RESEARCH ARTICLE



The oribatid mite subgenus Galumna (Galumna) (Acari, Oribatida, Galumnidae) in the Philippines

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Academic editor: V. Pesic R	eceived 14 September 2014 Accepted 13 October 2014 Published 4 November 2014
h	ttp://zoobank.org/9FEDDB41-5661-4EF8-BFAD-25247359CBBB

Citation: Ermilov SG, Corpuz-Raros L, Tolstikov AV (2014) The oribatid mite subgenus *Galumna* (*Galumna*) (Acari, Oribatida, Galumnidae) in the Philippines. ZooKeys 452: 1–13. doi: 10.3897/zooKeys.452.8212

Abstract

Five species of the subgenus *Galumna* (*Galumna*) (Acari, Oribatida, Galumnidae) are registered in the Philippine oribatid mite fauna. A new species, *G.* (*G.*) *makilingensis* **sp. n.**, is described; it is most similar morphologically to *G.* (*G.*) *tokyoensis* Aoki, 1966, but differs from the latter by the morphology of porose areas *Aa* and *Ap*, rostral setae, and length of interlamellar setae. Three species, *G.* (*G.*) *crenata* Deb & Raychaudhuri, 1975, *G.* (*G.*) cf. *exigua* Sellnick, 1925 and *G.* (*G.*) *khoii* Mahunka, 1989, are recorded in the Philippines for the first time. The species *G.* (*G.*) *crenata* is redescribed. An identification key to the Philippine species of *Galumna* (*Galumna*) is given.

Keywords

Oribatida, Galumnidae, Galumna (Galumna), new species, supplementary description, key, Philippines

Introduction

Galumna (*Galumna*) is the largest subgenus of *Galumna* Heyden, 1826, comprising 161 species, which have a cosmopolitan distribution (based on data by Subías 2004, updated 2014). In the course of taxonomic identification of oribatid mites from the Philippines, we found five species of this subgenus: one species is represented as a new to science and other four are already known ones (see *Checklist* section below). At present, only *G.* (*G.*) *flabellifera* Hammer, 1958 was reported from the Philippines (see Corpuz-Raros 1979; Corpuz-Raros and Gruèzo 2011).

The primary goal of the present paper is to describe and illustrate a new species. The secondary goal is to make a supplementary description of G. (G.) crenata based on the Philippine material, which was originally described by Deb and Raychaudhuri (1975) from India. The first description of G. (G.) crenata was incomplete, and lacks information on the length of morphological structures, leg setation, solenidia, gnathosoma, and the illustrations were insufficient.

In addition, we present an identification key to the Philippine species of *Galumna* (*Galumna*) below.

Material and methods

The species of Galumna (Galumna) were found in 11 sites:

- L-1 Philippines, Mindanao Island, Nasipit Lumber Company, Tungao, Agusan del Norte, in leaf litter, 28.V.1977, collected by J.M. Sotto and R.C. Garcia.
- L-3 Philippines, Luzon Island, Animal Science pasture, University of the Philippines Los Baños campus, College, Laguna, in litter from pasture, 28.VI.1975, collected by J.M. Sotto and R.C. Garcia.
- L-5 Philippines, Luzon Island, Mt. Makiling, Makiling Botanic Gardens, Los Baños, Laguna, in topsoil from plantation of Moluccan Sau (*Albizia falcataria*), 8.VI.1975, collected by J.M. Sotto and R.C. Garcia.
- L-16 Philippines, Luzon Island, Mt. Makiling, Makiling Botanic Gardens, Los Baños, Laguna, in litter from undistrurbed secondary forest, 1.VI.1975, collected by J.M. Sotto and R.C. Garcia.
- L-20 Philippines, Luzon Island, Mt. Makiling, Makiling Botanic Gardens, Los Baños, Laguna, in litter from plantation of molave (*Vitex parviflora*), 19.VII.1975, collected by J.M. Sotto and R.C. Garcia.
- L-21 Philippines, Luzon Island, Maddela, Quirino, in bamboo leaf litter, 11.XI.1975, collected by P.S. Raros.
- L-23 Philippines, Panay Island, Panay State Polytechnic College campus, Mambusao, Capiz, in grass litter, 12.X.1990, collected by A.M. Almeroda.
- L-34 Philippines, Luzon Island, Tagga, Tuguegarao, Cagayan, in forest litter, 14.XI.1975, collected by P.S. Raros.
- L-40 Philippines, Luzon Island, Animal Science pasture, University of the Philippines
 Los Baños campus, College, Laguna, in litter at base cogon (*Imperata cylindrica*),
 16.IX.1975, collected by J.M. Sotto and R.C. Garcia.
- L-43 Philippines, Mindanao Island, Nasipit Lumber Company, Tagpange, Tungao, Agusan del Norte, in litter from *Albizia falcataria*–Ipomoea sp., fern vegetation, 28.IV.1975, collected by R.S. Raros.
- L-45 Philippines, Luzon Island, Mt. Makiling, on north trail to peak, Los Baños in litter under pakong-lawit (*Goniophlebium percusum*, Polypodiaceae, fern), 4.V.1975, collected by J.M. Sotto.

