhyssa howdenorum</i>	USA	MN556947	Landry and Landry 2019 unpublished
	<i>Rhyssa persuasoria</i>	Norway	OQ272128	
	<i>Rhyssella humida</i>	Canada	KM997713	Eagalle 2014
	<i>Rhyssella nitida</i>	Canada	KM996159	Eagalle 2014
	<i>Rhyssella furanna</i>	Japan	MW056244	Spasojevic et al. 2021 9
	<i>Rhyssella approximata</i>	Finland	MZ625985	Roslin et al. 2022
	<i>Triancyra galloisi</i>	South Korea	KU753388	Suk and Won 2016 unpublished
	<i>Triancyra tricolorata</i>	South Korea	KU753389	Suk and Won 2016 unpublished

logenic analyses were carried out using RAxML version 8.2.12 (Stamatakis 2006) with a GTRGAMMA substitution model and rapid bootstrap option with 100 randomizations (-f a -# 100). The three-codon position were treated as separated data partitions.

Results

Parasitoid behaviour

As in many rhyssine wasps, males *C. moellerii* emerge before females and they can detect where the female will emerge from. Some males were observed performing a

Table 2. List of erotylid beetles, including Thai *E. opaca* and outgroups, with their provenance, GenBank Accession Number and references.

Family	Species	Provenance	GenBank accession No.	Reference
Erotylidae	<i>Dacne bipustulata</i>	Germany	HQ954205	iBOL 2011 unpublished
	<i>Dacne picta</i>	NA	KC510126	Cho et al. 2013 unpublished
	<i>Dacne quadrimaculata</i>	Canada	JN288175	iBOL 2011 unpublished
	<i>Dacne ruffifrons</i>	France	MN182895	Sire et al. 2019
	<i>Dacne</i> sp.	France	MN182938	Sire et al. 2019
	<i>Aulacochilus quadripustulatus</i>	NA	MN603446	Liu 2019 unpublished
	<i>Aulacochilus xingtaiensis</i>	NA	MN615269	Li 2019 unpublished
	<i>Encaustes cruenta ormosana</i>	NA	MN615271	Li 2019 unpublished
	<i>Encaustes opaca</i>	Thailand	OQ272139	present study
	<i>Iphiclus</i> sp.	NA	KC966646	Cline et al. 2014
	<i>Iphiclus sedecimmaculatus</i>	NA	KP134126	McElrath et al. 2014 unpublished
	<i>Episcapha fortunii</i>	Japan	LC619112	Saito et al. 2021 unpublished
	<i>Megalodacne fasciata</i>	Canada	GU013623	Park et al. 2010
	<i>Ischyryus quadripunctatus</i>	United States	HM433801	iBOL 2010 unpublished
	<i>Triplax aenea</i>	Finland	MZ659828	Roslin et al. 2022
	<i>Triplax dissimulator</i>	Canada	KM843753	Hebert et al. 2014 unpublished
	<i>Triplax frosti</i>	Canada	KR487395	Hebert et al. 2016
	<i>Triplax lacordairei</i>	France	MN182940	Sire et al. 2019
	<i>Triplax lepida</i>	France	KM285906	Rougerie 2014 unpublished
	<i>Triplax rufipes</i>	Belgium	HQ954016	iBOL 2011 unpublished
	<i>Triplax russica</i>	Poland	MH115489	Kolasa et al. 2014 unpublished
	<i>Triplax scutellaris</i>	Finland	MZ631796	Roslin et al. 2022
	<i>Triplax thoracica</i>	Canada	KT706260	Telfer et al. 2015
	<i>Tritoma bipustulata</i>	Finland	KJ964373	Pentinsaari et al. 2014
	<i>Tritoma pulchra</i>	Canada	KR489305	Hebert et al. 2016
Languriidae	<i>Acropteroxys gracilis</i>	Canada	MG059564	Dewaard 2017 unpublished
	<i>Languria mozardi mozardi</i>	Canada	MF635178	deWaard et al. 2019

tergal stroking behaviour (which is thought to be involved in marking the location of a conspecific that was nearing emergence). Male aggregation behaviour (Fig. 2C, Suppl. material 1) was observed, with some individuals showing aggressive guarding behaviour of their marked location. Mating behaviour was also video-recorded (Fig. 2D, Suppl. material 2). Even though the metasoma of male is not extremely slender, larger males inserted their metasomas into the chewed tunnel of female before her completing emergence process (Fig. 2E, Suppl. material 3). The smaller males that did not insert their metasomas into the wood still waited nearby and tried to mate with females when they exited the tree (Fig. 2F, Suppl. material 4).

Host-parasitoid interaction

The adult host beetles were identified as the pleasing fungus beetles, *Encaustes opaca* Crotch, 1876 (Coleoptera, Erotylidae) (Fig. 4A–C) (Crotch 1876; Deelder 1942; Chujo 1968b). Of 12 parasitoid wasp cocoons found, seven were empty and had a

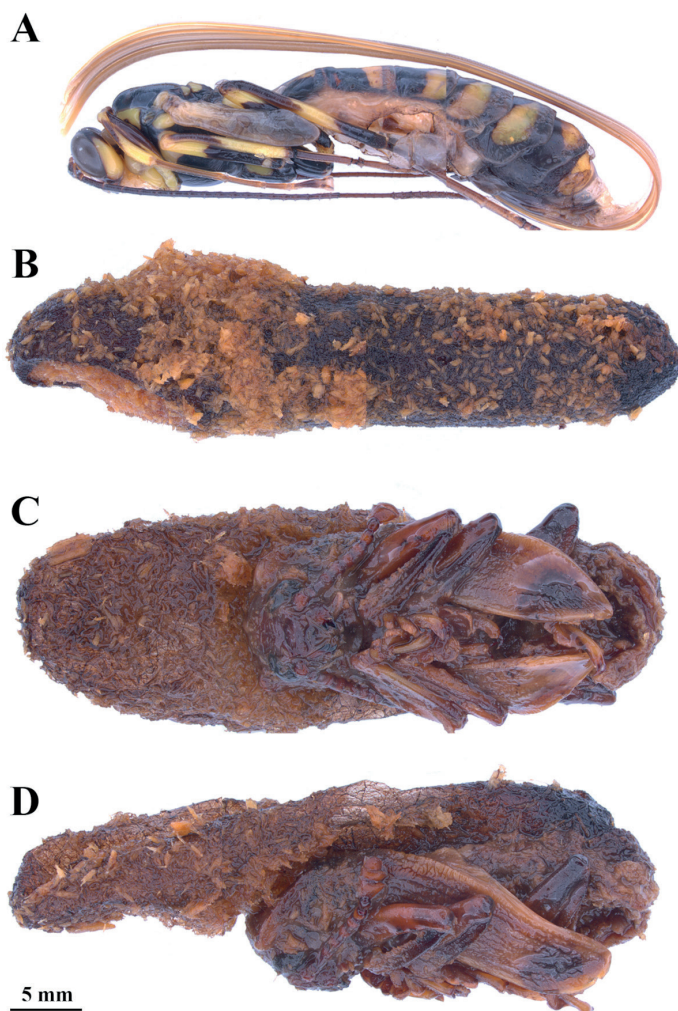


Figure 3. Pupa of female *C. moellerii* (A), empty pupa cocoon of *C. moellerii* with one emergence hole (B), pupa cocoon of *C. moellerii* fused with the teneral stage carcass of *E. opaca*, ventral view (C), lateral view (D).

sub-apical emergence hole (Fig. 3B). The others five cocoons contained living parasitoid pupae. Two of these were opened to allow observation of parasitoid development (Fig. 3A) and three were reared until the wasps (one male and two females) emerged. The captive longevity for the virgin male and two females when fed with 50% honey solution were 14, 16 and 31 days, respectively.

