Paratelenomus anu Rajmohana, Sachin & Talamas
(Hymenoptera, Scelionidae): description and biology of a new species of phoretic egg parasitoid of Megacopta cribraria (Fab.) (Hemiptera, Plataspidae)

Keloth Rajmohana1, James P. Sachin2, Elijah J. Talamas3,
Mukundan S. Shamyasree2, S. K. Jalali4, Ojha Rakshit4

1 Zoological Survey of India, P.O New Alipore, Kolkata-700053, West Bengal, India 2 PG & Research Department of Zoology, Malabar Christian College, Calicut-673001, Kerala, India 3 Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL, USA 4 National Bureau of Agriculturally Important Insects, Bangalore 560024, India

Corresponding author: Elijah J. Talamas (talamas.1@osu.edu)

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Abstract
Paratelenomus anu Rajmohana, Sachin & Talamas, sp. nov. (Hymenoptera: Scelionidae) is an egg parasitoid of the kudzu bug, Megacopta cribraria (Fab.) (Hemiptera: Plataspidae). It is morphologically and genetically distinct from P. saccharalis (Dodd), a well-known egg parasitoid of the same host. Paratelenomus anu is here described from India and diagnosed from other species of Paratelenomus Dodd. This parasitoid can be reared easily, has high rates of parasitism, and thus may be significant for the biological control of M. cribraria. Phoresy is documented in P. anu and provides the first known example of this behavior in Paratelenomus. Paratelenomus longus (Kozlov & Lê) syn. nov. and P. mangrovus Rajmohana & Narendran, syn. nov. are treated as junior synonyms of P. tetartus (Crawford), and P. obtusus (Lê) syn. nov. is treated as a junior synonym of P. saccharalis.

Keywords
kudzu bug, phoresy, India, invasive species, biological control
Introduction

*Megacopta cribraria* (Fab.) (Hemiptera: Plataspidae), commonly called the kudzu bug, the lablab bug, the bean plataspid, or the globular stink bug, is native to Asia, including the Indian subcontinent, and Australia (Srinivasaperumal et al. 1992, Hua 2000, Eger et al. 2010). This bug is a voracious feeder on kudzu and numerous agricultural crops including soy bean (Zhang 1985), lablab bean (Schaeffer and Panizzi 2000), pigeon pea (Hoffmann 1932), *Phaseolus* group (Hoffmann 1931, Easton and Pun 1997), broad beans (Ishihara 1950), peach (*Amygdalus persica* L.), and jujube (*Ziziphus jujube* Mill.) (Wang et al. 1996, Li et al. 2001, Wang et al. 2004). The invaded range now includes the United States where it feeds on the kudzu plant, *Pueraria montana* (Merr.) (Zhang et al. 2012), an economically important invasive weed (Suiter et al. 2010, Gardner et al. 2013b).

Eger et al. (2010) reported several hymenopteran parasitoids from the eggs of *M. cribraria*, including *Encarsia boswelli* (Girault) (Aphelinidae), *Ablerus* Howard (Aphelinidae), *Ooencyrtus nezarae* Ishi (Encyrtidae), *Trissolcus latisulcus* (Crawford) (Scelionidae), and *Paratelenomus saccharalis* (Dodd) (Scelionidae). *Paratelenomus saccharalis*, which was once restricted to the eastern Hemisphere, has been found in the United States parasitizing the eggs of *M. cribraria* (Gardner et al. 2013a, Medal et al. 2013). In this paper we describe another egg parasitoid from India, *Paratelenomus anu* Rajmohana, Sachin and Talamas sp. nov., from the same host, and provide notes on its behavior, distribution and parasitism rate, and provide updates to the taxonomy of other species of *Paratelenomus*.