Specimens were mounted in lactic acid on temporary cavity slides for measurement and illustration. The body length was measured in lateral view, from the tip of the rostrum to the posterior edge of the ventral plate. The notogastral width refers to the maximum width in dorsal aspect. Lengths of body setae were measured in lateral aspect. All body measurements are presented in micrometers. Formulae for leg setation are given in parentheses according to the sequence trochanter–femur–genu–tibia–tarsus (famulus included). Formulae for leg solenidia are given in square brackets according to the sequence genu–tibia–tarsus. General terminology used in this paper follows that of Grandjean (summarized by Norton and Behan-Pelletier 2009). Drawings were made with the drawing tube using the Carl Zeiss transmission light microscope "Axioskop-2 Plus" at Tyumen State University, Russia.

Checklist of registered Galumna (Galumna) species

Galumna (*Galumna*) *crenata* Deb & Raychaudhuri, 1975. Distribution: India. Locality: L-1. First record in the Philippines.

- *Galumna* (*Galumna*) cf. *exigua* Sellnick, 1925. Distribution: Sumatra. Localities: L-1, L-20, L-21, L-23, L-43, L-45. First record in the Philippines.
- *Galumna (Galumna) flabellifera* Hammer, 1958. Distribution: Pantropics and Subtropics. Localities: L-1, L-3, L-5, L-21, L-23, L-34.

Galumna (*Galumna*) *khoii* Mahunka, 1989¹. Distribution: Vietnam. Localities: L-3, L-16, L-23, L-40. First record in the Philippines.

Galumna (Galumna) makilingensis sp. n.: Locality: L-45

Results

Description of Galumna (Galumna) makilingensis sp. n.

http://zoobank.org/4BC74FB7-37C4-4670-8C6D-0BB8BA67C262 Figs 1–4

Diagnosis. With generic characters of *Galumna* as summarized by Ermilov et al. (2013). Body size: $647-680 \times 498-547$. Rostrum pointed. Rostral setae of medium size, ciliate. Lamellar and interlamellar setae long, slightly barbed. Bothridial setae spindle-form. Lamellar lines very strong, divergent in distal parts to sublamellar lines. Anterior notogastral margin developed. Four pairs of porose areas present; *Aa* boomerang-like, other rounded or oval. Median pore present. Postanal porose area long, elongated.

Description. *Measurements.* Body length: 647 (holotype, female), 680 (one paratype, female); notogaster width: 498 (holotype), 547 (one paratype).

¹ It is possible that *Galumna* (*Galumna*) khoii Mahunka, 1989 is a junior synonym of *G*. (*G*.) lanceata (Oudemans, 1900) (see Ermilov and Anichkin 2014).



Figure I. Galumna (Galumna) makilingensis sp. n., adult: dorsal view. Scale bar 200 µm.

Integument. Body color brown. Body surface smooth.

Prodorsum. Rostrum pointed. Rostral setae (ro, 49–57) setiform, ciliate unilaterally. Lamellar (le, 118–127) and interlamellar (in, 172–184) setae setiform, slightly barbed. Bothridial setae (ss, 135–147) spindle-form, with long stalk and short, slightly barbed head. Exobothridial setae and their alveoli absent. Porose areas Ad oval, transversally oriented (24–32 × 6–10). Sublamellar lines (S) distinct, thin, curving backwards. Lamellar lines (L) very strong, parallel in basal parts and divergent in distal parts to sublamellar lines.