Two *C. moellerii* cocoons were found firmly attached to dried carcasses of a teneral adult of the host beetle (Fig. 3C, D). We concluded that *C. moellerii* is at least facultatively able to develop on teneral adults of *E. opaca*.

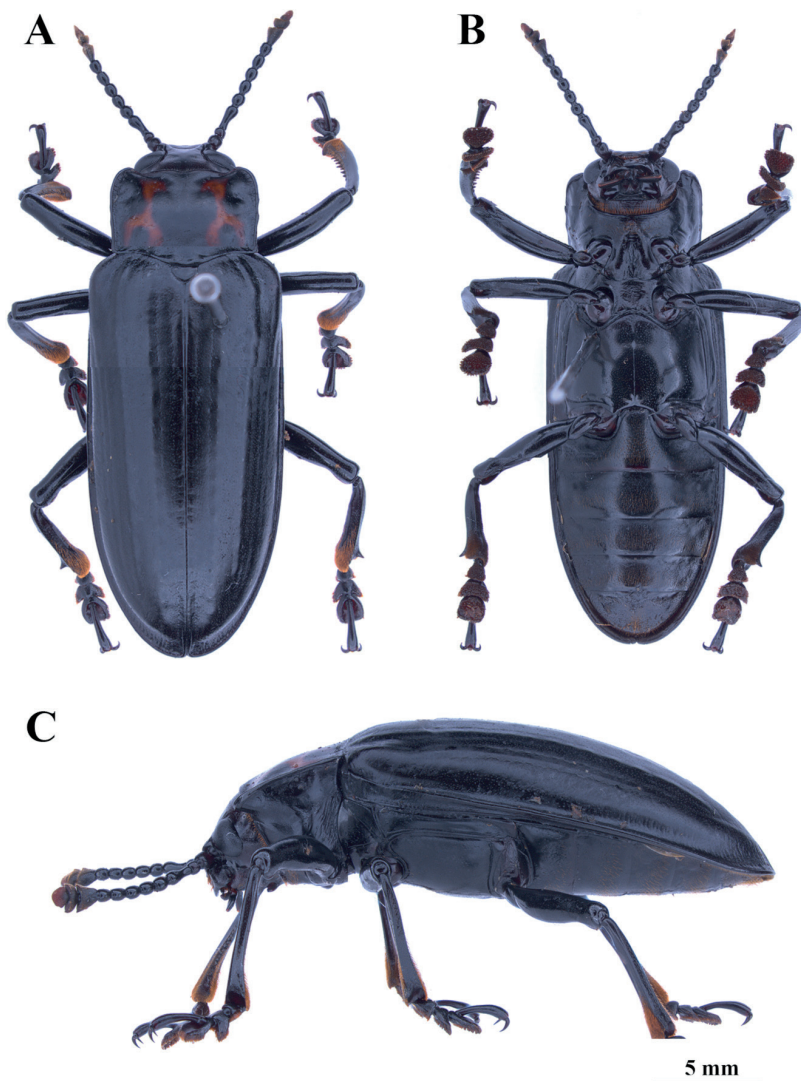


Figure 4. Adult male specimen of *E. opaca* dorsal view(A), ventral view (B), lateral view (C).

Key to species of *Cyrtorhyssa*

- 1 Female2
- Male4
- 2 Face yellow with a black longitudinal line; frons with a long tapered ridge that is depressed in the center; fore wing areolet absent; ovipositor sheaths 1.3× length of the body.....*C. xishuangensis* Wang, 1982
- Face all yellow; frons with a median carina and with a semicircular groove around ocellar triangle or a shallow furrow on either side; fore wing areolet present; ovipositor sheaths less than 1.3× length of the body.....3

- 3 Face strongly, transversely striated on its upper 0.6, lower 0.3 coarsely punctate; frons with a median carina and with a semicircular groove around ocellar triangle; clypeus broadly concave at apex; epicnemial carina weakly curved towards anterior edge, about $0.6\times$ height of mesopleuron; propodeum with median longitudinal shallow groove on basal 0.8; fore wing areolet short triangular; abdominal tergites black with broad apical yellow bands; ovipositor sheaths $1.05\text{--}1.1\times$ length of the body ***C. moellerii* Bingham, 1898**
- Face strongly transversely striated on its upper 0.3; frons with a shallow furrow on either side; clypeus strongly concave; epicnemial carina less than $0.5 (0.3) \times$ height of mesopleuron, weakly sinuate; propodeum median longitudinal shallow groove present or absent (if present weakly impressed on basal $0.3\text{--}0.5$); fore wing areolet widely triangular; first to third abdominal tergites reddish, tergite 2 and 3 without any apical yellow bands; ovipositor sheaths $1\text{--}1.2\times$ length of the body..... ***C. mesopyrrha* Mocsary, 1905**
- 4 Tubercle on metapleuron well developed; fore wing areolet shortly triangular or absent; fifth tergite without any broad transverse apical yellow band ***C. moellerii* Bingham, 1898**
- Tubercle on metapleuron not so well developed and weak; fore wing areolet present or absent; fifth tergite with a broad transverse apical yellow band ***C. mesopyrrha* Mocsary, 1905**

Redescription of *Cyrtorhyssa moellerii* Bingham, 1898 modified from Kamath and Gupta 1972 (adding male details)

Cyrtorhyssa moellerii

Material examined. Five females, twenty males. THAILAND, Nakhon Ratchasima, Wang Nam Khiao district, Udom Sap subdistrict, Sakaerat Environmental Research Station, dry evergreen forest, $14^{\circ}29.8'N$, $101^{\circ}54.96'E$, 496 m, aerial net, col. K. Chansri (CUMZ) (1♀ 27.i.2021, 1♀ 28.i.2021, 2♀ 15.ii.2021, 1♀ 16.ii.2021, 2♂ 21.i.2021, 2♂ 22.i.2021, 2♂ 23.i.2021, 2♂ 24.i.2021, 1♂ 25.i.2021, 1♂ 27.i.2021, 1♂ 29.i.2021, 5♂ 8.ii.2021, 1♂ 15.ii.2021, 3♂ 25.ii.2021).

Diagnosis. *Cyrtorhyssa moellerii* is clearly different from *C. xishuangensis* in which face with black longitudinal line. In addition, frons of *C. xishuangensis* has a tapered ridge rather than distinct carina, and female fore wing has no areolet. *Cyrtorhyssa moellerii* can be separated from *C. mesopyrrha* because the fore wing areolet of *C. moellerii*, when present, is quite short, whereas the fore wing areolet of female *C. mesopyrrha* is wider (Kamath and Gupta, (1972). The ground colour of tergites 1–3 of female *C. moellerii* is black with yellow bands, while in female *C. mesopyrrha* it is reddish without yellow bands.

Description. Female (Figs 5, 6). Body length, mean = 38.0 mm (range = 36.5–39.0 mm); fore wing length, mean = 27.8 (range = 27.0–28.0 mm); ovipositor sheath length, mean = 40.5 mm (range = 40.0–41.0 mm) (Fig. 5A).