Materials and methods

Collection of parasitoid and host

Surveys were conducted at five different localities in Calicut district, in the South Indian state of Kerala, from June 2015 until 2017, where *Lablab purpureus* (L.) plants were grown and incidence of *M. cribraria* was noticed (Table 1). Adult bugs, on which the parasitoids were phoretic, were also collected from the field (Fig.1). Several egg masses of *M. cribraria* were collected from these localities and brought into the laboratory. The egg masses were kept for rearing in small transparent plastic containers (8×11 cm) and kept at ambient temperature. The number of host bugs and the parasitoids that emerged from the eggs were recorded. The parasitoids recovered were preserved in ethyl alcohol (100%) for taxonomic study or kept alive in the laboratory by providing 20% honey solution as food. The rates of parasitism and bug emergence were calculated from field-collected and laboratory-reared eggs by comparing the number of parasitoids and nymphs that emerged from each egg mass to the number of eggs collected, respectively. The identity of *M. cribraria* was confirmed by the expertise available at the University of Agricultural Sciences, Bangalore, Karnataka, and the identity of the legumes were confirmed by the Department of Botany, University of Calicut, Kerala.
Microscopy

The preserved wasps were glued to the tip of point cards and examined with Leica M 205A and Zeiss V8 stereo microscopes. Extended-focus images were produced with two systems: a Leica DFC 500 camera attached to a Leica M 205 A stereomicroscope with images combined using the Leica Application Suite, and a Macroscopic Solutions Macropod Micro Kit with images combined in Helicon Focus. Scanning electron microscopy was performed with a Hitachi SU6600 Variable Pressure Field Emission Scanning Electron Microscope (FESEM) and a Hitachi TM3000 Tabletop Microscope.

Cybertaxonomy

The data associated with these specimens is deposited in the Hymenoptera Online Database (hol.osu.edu). The online systematics and taxonomy tool, vSysLab (vsyslab.osu.edu), was used to generate the material examined sections and taxonomic synopses. Morphological terms were matched to concepts in the Hymenoptera Anatomy Ontology using the text analyzer function and a table of these terms and URI links is provided in Suppl. material 1.

Collections

This study is based on specimens deposited in the following institutions.

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<th>Code</th>
<th>Institution</th>
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<td>Canadian National Collection of Insects, Ottawa, Canada</td>
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<td>IEBR</td>
<td>Institute for Ecology and Biological Resources, Hanoi, Vietnam</td>
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DNA barcoding

DNA was extracted from the whole insect using Qiagen DNeasy kit, following the manufacturer’s protocols. The extracts were subjected to PCR amplification of a 658 bp region near the 5’ terminus of the CO1 gene following standard protocol (Hebert et al. 2004). Primers used were: forward primer (LCO 1490: 5’-GGTCAACAATATCAT-AAAGATATTTGG-3’), and reverse primer (HCO 2198: 5’-TAAACTTCAAGGGT-GACAAAAATATCA-3’). PCR reactions were carried out in 96-well plates, 50 μL reaction volume containing: 5 μL GeNeiTM Taq buffer, 1 μL GeNeiT M 10mM dNTP mix, 2.5 μL (20 pmol/μL) forward primer, 2.5 μL (20 pmol/μL) reverse primer, 1 μL GeNeiT M Taq DNA polymerase (1 U/μL), 2 μL DNA (50 ng/μL), and 36 μL sterile...
Table 1. Egg emergence data for nymphs of *Megacopta cribraria* and adults of *Paratelenomus anu* by egg mass.

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water. Thermo cycling consisted of an initial denaturation of 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute and extension at 72 °C for 1 minute using a C1000™ Thermal Cycler. The amplified products were analyzed on a 1.5% agarose gel electrophoresis as described by Sambrook and Russell (2001), sequenced, and uploaded to Genbank (accession number KT896660.1, see Suppl. material 2).

### Results

#### Taxonomy

*Paratelenomus anu* Rajmohana, Sachin & Talamas, sp. nov.

http://zoobank.org/B367648F-1696-4487-883D-4CD455D316B0

http://bioguid.osu.edu/xbiod_concepts/486304

Figures 1–8

### Description.

Body length. Female: 0.65–0.71mm. Male: 0.66–0.68mm.