Notogaster. Anterior notogastral margin developed. Dorsophragmata (*D*) of medium size, longitudinally elongated. Notogastral setae represented by 10 pairs of alveoli. Four pairs of porose areas with distinct borders: *Aa* boomerang-like (90–102 × 8–16), other porose areas rounded or oval; *A1* (20–24 × 12–16 or diameter 16–20), *A2* (24–32 × 12–16 or diameter 16–20) and *A3* (24–45 × 20–24). Alveoli *la* inserted posteriorly to *Aa*. Lyrifissures *im* and opisthonotal gland openings (*gla*) located laterally to *A1*. Median pore (*mp*) present, located little posterior to virtual line connecting porose areas *A2*.



Figure 2. *Galumna* (*Galumna*) *makilingensis* sp. n., adult: ventral view (gnathosoma and legs not illustrated). Scale bar 200 µm.

Gnathosoma. Morphology of subcapitulum, palps and chelicerae generally typical for species of the subgenus *Galumna* (*Galumna*) (for example, Engelbrecht 1969; Ermilov and Anichkin 2011; Ermilov et al. 2011). Subcapitulum longer than wide (184 × 155). Subcapitular setae simple, slightly barbed: *a* (32–36) longer than *m* and *h* (both 24–28). Two pairs of adoral setae (20) setiform, barbed. Palps (135–139) with setation $0-2-1-3-9(+\omega)$. Solenidion straight, thickened, blunt-ended, attached to eupathidium. Chelicerae (229) with two setiform, barbed setae; *cha* (57) longer than *chb* (32). Trägårdh's organ distinct, tapered.

Epimeral and lateral podosomal regions. Apodemes (1, 2, sejugal, 3) well visible. Four pairs of setiform, slightly barbed epimeral setae present; 1a and 3b (41–49) longer than 4a and 4b (24–32). Pedotecta II rectangular, rounded anteriorly in ventral view. Discidia (*dis*) rounded distally. Circumpedal carinae (*cp*) of medium length, directed to 3b.



Figures 3–4. *Galumna (Galumna) makilingensis* sp. n., adult: **3** dorso-lateral view of prodorsum, left pteromorph and anterior part of notogaster (gnathosoma and leg I not illustrated) **4** posterior view of notogaster. Scale bar 200 µm.

Anogenital region. Six pairs of genital $(g_1-g_6, 20-24)$, one pair of aggenital (ag, 20-24), two pairs of anal $(an_1, an_2, 28-32)$ and three pairs of adamal $(ad_1-ad_3, 28-32)$ setae setiform, slightly barbed. Anal and adamal setae slightly thicker than genital and

Leg	Trochanter	Femur	Genu	Tibia	Tarsus
Ι	v	d, (l), bv"	<i>(l)</i> , ν', σ	(<i>l</i>), (<i>v</i>), φ_1, φ_2	(ft), (tc), (it), (p), (u), (a), s, (pv), v', (pl), l", ε , ω_1 , ω_2
II	v	d, (l), bv"	<i>(l)</i> , ν', σ	<i>(l)</i> , <i>(v)</i> , φ	(ft), (tc), (it), (p), (u), (a), s, (pv), ω_1, ω_2
III	v	d, ev'	<i>l</i> ', σ	<i>l'</i> , <i>(v)</i> , φ	(ft), (tc), (it), (p), (u), (a), s, (pv)
IV	v	<i>d</i> , <i>ev</i> '	d, l'	<i>l'</i> , <i>(v)</i> , φ	ft", (tc), (p), (u), (a), s, (pv)

Table 1. Leg setation and solenidia of *Galumna* (*Galumna*) *makilingensis* sp. n. (same data for *G.* (*G.*) *crenata* Deb & Raychaudhuri, 1975).

Roman letters refer to normal setae (ϵ to famulus), Greek letters to solenidia. Single prime (') marks setae on anterior and double prime (') setae on posterior side of the given leg segment. Parentheses refer to a pair of setae.

aggenital setae. Anterior edge of genital plates with two setae. Adanal setae ad_3 inserted laterally to adanal lyrifissures *iad*. Postanal porose area (*Ap*) long, elongated, transversally oriented (61–77 × 8–12).