Head. Antenna with 40–41 flagellomeres, terminal flagellomere acuminate; face strongly, transversely striated on its upper 0.6, lower 0.3 coarsely punctate, interspaces 0.5 their diameter, towards orbits punctures finer and sparser; clypeus minutely, finely punctate, broadly concave at apex (Fig. 5B); malar space mat, $0.4\times$ basal width of mandible (Fig. 5C); frons smooth, subpolished with a median carina and with a semi-circular groove around ocellar triangle bordered laterally by fine striations; vertex with a few scattered punctures, smooth and polished; interocellar distance 0.5 ocello-ocular distance; occiput without a median groove dorsally (Fig. 5D). Occipital carina absent medio-dorsally.

Mesosoma. Mesoscutum coarsely transversely scutellum rugose, notauli meeting approximately 0.4 distance from anterior of mesoscutum; scutellum strongly, coarsely punctate; median area of metanotum smooth and polished (Fig. 6A); mesopleuron sparsely punctate, punctures on lower 0.3 separated by $2\text{--}3\times$ their diameter, epicnemium with more crowded punctures, interspace $0.5\text{--}1.0$ their diameter; epicnemial carina weakly curved towards anterior edge, about 0.6 the height of mesopleuron; mesosternum coarsely punctate, punctures sometimes coalescent; metapleuron punctate, interspaces $1\text{--}2\times$ their diameter (Fig. 5E); propodeum largely smooth and polished, with very sparse, minute punctures dorsally, dorsolateral corners and lateral sides shallowly punctate, interspace $4\text{--}6\times$ their diameter, broadly depressed at extreme base in middle, and medially with a distinct, shallow groove on basal 0.8 (Fig. 6A, C).

Wing. Areolet of fore wing short triangular, lengths of veins 2RS (=2rs-m): 1M: rs-m (= 3rs-m) = 0.6: 0.8: 1.0; vein 2m-cu joining M interstitial with rs-m (= 3rs-m) (Fig. 6B).

Metasoma. First tergite smooth and shiny $2.1\times$ its apical width; second tergite weakly mat at base, with few scattered punctures; third tergite with basal 0.5 distinctly punctate medially, interspace $0.5\text{--}1\times$ diameter of punctures, rest smoother (Fig. 6C, E); fourth tergite with basal 0.5 coarsely punctate, elsewhere punctures minute, becoming smoother towards apex with moderately dense, brownish pubescence; basal 0.7 of fifth and following tergites punctate, punctures becoming finer on succeeding segments and with dense brownish pubescence (Fig. 6D, E); ovipositor sheath $2.1\times$ the length of fore wing.

Coloration. Black. Face and clypeus yellow, malar area black; mandibles basally reddish-brown with a yellow macula in middle, teeth black; malar space black; temple yellow; frons with two broad lateral spots touching eye margin, median carina on frons yellow; antenna with scape yellowish in front, flagellum dark brown; occiput largely yellow, dorsally black; pronotum, yellow with black band curving from postero-ventral to anterior margin, and anteriorly pointed mediodorsal mark; mesoscutum black with narrow yellow mark alongside notauli medially; tegula, subtegular tubercle and anterior 0.5 of mesopleuron, and metapleuron with posterior 0.6 including tubercle, yellow; axillae yellow, scutellum, with yellow patch antero-medially; metascutellum black except for small yellow spot medio-dorsally; propodeum yellow except extreme dorsolateral corners, spiracular region and extreme apical margin, black; fore legs yellow ventrally from coxa to tibia, coxa dorsally black; middle leg, coxa black with yellow

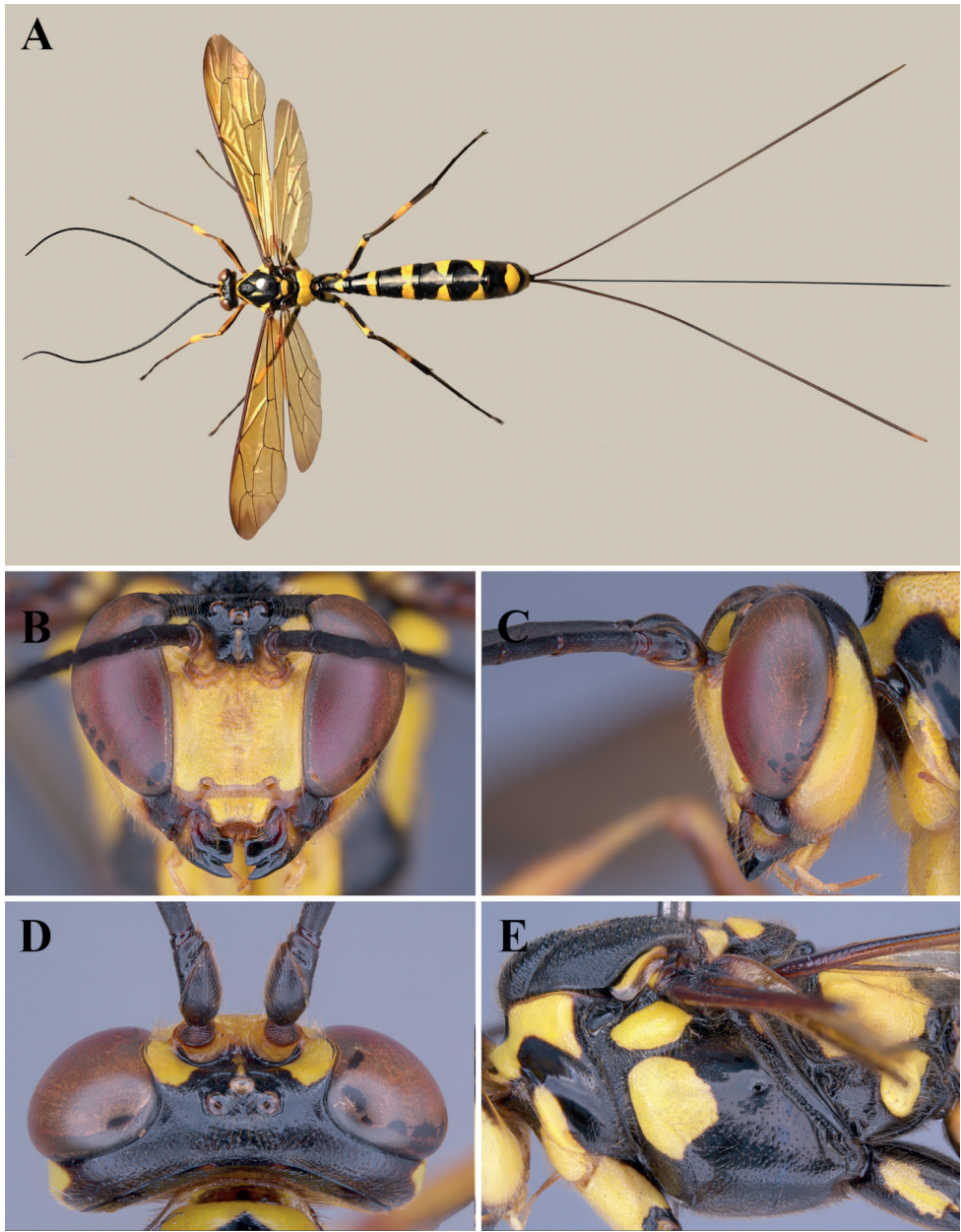


Figure 5. Light micrograph of female *C. moellerii* dorsal view of habitus (A), face (B), lateral view of head (C), dorsal view of head (D), lateral view of mesosoma (E).