*Color.* Body black to honey brown; first metasomal tergite slightly xanthic, weakly contrasting with posterior metasomal segments; antenna and legs yellow to brown; wings hyaline; wing venation brown.
Figures 1–3. 1 Megacopta cribraria with phoretic Paratelenomus anu 2 female of P. anu on eggs of M. cribraria 3 individuals of P. anu prior to emergence from eggs of M. cribraria.

Head. Frons mostly smooth with coriaceous sculpture dorsally; central keel attenuated dorsally, not bifurcating around median ocellus; submedian carina absent; orbital carina present; a single row of equidistant setae present along orbital carina; gena dorsally coriaceous, as on vertex, but smooth toward mandibular articulation; occipital carina incomplete medially; crenulae arising from occipital carina short; labrum pentagonal, slightly more than 2x wider than long, apex bidentate medially; antennal clava 4-merous; claval formula A11–A8: 1-2-2-1; A5 in males with tyloid.

Mesosoma. Notauli absent to weakly present posteriorly; mesoscutum with coriaceous sculpture; parapsidal lines present; mesoscutal humeral sulcus and mesoscutal suprahumeral sulcus indicated by cells; transscutellar articulation narrowed medially, wider and crenulate laterally; foveae of posterior mesoscutellar sulcus of uniform size; mesoscutellum abutting mesoscutum medially; disc of mesoscutellum semicircular, with coriaceous sculpture; setal bases on mesoscutellum simple, not pustulate; metascutellum rugulose; mesopleural carina absent; intercoxal space narrow, not completely occluded by postacetabular and mesopleural epicoxal sulci; acetabular field small, finely setose, and coriaceous; episternal fovea present; femoral depression weakly indicated; prespecular sulcus present; metapleural triangle present; metapleural carina present; paracoxal sulcus absent; posterodorsal metapleural sulcus present.

Metasoma. T1 longitudinally costate, with two lateral setae; T2 striate, striae absent in lateral and posterior portions of tergite.

Male. Similar to female, except antennae filiform and metasoma with 8 external tergites and 7 external sternites.

Diagnosis. Paratelenomus anu does not fully follow either lead of the first couplet in the key to species of Paratelenomus by Johnson (1996) because the notauli are weakly present at the posterior margin of the metasoma (best seen in anterodorsal view) and may appear absent. Otherwise, P. anu matches the second lead based on the medially narrowed transscutal articulation and the presence of just two lateral setae on T1. By following the second lead of the couplet one would arrive at P. saccharalis, which is morphologically very similar to P. anu. They can be separated by the notaulus, which is well developed in P. saccharalis and extends for more than half the length of the mesoscutum; the central keel,
which does not bifurcate around the median ocellus in \textit{P. anu}; and the interorbital space, which in \textit{P. anu} is $1.25 \times$ eye height and in \textit{P. saccharalis} is slightly less than eye height.

\textbf{Etymology.} The species is named ‘\textit{anu}’ because of its small size. (In Sanskrit ‘\textit{anu}’ is the equivalent for the smallest unit of matter). The name is treated as a noun in apposition.

Comments. A central keel that dorsally bifurcates around the medial ocellus was listed by Johnson (1996) as a generic character for *Paratelenomus* and was used to distinguish it from *Psix* Kozlov & Lê. In *P. anu*, the central keel dorsally attenuates and does not bifurcate around the median ocellus and thus this character does not unambiguously separate *Psix* from *Paratelenomus*, although it remains useful for identifying other species of *Paratelenomus*. The other characters presented by Johnson (1996) remain valid for *Paratelenomus* and it is based on these that we are confident in the generic placement of *P. anu*: head and mesosoma without rugose-reticulate sculpture; mandibles narrow, sicklelike, unidentate and broadly overlapping; paracoxal and metapleural sulci absent.