Legs. Morphology of leg segments, setae and solenidia generally typical for species of the subgenus *Galumna* (*Galumna*) (for example, Engelbrecht 1969; Ermilov and Anichkin 2011; Ermilov et al. 2011; Bayartogtokh and Akrami 2014). Formulae of leg setation and solenidia: I (1-4-3-4-20) [1-2-2], II (1-4-3-4-15) [1-1-2], III (1-2-1-3-15) [1-1-0], IV (1-2-2-3-12) [0-1-0]; homology of setae and solenidia indicated in Table 1.

Material examined. Holotype (female) and one paratype (female): L-45.

Type deposition. The holotype is deposited in the collection of the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia; one paratype (dissected) is deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

Etymology. The specific name "*makilingensis*" refers to the type locality, Mt. Makiling, the forest reservation of the University of the Philippines Los Baños.

Comparison. In having large body size, pointed rostrum, spindle-form bothridial setae, long prodorsal setae, anterior notogastral margin, four pairs of notogastral porose areas, *Galumna* (*Galumna*) makilingensis sp. n. is most similar to *Galumna* (*Galumna*) tokyoensis Aoki, 1966 from the Palaearctic region (Aoki 1966). However, it clearly differs from the latter by the boomerang-like porose areas *Aa* and long, elongated postanal porose area (versus both oval in *G.* (*G.*) tokyoensis), ciliate rostral setae (versus smooth in *G.* (*G.*) tokyoensis) and interlamellar setae longer than lamellar setae (versus similar in length in *G.* (*G.*) tokyoensis).

Also, in having large body size, bothridial setae with dilated head, long prodorsal setae, anterior notogastral margin, four pairs of notogastral porose areas, *Aa* boomerang-like, *Galumna* (*Galumna*) makilingensis sp. n. is most similar to *Galumna* (*Galumna*) cuneata Aoki, 1961 from the Palaearctic region (Aoki 1961). However, it clearly differs from the latter by the pointed rostrum (versus rounded in *G.* (*G.*) cuneata), ciliate rostral setae (versus smooth in *G.* (*G.*) cuneata), interlamellar setae longer than lamellar setae (versus similar in length in *G.* (*G.*) cuneata) and spindle-form bothridial setae (versus clavate in *G.* (*G.*) cuneata).

Supplementary description of *Galumna* (*Galumna*) crenata Deb & Raychaudhuri, 1975

Figs 5-8

Description. *Measurements.* Body length: 348–390 (four specimens, two females and two males); notogaster width: 258–290 (four specimens).

Integument. Body color brown. Body surface smooth, but ventral side covered by the microgranular cerotegument (diameter of granules less than 1), visible only under high magnification (×1000) in dissected specimens. Genital plates with one longitudinal stria in medial parts.

Prodorsum. Rostrum rounded. Rostral setae (24–32) setiform, smooth. Lamellar and interlamellar setae minute (both 6–8), thin, smooth. Bothridial setae (49–57) clavate, with long stalk and shorter, rounded and weakly barbed distally head. Exobothridial



Figure 5. Galumna (Galumna) crenata Deb & Raychaudhuri, 1975, adult: dorsal view. Scale bar 100 µm.



Figure 6. *Galumna* (*Galumna*) *crenata* Deb & Raychaudhuri, 1975, adult: ventral view (legs not illustrated). Scale bar 100 µm.

setae and their alveoli absent. Porose areas Ad large, oval, transversally oriented (20–22 × 6–8). Lamellar and sublamellar lines distinct, thin, parallel, curving backwards.

Notogaster. Anterior notogastral margin developed, but sometimes poorly visible. Dorsophragmata of medium size, longitudinally elongated. Notogastral setae represented by 10 pairs of alveoli. Four pairs of porose areas with distinct borders: Aa large, boot-shaped or weakly triangular, transversally oriented (32–36 × 12–16); A1, A2 (diameter of both 8–16) and A3 (diameter of 14–20) rounded. Alveoli *la* inserted posteriorly to Aa. Lyrifissures *im* located between *lm* and *lp*. Opisthonotal gland openings located laterally to A1. Median pore present, located little anterior to virtual line connecting porose areas A3.