low dorsal patch, trochanter black except small dorsal yellow spot, and brownish distal margin, trochantellus black with brownish dorsal part, tibia without apical black, femur black basally, apical 0.3, tibia yellow with dorsal blackish mark on basal 0.5, tarsus black; hind legs as middle leg except trochanter largely yellow, tibia black with medial

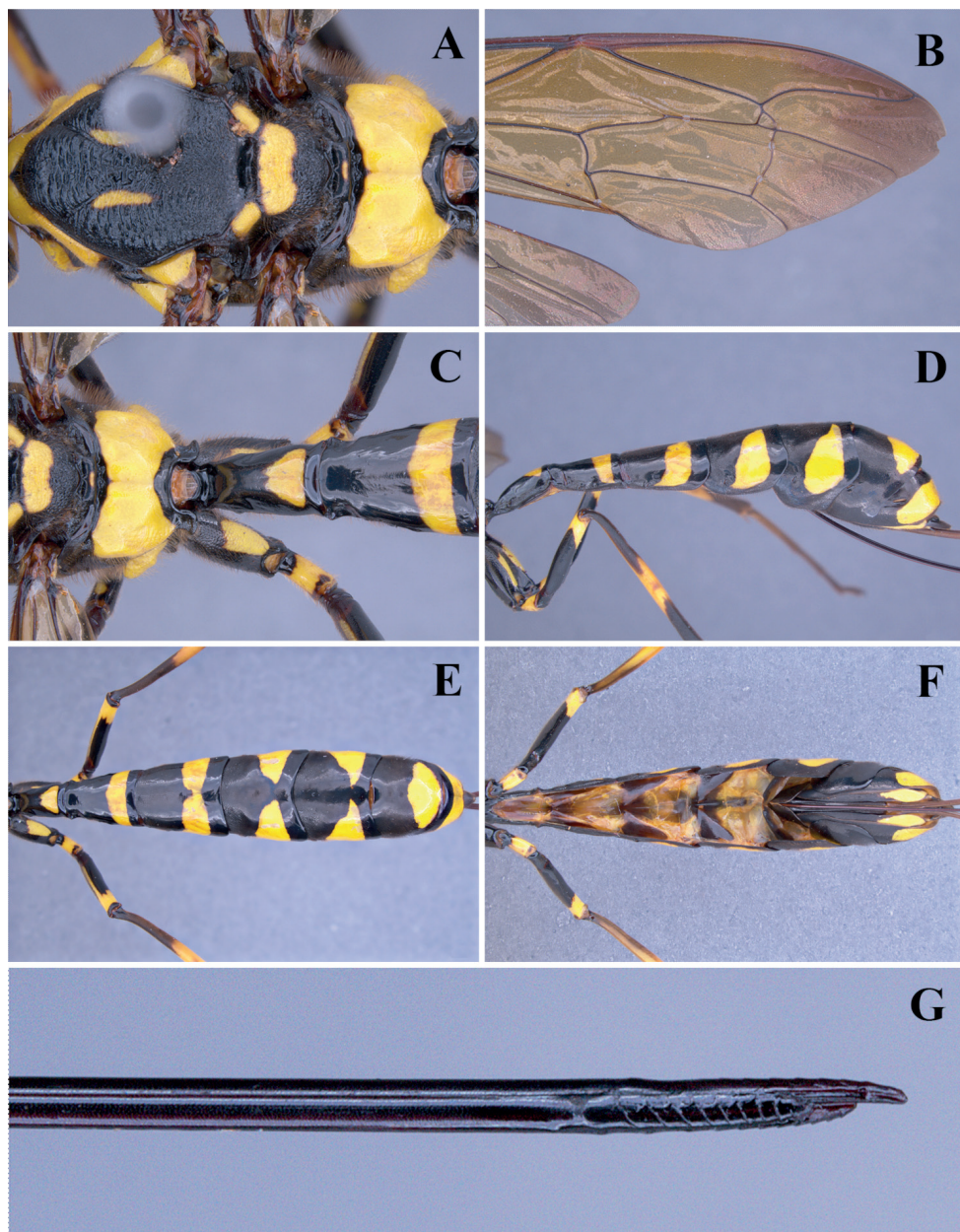


Figure 6. Light micrograph of female *C. moellerii* dorsal view of mesosoma and propodeum (A), fore wing (B), dorsal view of propodeum (C), lateral view of metasoma (D), dorsal view of metasoma (E), ventral view of metasoma (F), lateral view of ovipositor (G).

0.3 brown-yellow around subgenital organ, femur with narrow longitudinal yellow line except basal 0.1; wings yellowish-hyaline with apical margins infuscate; stigma brownish and vein dark brown; metasomal tergites black with the following yellow: tergite

1 subposterior dorsal patch, tergites 2 and three, complete (except laterally) transverse subposterior band, tergites 4 and 5 with large triangular sub posterior patches, tergite 6 large lozenge-shaped postero-dorsal patch, tergite 7 broad yellow posterior transverse band. Ovipositor sheaths black with reddish tinge.

Male (Figs 7, 8). Body length, mean = 23.9 mm (range = 14.0–32.0 mm); fore wing length, mean = 16.8 mm (range = 11.5–24.0 mm) (Fig. 7A).

Head. Antennae with 34–41 flagellomeres, terminal flagellomere acuminate; face strongly transversely striated on its upper 0.8, lower 0.2 coarsely punctate, interspaces 0.5 their diameter, towards orbits punctures finer and sparser; clypeus minutely, finely punctate, broadly concave at apex (Fig. 7B); malar space mat, $0.6\times$ basal width of mandible (Fig. 7D); frons smooth, subpolished with a median carina and with a semi-circular groove around ocellar triangle, vertex with a few scatter punctures, smooth and polished; interocellar distance $0.5\times$ ocello-ocular distance; occiput without a median groove dorsally (Fig. 7C).

Mesosoma. Scutellum strongly, coarsely punctate; median area of metanotum smooth and polished (Fig. 7F); mesopleuron sparsely punctate, punctures on lower 0.3 separated by $2\text{--}3\times$ their diameter, epicnemium with more crowded punctures, interspace $0.5\text{--}1.0\times$ diameter; epicnemial carina weakly curved towards anterior edge, about 0.5 the height of mesopleuron; mesosternum coarsely punctate, punctures sometime coalescent; metapleuron punctate, interspaces $1\text{--}2\times$ their diameter (Fig. 7E); propodeum largely smooth and polished, with very sparse, minute punctures dorsally, dorsolateral corners and lateral sides shallowly punctate, interspace $4\text{--}6\times$ their diameter, broadly depressed at extreme base in middle, and medially with a distinct, shallow groove on basal 0.8 (Figs 7F, 8C).

Wing. Areolet of fore wing short triangular, length of veins 2RS (=2rs-m): 1-M: rs-m (=3rs-m) = 0.6: 0.8: 1.0 (Fig. 8A) or absent (Fig. 8B); vein 2m-cu joining M interstitial with rs-m (= 3rs-m).

Metasoma. First tergite smooth and shiny $2.0\times$ its apical width (Fig. 8C); second and third tergites smooth and polished with few scattered punctures; fourth tergite with basal 0.2 coarsely punctate, elsewhere punctures minute, becoming smoother towards apex with moderately dense, brownish pubescence; fifth to seventh third tergites smooth and polished with few scattered punctures with dense brownish pubescence (Fig. 8D, E).