Sequence analysis. The CO1 sequence of *Paratelenomus anu* (KT896660.1) was analyzed using the online BLAST tool of NCBI for comparison with other sequences in the GenBank database. We found *P. anu* showed 85% sequence identity with *P. saccharalis* (KC778442.1) with 520/628 identities, and 7 gaps that accounted for about 1% of the total alignment length. This degree of sequence divergence is congruent with treatment of *P. saccharalis* and *P. anu* as separate species.

Parasitism. The host eggs collected from all locations contained both parasitized and unparasitized eggs. In the laboratory, *M. cribraria* nymphs emerged from almost all unparasitized eggs within five days of collection, while the parasitoids emerged within 11–13 days. Male wasps were usually the first to emerge and remained on the egg mass for emergence of the females, with which they immediately mated for 12–15 sec. Following copulation, males continued waiting for the emergence of additional females. Among all the egg batches collected, the maximum number of males that emerged from an egg mass was four. Each egg mass had an average of 22.97 ± 6.41 eggs. The percent emergence of male and female parasitoids was 10.3 % and 63.2 % whereas the remainder (26.4%) were nymphs. This female-biased sex ratio enhances the potential of this parasitoid to be developed as a biocontrol agent against *M. cribraria* (Ode and Hardy 2008) (Table 2). It was also observed in the laboratory that, immediately after mating, the parasitoid females mounted the dorsal abdomen of *M. cribraria* in the vicinity and remained phoretic.

Parasitoid efficiency. The parasitism rate was 73.7 ± 7.3% for field-collected egg masses and 75.9 ± 3.5% for egg masses reared in the laboratory. Among the parasitized egg masses, nymph emergence was 10.39% for field-collected eggs and 7.58% in the laboratory (Table 2).

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<tr>
<th>Locations</th>
<th># of egg masses</th>
<th>total # of eggs</th>
<th>total # of nymphs emerged</th>
<th>total # of <em>P. anu</em> emerged</th>
<th>total # of male egg parasitoids emerged</th>
<th>total # of female egg parasitoids emerged</th>
<th>total # of unhatched eggs</th>
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<td>31</td>
<td>686</td>
<td>52 (7.58%)</td>
<td>521 (75.95%)</td>
<td>67 (9.77%)</td>
<td>454 (66.18%)</td>
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<td>Field</td>
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<td>1473</td>
<td>153 (10.39%)</td>
<td>1086 (73.73%)</td>
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</tbody>
</table>

Table 2. Comparison of emergence rates of *Megacopta cribraria* nymphs and adults of *Paratelenomus anu* from laboratory-reared and field-collected egg masses. Percentages in parentheses are based on total number of eggs.
New Synonymies

*Paratelenomus saccharalis* (Dodd)

http://bioguid.osu.edu/xbiod_concepts/3343

Figures 9–16

*Telenomus saccharalis* Dodd, 1914: 293 (original description).

*Aphanurus Graeffei* Kieffer, 1917: 343 (original description); Johnson 1996: 282 (junior synonym of *Telenomus saccharalis* Dodd); Johnson 1996: 282 (junior synonym of *Paratelenomus saccharalis* (Dodd)).

*Liophanurus saccharalis* (Dodd): Kieffer 1926: 64, 71 (description, generic transfer, keyed).

*Microphanurus graeffei* (Kieffer): Kieffer 1926: 91, 100 (description, generic transfer, keyed).

*Asolcus minor* Watanabe, 1954: 20, 21 (original description. Keyed); Johnson 1996: 282 (junior synonym of *Telenomus saccharalis* Dodd); Johnson 1996: 282 (junior synonym of *Paratelenomus saccharalis* (Dodd)).


*Archiphanurus obtusus* Lê, 1982: 145 (original description); Lê 1997: 24 (keyed); Lê 2000: 249, 252 (description, keyed, type information).