Gnathosoma. Morphology of subcapitulum, palps and chelicerae generally typical for species of the subgenus *Galumna* (*Galumna*) (for example, Engelbrecht 1969; Ermilov and Anichkin 2011; Ermilov et al. 2011). Subcapitulum longer than wide $(90-94 \times 82-68)$. Subcapitular setae simple, smooth: *a* (14–16) longer and thicker



Figures 7–8. *Galumna* (*Galumna*) *crenata* Deb & Raychaudhuri, 1975, adult: **7** lateral view of prodorsum, left pteromorph and anterior part of notogaster (gnathosoma and leg I not illustrated) **8** posterior view of notogaster and adanal setae ad_1 and ad_2 . Scale bar 100 µm.

than *m* and *h* (both 6–8). Two pairs of adoral setae (8) setiform, slightly barbed. Palps (57) with setation $0-2-1-3-9(+\omega)$. Solenidion straight, thickened, blunt-ended, attached to eupathidium. Chelicerae (106–110) with two setiform, barbed setae; *cha* (36) longer than *chb* (20). Trägårdh's organ distinct, tapered.

Epimeral and lateral podosomal regions. Anterior tectum of epimere I with numerous rectangular teeth. Apodemes (1, 2, sejugal, 3) well visible. Six pairs of thin, smooth epimeral setae (8–12) present. Setae *4c* inserted on tubercle. Pedotecta II rectangular, rounded anteriorly in ventral view. Discidia triangular. Circumpedal carinae long, directed to pedotecta I.

Anogenital region. Six pairs of genital setae $(g_1, g_2, 8, g_3-g_6, 4-6)$, one pair of aggenital (ag, 6-8), two pairs of anal $(an_1, an_2, 8)$ and three pairs of adanal $(ad_1-ad_3, 8)$ setae thin, smooth. Anterior edge of genital plates with two setae. Adanal setae ad_3 inserted laterally to adanal lyrifissures *iad*. Postanal porose area oval, transversally oriented $(12-20 \times 6-8)$.

Legs. Morphology of leg segments, setae and solenidia generally typical for species of the subgenus *Galumna* (*Galumna*) (for example, Engelbrecht 1969; Ermilov and Anichkin 2011; Ermilov et al. 2011; Bayartogtokh and Akrami 2014). Formulae of leg setation and solenidia: I (1-4-3-4-20) [1-2-2], II (1-4-3-4-15) [1-1-2], III (1-2-1-3-15) [1-1-0], IV (1-2-2-3-12) [0-1-0]; homology of setae and solenidia indicated in Table 1.

Material examined. Four specimens (two females and two males): L-1.

Remarks. Galumna (Galumna) crenata distinctly differs from other species of the sugenus by the presence of dentate anterior tectum of epimere I. The available Philippine specimens of this species are morphologically and in general appearance similar to the Indian specimens (Deb and Raychaudhuri, 1975). Three main differences are as follows:

1) Body longer (348–390 versus 319–325 in Indian specimens). We believe these differences represent intraspecific (perhaps geographical) variability.

2) Anterior notogastral margin is well visible (versus completely absent in Indian specimens); also, the text of other paper (Sarkar et al. 2007) on G. (G.) crenata assert that it is present.

3) Rostral, lamellar and interlamellar setae developed (versus absent in Indian specimens). We believe these differences can be erroneous. The reason is that Deb and Raychaudhuri (1975) inadequately described this species and, probably, they overlooked these setae, because the rostral setae are usually strongly pressed to the prodorsum surface and are often not visible in dorsal and ventral views, and the lamellar and interlamellar setae are minute, well visible only under high magnification.