Coloration. Yellow. Mandibles basally with brownish and with a yellow macula in middle, teeth black; malar space brownish-yellow; frons with two broad lateral spots touching eye margin, antenna with scape yellowish in front, flagellum dark brown; occiput dorsally brownish; pronotum, yellow with a curved incomplete black band dorsally and with a reddish-brown stripe in centre; median and lateral lobe of mesoscutum reddish-brown, posterior of merging notauli extending into a black midlongitudinal stripe; scutellum largely dull yellow with posterior 0.2 of piceous; median area of metanotum yellow; tegula, epicnemium, posterior transverse carina of mesosternum, juxtacoxal carina black; propodeum yellow except extreme dorsolateral base, spiracular region and extreme apical margin black; Legs yellow with tarsi gradually infuscate towards apex except: fore femur ventrally brown; fore tibia narrowly brown dorsally on

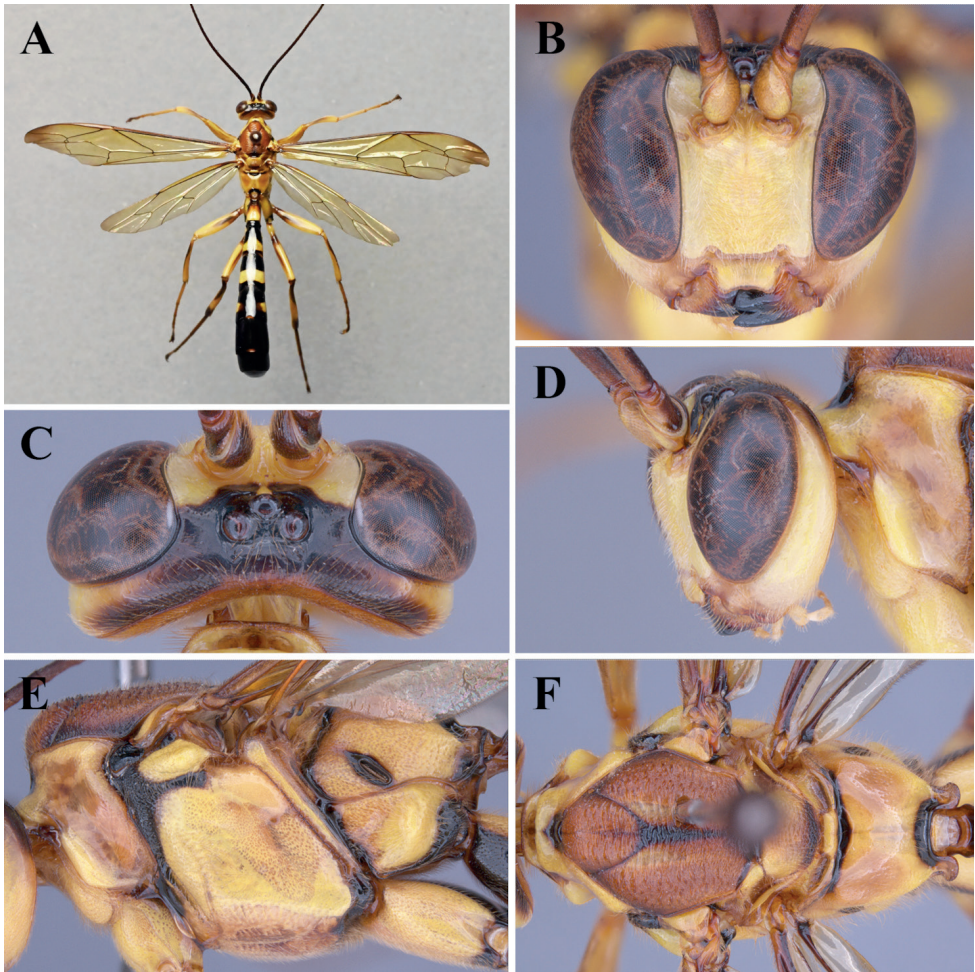


Figure 7. Light micrograph of male *C. moellerii* dorsal view of habitus (A), face (B), dorsal view of head (C), lateral view of head (D), lateral view of mesosoma (E), dorsal view of mesosoma and propodeum (F).

basal 0.7, fore; middle leg similar to fore leg except dark mark on femur on medial side; hind coxa ventrally black, hind femur brown-black basally and medioventrally, hind tibia dorsally with basal 0.5 and distal 0.2 posteriorly dark brown; wing yellowish-hyaline with apical margin infuscate, stigma brownish and vein dark brown; metasomal tergites black with yellow marks as follows: tergite 1 with large, sub-posterior yellow patch, tergites 2 and 3 with broad sub-posterior yellow bands, tergite 4 with yellow patches mediolaterally.

Comment. Male and female of the *C. moellerii* display sexual dimorphism with different colour patterns. Fore wing areolet of female always present according to the keys to species of this genus by Kamath and Gupta, (1972) (Fig. 6B), however, male of *C. moellerii* shows variation of fore wing areolet, either present or absent (Fig. 8A, B) same as male *C. mesopyrrha* (Kamath and Gupta 1972).

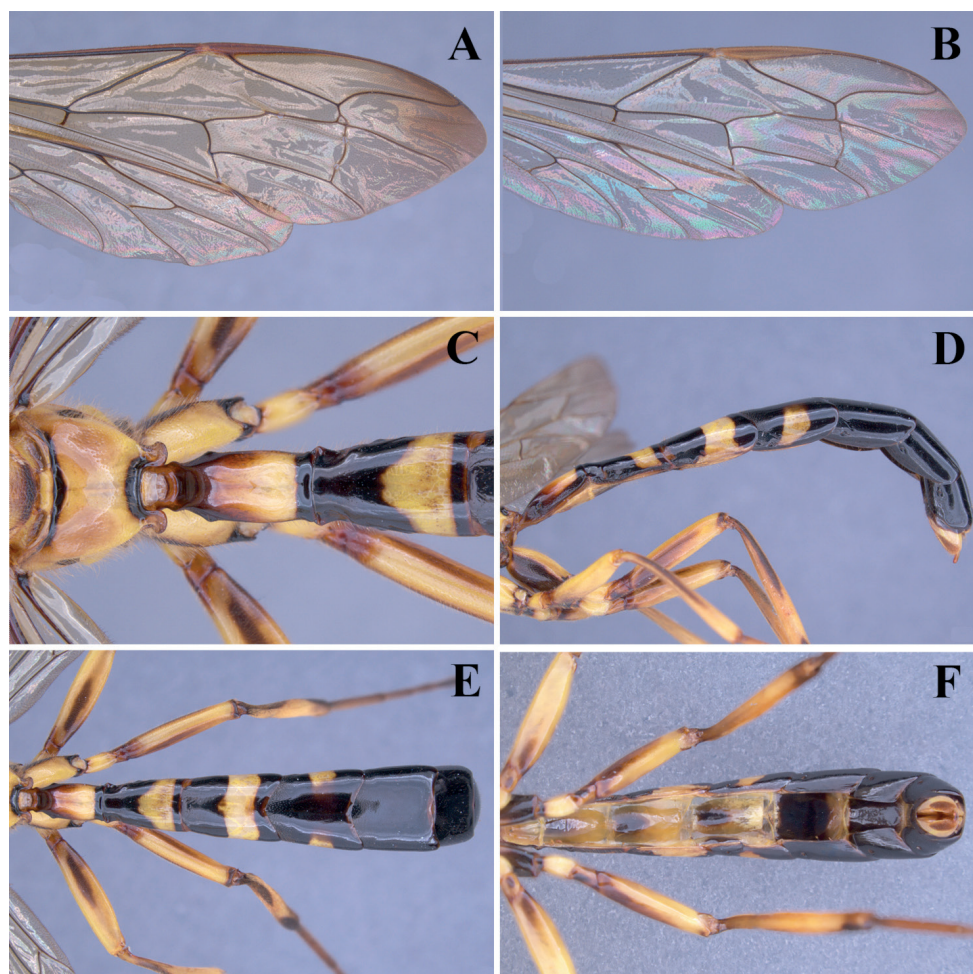


Figure 8. Light micrograph of male *C. moellerii* fore wing areolet present morph (A), fore wing areolet absent morph (B), dorsal view of propodeum (C), lateral view of metasoma (D), dorsal view of metasoma (E), ventral view of metasoma (F).