*Paratelenomus saccharalis* (Dodd): Johnson 1988: 231 (type information, generic transfer); Johnson 1992: 564 (cataloged, type information); Johnson 1996: 278, 282 (description, synonymy, keyed); Johnson 1996: 278, 282 (description, synonymy, keyed); Rajmohana and Narendran 2007: 2523 (keyed); Saminet al. 2012: 19 (new distribution record for Iran); Rajmohana K. & Peter 2013: 22 (description); Talamas et al. 2015: 52 (keyed).


*Paratelenomus obtusus* (Lê) syn. nov.: Johnson 1992: 564 (cataloged, type information).


**Comments.** Our synonymy of *Paratelenomus obtusus* (Lê) is based on photographs of the holotype specimens provided by Talamas and Pham (2017) (Figs 12–16). This specimen matches the concept of *P. saccharalis* in the description and identification key of Johnson (1996) and likely was not considered by Lê in his later treatments of *Archiphanurus Szabó (=Paratelenomus*) (Lê 1997, 2000).

*Paratelenomus tetartus* (Crawford)

http://bioguid.osu.edu/xbiod_concepts/3345

Figures 17–27

*Dissolcus tetartus* Crawford, 1911: 270 (original description); Kieffer 1926: 124, 125 (description, keyed); Wall 1931: 381 (repetition of Crawford (1911), variation).


*Paratelenomus tetartus* (Crawford): Johnson 1992: 564 (cataloged, type information);
Johnson 1996: 277, 286 (description, keyed); Johnson 1996: 277, 286 (description, keyed).

*Archiphanurus longus* Kozlov & Lê syn. nov.: Lê 1997: 24, 27 (original description, keyed); Lê 2000: 251 (description, type information).


Figures 17–21. *Paratelenomus tetartus* 17 female holotype of *P. tetartus* (USNMENT00989067), anterolateral view 18 female holotype of *P. longus* (IEBR 0049), head, anterolateral view 19 female holotype of *P. longus* (IEBR 0049) head and mesosoma, lateral view 20 female holotype of *P. longus* (IEBR 0049), head and mesosoma, dorsolateral view 21 female holotype of *P. longus* (IEBR 0049), head, mesosoma, metasoma, dorsal view. Scale bars: in millimeters.


**Comments.** The synonymy of *Paratelenomus longus* (Kozlov & Lê) is based on photographs of the holotype specimen provided by Talamas and Pham (2017) (Figs 18–

21) and images of the holotype of *P. tetartus* provided by Talamas et al. (2017) (Fig. 17). The mesopleuron on the holotype of *P. longus* is obscured by its foreleg and by glue (Fig. 19), making it impossible to confidently assess the characters of couplet 3 in the identification key of Johnson (1996). However, we can exclude the species that follow the first lead of the couplet because this specimen has microsculpture throughout the mesoscutum and mesoscutellum, well-developed submedian carinae on the frons, and T1 is xanthic and distinctly contrasting in color with the following metasomal segments. This combination of characters is not found in *P. angor* Johnson (Figs 28, 29),
Figure 27. Paratelenomus tetartus, female (OSUC 398251), head, mesosoma, metasoma, lateral view. Scale bar: in millimeters.

*P. matinalis* Johnson (Figs 30, 31), or *P. striativentris* (Risbec) (Figs 32, 33). Following the second lead of the couplet results in an identification of *P. tetartus*, which matches the visible morphology and collection locality of *P. longus*.

Treatment of *P. mangrovus* as a junior synonym follows reexamination of the holotype of this species and reevaluation of the characters used by Rajmohana & Narendran (2007) to separate it from *P. tetartus*. Specifically, the length of the orbital carina and the proximity of the cells of the postacetabular and mesopleural epicoxal sulci are variable. The lateral habitus image of the holotype of *P. mangrovus* (Fig. 26) does not illustrate the presence of 4–5 episternal foveae, but instead illustrates the presence of 4–5 foveae immediately dorsal to the mesopleural carina, which is known to exist in *P. tetartus* (Fig. 27).