Key to species of Galumna (Galumna) of the Philippines

2 Lamellar and interlamellar setae well developed, long; bothridial setae lanceolate; body size: 425–482 × 305–344 G. (G.) khoii Mahunka, 1989. Distribution: Vietnam and Pilippines. Lamellar and interlamellar setae minute or absent; bothridial setae clavate....3 3 Porose areas Aa elongated, transversally oriented, boot-shaped or weakly triangular; anterior tectum of epimere I dentate; body size: 319-390 × 249-290......G. (G.) crenata Deb & Raychaudhuri, 1975 (including our data). Distribution: India and Philippines. 4 Bothridial heads densely ciliale; anterior notogastral margin developed; body size: 303–348 × 204–220.....G. (G.) flabellifera Hammer, 1958 (see also Aoki 1964, 1965, 1982). Distribution: Pantropics and Subtropics. Bothridial head smooth; anterior notogastral margin not developed; body size: 330 × 264 G. (G.) cf. exigua Sellnick, 1925. Distribution: Sumatra and Philippines.

Acknowledgements

We cordially thank Prof. Dr. Badamdorj Bayartogtokh (National University of Mongolia, Ulaanbaatar, Mongolia) and one anonymous reviewer for the valuable comments, Mr. Orlando Eusebio and Jeremy Naredo (Museum of Natural History, University of the Philippines Los Baños, College, Laguna, Philippines) for facilitating dispatchment of the specimens, together with other oribatids, to Russia.

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RESEARCH ARTICLE



Systematics of the freshwater leech genus Hirudinaria Whitman, 1886 (Arhynchobdellida, Hirudinidae) from northeastern Thailand

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Academic editor: F. Govedich Received 16 March 2014 Acce	epted 1 October 2014 Published 4 November 2014
http://zoobank.org/0EC15CFA-3308-40	

Citation: Tubtimon J, Jeratthitikul E, Sutcharit C, Kongim B, Panha S (2014) Systematics of the freshwater leech genus *Hirudinaria* Whitman, 1886 (Arhynchobdellida, Hirudinidae) from northeastern Thailand. ZooKeys 452: 15–33. doi: 10.3897/zooKeys.452.7528

Abstract

In total, 435 specimens of the Southeast Asian freshwater leech species within the Hirudinidae family were collected from 17 locations of various types of aquatic habitats in northeastern Thailand. They were all morphologically placed within the genus Hirudinaria Whitman, 1886 and there were three distinct species: the common Hirudinaria manillensis, 78.2% of all collected specimens and at all 17 locations, Hirudinaria javanica at 20.3% of collected samples and from five locations and a rarer unidentified morphospecies (Hirudinaria sp.) with six samples from only two locations. The karyotypes of these three species were examined across their range in this study area for 38, 11 and 6 adult specimens of Hirudinaria manillensis, Hirudinaria javanica and Hirudinaria sp., respectively. This revealed different chromosome numbers among all three species, with Hirudinaria javanica having n = 13, 2n = 26, Hirudinaria manillensis lacked one small chromosome pair with n = 12, 2n = 24, and the unknown Hirudinaria sp. differed from any known Hirudinaria karyotypes in exhibiting a higher chromosome number (n = 14, 2n = 28) and a gradual change in size from large to small chromosomes. This suggests that the unknown Hirudinaria sp. is a new biological species. However, phylogenetic analysis based upon a 658 bp fragment of the cytochrome oxidase subunit I gene placed this unknown morphospecies within the *Hirudinaria* manillensis clade, perhaps then suggesting a recent sympatric speciation, although this requires further confirmation. Regardless, the chromosomes of all three species were asymmetric, most with telocentric

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elements. A distinct bi-armed chromosome marker was present on the first chromosome pair in *Hirudi-naria javanica*, whilst it was on pairs 1, 2, 3 and 5 in *Hirudinaria manillensis*, and on pairs 3 and 5 for the unknown *Hirudinaria* sp.