Phylogenetic analyses

A preliminary molecular phylogeny based on the available DNA barcodes of the rhyssines is shown in Fig. 9. *Cyrtorhyssa* was recovered as sister group to *Myllenyxis* but with low bootstrap support (37%), and the two together as derived from within *Epirhyssa*, with 75% support.

The ML phylogeny including the new sequence from the host *Encaustes opaca* with other available erotylid sequences is shown in Fig. 10. Dacninae, Eucastinae, Megalodacninae and Trominae+Erotulinae were each recovered as monophyletic with strong bootstrap support (84–100%). *Encaustes opaca* was recovered in a polytomy with the only other represented congener *Encaustes cruenta formosama*, and the genus *Aulacochilus*, the only other member of Eucastinae included represented by two species (Fig. 10).

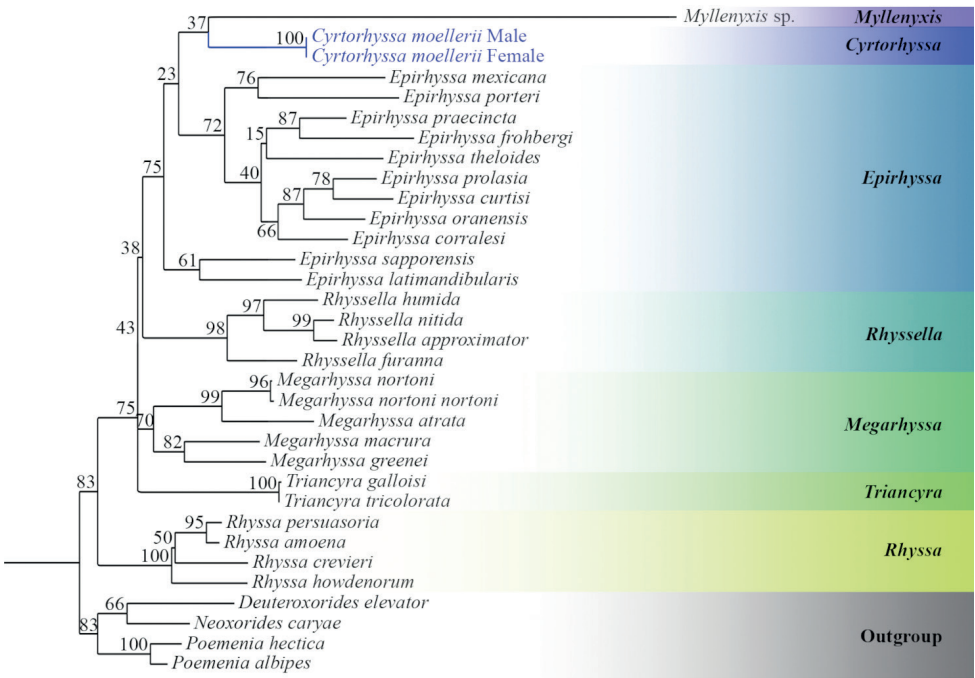


Figure 9. Maximum likelihood tree of *C. moellerii* and other rhyssine wasps based on the barcoding region of cytochrome oxidase subunit 1 (COI) with RaxML rapid bootstrap support values.

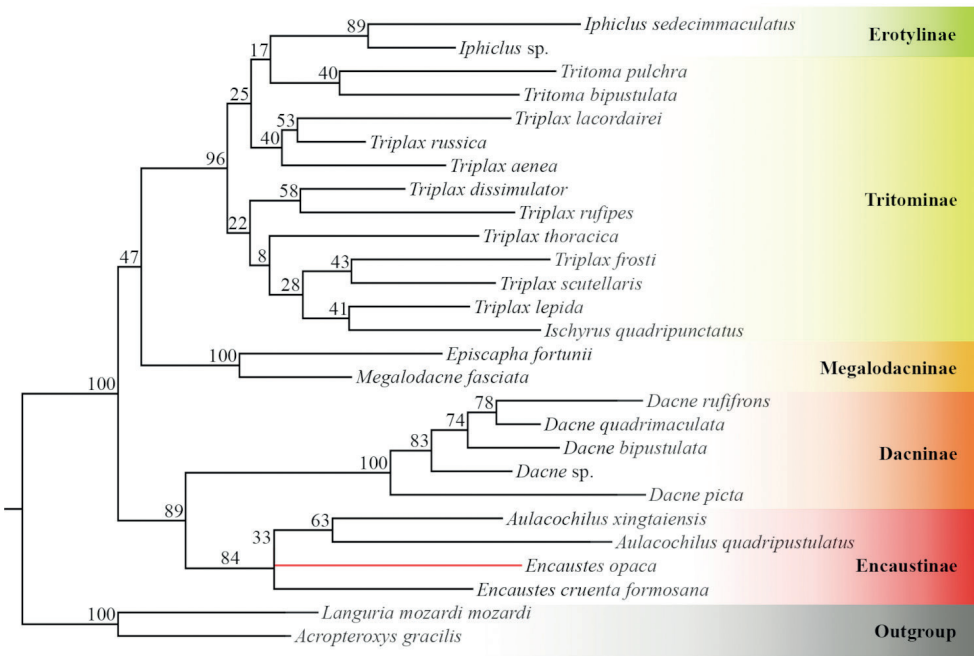


Figure 10. Maximum likelihood tree of *E. opaca* (Encaustinae) and other erotyloid beetles based on the barcoding region of cytochrome oxidase subunit 1 (COI) with RaxML rapid bootstrap support values.

Discussion

The protandry observed in *C. moellerii* is similar to what is known for other rhyssine wasps. Most males were observed aggregating in the area where a female was about to emerge (Baker 1992; Eggleton 1991). Tergal stroking behaviour has previously been reported for the males of the genus *Megarhyssa* (Matthews et al. 1979) and here we report it for the first time in *Cyrtorhyssa*. Male rhyssines that have long slender metasomas, e.g. *Megarhyssa rixator* (Schellenberg, 1802), are able to insert their abdomens into the female emergence tunnel for mating (Quicke 2015). However, males of *C. moellerii* are not so slender. Further, in *C. moellerii* only the larger males have a sufficiently long metasoma that can be inserted into the emergence tunnel to achieve copulation. The observed adult lifespan of *C. moellerii* is comparable to that reported for *Megarhyssa* spp. which can live at least 27 days (Heatwole and Davis 1965).