Comments on taxonomy of *Paratelenomus*

There remain two species of *Paratelenomus*, *P. aculus* (Lê) and *P. irritus* (Lê), for which the species concepts are unclear. Lê (1980) stated that *P. irritus* (=*Archiphanurus irritus*) had “notauli absent” yet illustrated this species with short notauli. Lê (1980) also stated “notauli usually short” for *P. aculus* (=*Archiphanurus aculus*), and the notauli for this species are illustrated in Lê (2000) as extending for half the length of the mesoscutum. Based on this evidence, we conclude that both species have notauli, which exclude these names as possibilities for the species we here describe as new. Treatment of *P. irritus* and *P. aculus* will require examination of additional type material, and we note that the holotype specimens of these species were not present in the Institute for Ecology and Biological Resources, Hanoi, Vietnam, during EJT’s visit to this collection in 2016.
Discussion

Phoresy is common in the arthropod world (Ferrière 1926, Clausen 1976) but among insect parasitoids, it is mostly restricted to those that parasitize eggs (Clausen 1976). Females of about 35 egg parasitoid species are known to hitchhike on the adult hosts...
to reach their egg-laying sites (Huigens and Fatouros 2013). Phoresy is a highly specialized strategy exploited by egg parasitoids to reduce the spatial and temporal discontinuity between where hosts mate and where host females oviposit (Clausen 1976, Vinson 1998, Fatourous and Huigens 2012) and facilitates dispersal of the parasitoids with their hosts. In phoretic species, the age and quality of the host eggs can be crucial for the success of parasitism.

Phoresy has now been documented in several scelionid genera: Synoditella Muesebeck, Sceliocerdo Muesebeck, and Scelio Latreille on grasshoppers (Acrididae) (Lanham and Evans 1958, Brues 1917, Veenakumari et al. 2012, Noble 1935), Thoronella Masner on an aeshnid dragonfly, Epiaeschna heros (Fabr.) (Carlow 1992), Mantibaria Kirby on Mantodea (Kirby 1900), and Protelenomus on coreid bugs (Kohno 2002). This is the first report of phoretic behavior in Paratelenomus, which may be present in species other than P. anu. Both in captivity and in the field, females of P. anu were found to be phoretic on their bug hosts, with up to five wasps on a single host bug. Thus, the absence of reported phoresy in P. saccharalis, combined with the amount of attention it has received as a biological control agent, provides reasonable evidence that P. saccharalis is not phoretic. Biological data and a species-level phylogeny are needed to determine if phoresy in P. anu is an independent derivation or if it is phenomenon that arose earlier in the evolution of Paratelenomus. We surmise that the phoretic behavior of P. anu gives it a competitive advantage by enabling it to parasitize the eggs at the earliest possible moment. However, a cost to phoresy may exist because females of P. anu spend time attached to adult bugs instead of searching for egg masses, thus limiting their dispersal ability. In a comparison of P. saccharalis and P. anu, this idea is supported by the distributional data of the species: P. saccharalis is extremely widespread (Europe, Africa, tropical Asia, northern Australia (Johnson 1996)) while P. anu is known only from India. However, it must also be considered that the absence of P. anu from collections may be an artifact of collecting methods that are biased toward free-living, non-phoretic insects, such as Malaise and yellow pan traps.

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### Supplementary material 1

**URI table of HAO morphological terms**

Authors: Keloth Rajmohana, James P. Sachin, Elijah J. Talamas, Mukundan S. Shamyasree, S. K. Jalali, Ojha Rakshit  
Data type: Microsoft Excel Spreadsheet (.xlsx)  
Explanation note: This table lists the morphological terms used in this publication and their associated concepts in the Hymenoptera Anatomy Ontology  
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Link: https://doi.org/10.3897/jhr.73.34262.suppl1

### Supplementary material 2

**Barcode sequence of CO1 for *Paratelenomus anu***  
Authors: Keloth Rajmohana, James P. Sachin, Elijah J. Talamas, Mukundan S. Shamyasree, S. K. Jalali, Ojha Rakshit  
Data type: Microsoft Word Document (.docx)  
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