Keywords

Freshwater leeches, Hirudinea, karyotypes, morphology, COI, sanguivorous

Introduction

The family Hirudinidae (Arhynchobdellida, Hirudiniformes) is comprised of mainly blood-sucking (sanguivorous) freshwater leeches, or medicinal leeches, although four terrestrial species are known. It includes approximately 60 hirudinids ranging across all continents, except for Antarctica, and from temperate to tropical regions (Elliott and Kutschera 2011). On the basis of the number of complete somites, the distance between the third and fourth pair of eyes, number of sensillae, position of the nephropore opening, and the presence or absence of auricular characters, the Hirudinidae family is divided into the two subfamilies of Hirudininae and Haemadipsinae. The Hirudininae, or buffalo leeches, contains 12 known species (Moore 1927) within six genera (Dinobdella, Hirudinaria, Hirudo, Limnatis, Myxobdella and Whitmania), and are distributed in temperate and tropical Asia, Africa and the Caribbean islands (Richardson 1969, Sket and Trontelj 2008, see Phillips and Siddall 2009 for alternative classification). The high species diversity and their wide geographic distribution make the hirudinid leeches attractive material for systematic and biogeographical studies. However, due to their conserved morphology, it is not easy to establish a reliable phylogenetic hypothesis for this group. There is only one recent published paper regarding their phylogenetic relationship that considered both morphological and molecular analyses and described a new species of the Asian buffalo leech, Hirudinaria bpling Phillips, 2012.

The genus *Hirudinaria* Whitman, 1886 consists of only three known species *Hirudinaria javanica* (Wahlberg, 1856), *Hirudinaria manillensis* (Lesson, 1842), and *Hirudinaria bpling* that are widely distributed over tropical South and Southeast Asia, being recorded from within Peninsular Malaysia, Thailand, Indo-China, Indonesia, Philippines, China, Myanmar, Bangladesh, India and Sri Lanka (Moore 1938, Lai and Chen 2010). Chromosomal data for hirudinid leeches have only been recorded for three species of *Hirudo* (Utevsky et al. 2009).

In this study, we examined the karyotypes of 38, 11 and 6 specimens of the three species (*H. manillensis*, *H. javanica*, and a third distinct and different morphospecies, *Hirudinaria* sp.) collected from across 17 locations in northeastern Thailand, representing 13.4%, 12% and 100% of the collected samples, respectively. Their systematic implications are then discussed in comparison with other previously reported hirudinid karyotypes. The phylogenetic analysis, based upon a 658 bp fragment of the cytochrome oxidase subunit I gene, was also conducted to clarify the systematics of all collected morphospecies.

Materials and methods

Locality, co-ordination and sample size for all collected species are given in Table 1. Species identification of each specimen was made on the basis of Lesson (1842), Wahlberg (1856), Whitman (1886), Moore (1927), Richardson (1969), Klemm (1972) and Lai and Chen (2010). Voucher specimens were deposited in the Museum of Zoology, Chulalongkorn University, Bangkok, Thailand (CUMZ).

Freshwater leeches were collected from 17 localities in northeastern Thailand (Fig. 1 and Table 1) during invertebrate faunal surveys performed from April 2012 to February 2014. In total, 435 adult specimens were collected and examined. Specimens were photographed and kept alive in a glass aquarium in order to observe the body color pattern and other external morphological characteristics, plus any behavioral traits. Most specimens were relaxed in 10% (v/v) ethanol and then fixed and kept in 95% (v/v) ethanol for further external and internal morphological studies. Some specimens were brought back alive to the laboratory for karyotypic analysis.

Jaws of some specimens were examined by scanning electron microscopy (SEM). The dried specimens were sputter coated with 35 nm of gold/palladium before being examined using a LEO/Zeiss DSM982 Geminifield emission scanning electron microscope located in the Scientific and Technological Research Equipment Centre, Chulalongkorn University.

Chromosome preparations were made from the testisac using hypotonic, fixation and air-drying techniques modified from Patterson and Burch (1978) and Kongim et al. (2013). Live leeches were injected with 0.1 mL of 0.1% (v/v) colchicine, left for 3–4 h and then dissected to remove the testisacs into 0.07% (w/v) KCl solution (hypotonic) for 30 min. Samples were then fixed in fresh Carnoy's fixative (3:1 (v/v) absolute ethanol: glacial acetic acid). The testisacs were cut into small pieces in fresh Carnoy's fixative and the separated cells were collected by centrifugation at 1,500 rpm for 10 min. The supernatant was removed and the cell pellet resuspended in 0.5 mL of fresh Carnoy's fixative. Cell suspensions were dropped onto clean pre-heated (60 °C) glass slides, air-dried and stained in 4% (v/v) Giemsa solution for 10 min. Photomicrographs of 10 to 15 well-spread metaphase cells were measured for their relative length and centromeric index. Mitotic karyotypes were arranged and numbered for chromosome pairs.