Our molecular study is the first to include a substantial number of representative rhyssines since Klopstein et al. (2019), which included representatives of only three genera. In agreement with that study, we recovered *Rhyssa* as sister group to the remaining included genera (Fig. 9). We recovered *Cyrtorhyssa* and *Myllenyxis* as sister groups nested within *Epirhyssa*, however, additional molecular data would be needed before any conclusions can be drawn about possible non-monophyly of the latter. Both are endemic Asian genera, being known from in India, southern China and both mainland and island of Southeast Asia (Kamath and Gupta 1972; Wang 1972). In contrast, the genera *Epirhyssa* and *Megarhyssa* have considerably more cosmopolitan distributions. *Myllenyxis* is the only genus of Rhyssinae that has the upper tooth of the mandible subdivided thus appearing tridentate. Moreover, fore wing areolet is always present in *Megarhyssa* and *Rhyssella* and always absent in *Epirhyssa*, but some variation in this character is found in males of *C. moellerii* and *C. mesopyrrha*.

The host genus *Encaustes* is widespread in the Old-World tropics and subtropics being reported from Africa, South Asia, East Asia, Southeast Asia to Australia (Chujo 1968a; Chujo 1968b; Chujo 1969; Chujo 1973; Chujo and Chujo 1987; Chujo and Chujo 1988; Chujo et al. 1993). Its distribution completely overlaps that of *Cyrtorhyssa*. Only two species of *Encaustes* have been recorded in Thailand previously, *E. cruenta montana* Schenkling, 1919 and *E. opaca* Crotch, 1876, both from Chiang Mai province in 1957 (Chujo 1968b; Chujo and Chujo 1988). The relationships between the subfamilies of Erotylidae recovered here using just COI (Fig. 10) are essentially identical to those obtained by Robertson (2004) using combined 16S and 28S data.

Acknowledgements

We are grateful to Mr Surachit Waengsothorn for providing facilities at the Sakaerat Environmental Research Station; Samai Sewakhonburi and Arthit Janthadee for identification of the tree; Sakaerat bird team for collecting insect specimens; and Dr Michael Geiser, The Natural History Museum (London) for confirming identification of the beetle. We also thank Shen-Horn Yen (National Sun Yat-sen University, Taiwan) and Zhipang

Huang (Insitute of Eastern-Himalaya Biodiversity Research) for help with literature. This research was funded by National Research Council of Thailand (NRCT) (N42A650262) and Chulalongkorn University, RSPG-Chula to BAB; DLJQ was supported by Rachadaphiseksomphot Fund, Graduate School, Chulalongkorn University; KC was supported by CU Graduate School Thesis Grant (GCUGR1225641025D), the Overseas Research Experience Scholarship for Graduate Student from CU Graduate School and Faculty of Science; and The Second Century Fund (C2F), Chulalongkorn University.

References

- Baker BR (1992) Emergence and pairing of *Rhyssa persuasoria* L. (Hymenoptera: Ichneumonidae). British Journal of Entomology and Natural History 5: 184–185.
- Bennett AMR, Cardinal S, Gauld ID, Wahl DB (2019) Phylogeny of the subfamilies of Ichneumonidae (Hymenoptera). Journal of Hymenoptera Research 71: 1–156. <https://doi.org/10.3897/jhr.71.32375>
- Bocak L, Barton C, Crampton-Platt ALEX, Chesters D, Ahrens D, Vogler AP (2014) Building the Coleoptera tree-of-life for > 8000 species: composition of public DNA data and fit with Linnaean classification. Systematic Entomology 39: 97–110. <https://doi.org/10.1111/syen.12037>
- Broad G, Shaw M, Fitton M (2018) The Ichneumonid Wasps of Britain and Ireland (Hymenoptera: Ichneumonidae). Royal Entomological Society, Telford.
- Cao LM, Achterberg CV, Tang YL, Wang XY, Yang ZQ (2020) Revision of parasitoids of *Massicus raddei* (Blessig Solsky) (Coleoptera, Cerambycidae) in China, with one new species and genus. Zootaxa 4881: 104–130. <https://doi.org/10.11646/zootaxa.4881.1.7>
- Chujo M (1968a) Erotylid beetles from south-China, Hainan, Taiwan and the Ryukyus. Studies on the erotyld beetles (20). Pacific Insects 10: 539–550.
- Chujo M (1968b) Erotylid beetles from Thailand, Laos and Viet-Nam. Studies on the erotyld beetles (21). Pacific Insects 10: 551–573.
- Chujo M (1969) Erotylidae (Insecta: Coleoptera). Fauna Japonica, Insecta Coleoptera Erotylidae. Academic Press of Japan, Tokyo.
- Chujo M (1973) Coleoptera: Erotylidae and Languriidae from Ceylon. Entomologica Scandinavica. Supplementum 4: 195–198.
- Chujo M, Chujo M (1987) Erotylidae from New Guinea and her adjacent islands I (Coleoptera). Esakia 25: 5–36. <https://doi.org/10.5109/2492>
- Chujo M, Chujo M (1988) A catalog of the Erotylidae (Insecta, Coleoptera) from the Old World (excl. the Ethiopian Region). Esakia 26: 139–185. <https://doi.org/10.5109/2510>
- Chujo M, Chujo M, Lee CE (1993) Erotylidae from Korea (Insecta, Coleoptera). Esakia 33: 99–108. <https://doi.org/10.5109/2567>
- Cline AR, Smith TR, Miller K, Moulton M, Whiting M, Audisio P (2014) Molecular phylogeny of Nitidulidae: assessment of subfamilial and tribal classification and formalization of the family Cybocephalidae (Coleoptera: Cucujoidea). Systematic Entomology 39: 758–772. <https://doi.org/10.1111/syen.12084>

- Couturier A (1949) Observations sur *Rhyssa approximata* F. cleptoparasite de *Rh. persuasoria* L. (Hym. Ichneumonidae). Bulletin de la Société Entomologique de France 54: 62–63. <https://doi.org/10.3406/bsef.1949.18366>
- Crotch GR (1876) A revision of the coleopterous family Erotylidae. Cistula Entomologica [1869–1876]: 359–572.
- Deelder CL (1942) Revision of the Erotylidae (Coleoptera) of the Leiden Museum. Zoologische Mededelingen 24: 49–115.
- DeWaard JR, Ratnasingham S, Zakharov EV, Borisenko AV, Steinke D, Telfer AC, Perez KH, Sones JE, Young MR, Levesque-Beaudin V, Sobel CN (2019) A reference library for Canadian invertebrates with 1.5 million barcodes, voucher specimens, and DNA samples. Scientific data 6: 1–12. <https://doi.org/10.1038/s41597-019-0320-2>
- Eagalle T (2014) Latitudinal Effects on Diversity and Body Size: A Case Study with Parasitoid and Parasitic Wasps. Unpublished Ph.D. thesis, University of Guelph.
- Eggleton P (1991) Patterns in male mating strategies of the Rhyssini: a holophyletic group of parasitoid wasps (Hymenoptera: Ichneumonidae). Animal Behaviour 41: 829–838. [https://doi.org/10.1016/S0003-3472\(05\)80350-1](https://doi.org/10.1016/S0003-3472(05)80350-1)
- Hanson HS (1939) Ecological notes on *Sirex* wood wasps and their parasites. Bulletin of Entomological Research 30: 27–65. <https://doi.org/10.1017/S0007485300004399>
- Heatwole H, Davis DM (1965) Ecology of three sympatric species of parasitic insects of the genus *Megarhyssa* (Hymenoptera: Ichneumonidae). Ecology 46: 140–150. <https://doi.org/10.2307/1935265>
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London, Series B: Biological Science 270(suppl_1): S96–S99. <https://doi.org/10.1098/rsbl.2003.0025>
- Hebert PDN, Ratnasingham S, Zakharov EV, Telfer AC, Levesque-Beaudin V, Milton MA, Pedersen S, Jannetta P, DeWaard JR (2016) Counting animal species with DNA barcodes: Canadian insects. Philosophical Transactions of the Royal Society B: Biological Sciences 371: e20150333. <https://doi.org/10.1098/rstb.2015.0333>
- Kamath MK, Gupta VK (1972) Ichneumonologia Orientalis, Part II. The Tribe Rhyssini (Hymenoptera: Ichneumonidae). Oriental Insects Monographs 2: 1–300.
- Klopfstein S, Langille B, Spasojevic T, Broad GR, Cooper SJ, Austin AD, Niehuis O (2019) Hybrid capture data unravel a rapid radiation of pimpliform parasitoid wasps (Hymenoptera: Ichneumonidae: Pimpliformes). Systematic Entomology 44: 361–383. <https://doi.org/10.1111/syen.12333>
- Matthews RW, Matthews JR, Crankshaw O (1979) Aggregation in male parasitic wasps of the genus *Megarhyssa*: I. Sexual discrimination, tergal stroking behavior, and description of associated anal structures. The Florida Entomologist 62: 3–8. <https://doi.org/10.2307/3494037>
- National Center for Biotechnology Information [NCBI] (2023) [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2023 Sep 14]. <https://www.ncbi.nlm.nih.gov/>

- Park DS, Suh SJ, Oh HW, Hebert PDN (2010) Recovery of the mitochondrial COI barcode region in diverse Hexapoda through tRNA-based primers. *BMC Genomics* 11: 1–7. <https://doi.org/10.1186/1471-2164-11-423>
- Pentinsaari M, Hebert PDN, Mutanen M (2014) Barcoding beetles: a regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. *PLoS ONE* 9: e108651. <https://doi.org/10.1371/journal.pone.0108651>
- Porter CC (1978) A revision of the genus *Epirhyssa* (Hymenoptera: Ichneumonidae). *Studia Entomologia* 20: 297–411.
- Quicke DLJ (2015) *The Braconid and Ichneumonid Parasitic Wasps: Biology, Systematics, Evolution and Ecology*. Wiley Blackwell, Chichester, 681 pp. <https://doi.org/10.1002/9781118907085>
- Quicke DLJ, Laureenne NM, Fitton, MG Broad GR (2009) A thousand and one wasps: a 28S rDNA and morphological phylogeny of the Ichneumonidae (Insecta: Hymenoptera) with an investigation into alignment parameter space and elision. *Journal of Natural History* 43: 1305–1421. <https://doi.org/10.1080/00222930902807783>
- Quicke DLJ, Smith MA, Janzen DH, Hallwachs W, Fernandez-Triana J, Laureenne NM, Zaldivar-Riveron A, Shaw MR, Broad GR, Klopstein S, Shaw SR, Hreck J, Hebert PD, Miller SE, Rodriguez JJ, Whitfield JB, Sharkey MJ, Sharanowski BJ, Jussila R, Gauld ID, Chesters D, Vogler AP (2012) Utility of the DNA barcoding gene fragment for parasitic wasp phylogeny (Hymenoptera: Ichneumonidae): data release and new measure of taxonomic congruence. *Molecular Ecology Resources* 12: 676–685. <https://doi.org/10.1111/j.1755-0998.2012.03143.x>
- Robertson JA, McHugh JV, Whiting MF (2004) A molecular phylogenetic analysis of the pleasing fungus beetles (Coleoptera: Erotylidae): evolution of colour patterns, gregariousness and mycophagy. *Systematic Entomology* 29: 173–187. <https://doi.org/10.1111/j.0307-6970.2004.00242.x>
- Roslin T, Somervuo P, Pentinsaari M, Hebert PD, Agda J, Ahlroth P, Anttonen P, Aspi J, Blagoev G, Blanco S, Chan D 2022. A molecular-based identification resource for the arthropods of Finland. *Molecular Ecology Resources* 22: 803–822. <https://doi.org/10.1111/1755-0998.13510>
- Sharkey MJ, Wharton RA (1997) Morphology and terminology. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) *Identification manual to the New World genera of Braconidae*. Special Publication of the International Society of Hymenopterists 1: 19–37.
- Sheng ML, Sun SP (2010) *Parasitic Ichneumonids on Woodborers in China* (Hymenoptera: Ichneumonidae). Science Press, Beijing, 338 pp. [in Chinese with English summary]
- Sikes DS, Bowser M, Morton JM, Bickford C, Meierotto S, Hildebrandt K (2017) Building a DNA barcode library of Alaska's non-marine arthropods. *Genome* 60: 248–259. <https://doi.org/10.1139/gen-2015-0203>
- Sire L, Gey D, Debruyne R, Noblecourt T, Soldati F, Barnouin T, Parmain G, Bouget C, Lopez-Vaamonde C, Rougerie R (2019) The challenge of DNA barcoding saproxylic beetles in natural history collections—exploring the potential of parallel multiplex sequencing with Illumina MiSeq. *Frontiers in Ecology and Evolution* 7: e495. <https://doi.org/10.3389/fevo.2019.00495>

- Spasojevic T, Broad GR, Sääksjärvi IE, Schwarz M, Ito M, Korenko S, Klopstein S (2021) Mind the outgroup and bare branches in total-evidence dating: a case study of pimpliform Darwin wasps (Hymenoptera, Ichneumonidae). *Systematic Biology* 70: 322–339. <https://doi.org/10.1093/sysbio/syaa079>
- Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>
- Telfer AC, Young MR, Quinn J, Perez K, Sobel CN, Sones JE, Levesque-Beaudin V, Derbyshire R, Fernandez-Triana J, Rougerie R, Thevanayagam A (2015) Biodiversity inventories in high gear: DNA barcoding facilitates a rapid biotic survey of a temperate nature reserve. *Biodiversity Data Journal* 3: e6313. <https://doi.org/10.3897/BDJ.3.e6313>
- Wang SF (1982) A new species of the genus *Cyrtorhyssa* (Ichneumonidae: Ephialtinae). *Sino-zoologia* 2: 59–60. [in Chinese with English summary]

Supplementary material I

Video Online Resource 1

Authors: Kittipum Chansri, Kanoktip Somsiri, Donald L. J. Quicke, Buntika A. Butcher

Data type: mp4

Explanation note: Tergal stroking behaviour of male *Cyrtorhyssa moellerii*.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/jhr.96.107196.suppl1>

Supplementary material 2

Video Online Resource 3

Authors: Kittipum Chansri, Kanoktip Somsiri, Donald L. J. Quicke, Buntika A. Butcher

Data type: mp4

Explanation note: Aggressive guarding behaviour of male *Cyrtorhyssa moellerii*.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/jhr.96.107196.suppl2>

Supplementary material 3

Video Online Resource 2

Authors: Kittipum Chansri, Kanoktip Somsiri, Donald L. J. Quicke, Buntika A. Butcher

Data type: mp4

Explanation note: Mating behaviour of male *Cyrtorhyssa moellerii*.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/jhr.96.107196.suppl3>

Supplementary material 4

Video Online Resource 4

Authors: Kittipum Chansri, Kanoktip Somsiri, Donald L. J. Quicke, Buntika A. Butcher

Data type: mp4

Explanation note: Emergence of female *Cyrtorhyssa moellerii*.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/jhr.96.107196.suppl4>