For the molecular analysis, the total genomic DNA was extracted from a part of the wall-body muscle to avoid contamination from the host DNA, following the standard protocol of the DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA). A fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'), which is the region used in animal DNA barcoding (Folmer et al. 1994). Polymerase chain reaction (PCR) of a 50 μ L final volume using 20 μ M of 2×Illustra hot starts master mix (GE Healthcare), plus 10 μ M of each primer and about 10 ng of DNA template was performed in an eppendorf Mastercycler[®] pro S PCR thermal cycler with the following



Figure 1. Map showing the locality of the sampling sites (collection of specimens from the genus *Hirudinaria*) in northeastern Thailand. Further details of sample numbers and locations are given in Table 1.

thermal cycling conditions: 3 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 45 °C and 150 s at 72 °C, before a final extension at 72 °C for 5 min. The PCR products were purified with a QIAquick PCR purification Kit (QIAGEN Inc.) before being commercially direct cycle-sequenced at Macrogen, Inc, Korea.

Sequence alignment and editing were performed using MEGA 6.06 (Tamura et al. 2013). The best-fit models of nucleotide substitution, as judged by the Akaike information criterion (AIC: Akaike 1974), were estimated using Kakusan4 (Tanabe 2007; with maximum likelihoods calculated in Treefinder, Jobb et al. 2004). The bestfit evolution model obtained was GTR+G. Phylogenetic trees based on maximum likelihood (ML) and Bayesian inference (BI) were constructed. The ML analysis was performed with Treefinder (Jobb et al. 2004), using the likelihood-ratchet method with 1000 bootstrap replicates. The BI tree was constructed using MrBayes v3.2.2 (Ronquist et al. 2012), which employs a Metropolis-coupled, Markov chain Monte Carlo (MC-MCMC) sampling approach. The BI analysis was run twice in parallel for one million generations (with default heating values), starting with a random tree, and trees were sampled every 100 generations. The remaining trees, after discarding 25% of "burn-in" samples, were used for calculation of the bipartition posterior probability (Ronquist et al. 2012). Tree topologies with bootstrap values of 70% or greater for ML and/or a bipartition posterior probability of 0.95 or greater for the BI were regarded as sufficiently resolved (Huelsenbeck and Hillis 1993, Larget and Simon 1999). Pairwise

No.	Locality	Coordinates	Numbe	r of specimens	examined
			Hirudinaria javanica	Hirudinaria manillensis	Hirudinaria sp.
1	Ban Donsala, Na Wa, Nakhon Phanom	17°34'27.22"N, 104°7'18.64"E	44	82	5
2	Ban Majang, Na Wa, Nakhon Phanom	17°36'53.4"N, 104°8'21.9"E	-	51	1
3	Ban Nongwang, Tao Ngoi, Sakon Nakhon	17°45'41.26"N, 103°44'42.00"E	9	4	-
4	Phang Khon, Sakon Nakhon	17°22'29.02"N, 103°40'26.81"E	-	2	-
5	Mueang, Sakon Nakhon	17°10'52.69"N, 104°7'50.94"E	-	2	-
6	Phu Phan, Sakon Nakhon	16°54'14.64"N, 103°54'7.50"E	-	6	-
7	Ban Janpen, Tao Ngoi, Sakon Nakhon	16°55'32.59"N, 104°10'9.31"E	16	1	-
8	Ban Nonghai, Khamcha-i, Mukdahan	16°34'53.92"N, 104°29'29.00"E	13	13	-
9	Khong Chai, Kalasin	16°15'44.76"N, 103°27'22.91"E	-	28	-
10	Ban Thatoom, Mueang, Mahasarakham	16°10'48.40"N, 103°26'59.30"E	-	4	-
11	Huai E-pong, Phu Wiang, Khon Kaen	16°43'51.30"N, 102°17'17.00"E	-	11	-
12	Tumbon Bung, Mueang Amnat Charoen	15°50'21.48"N, 104°27'33.95"E	-	30	-
13	Pa Tio, Yasothon	15°57'2.81"N, 104°25'12.78"E	-	3	-
14	Khemarat, Ubon Ratchathani	15°59'11.82"N, 105°8'20.53"E	-	26	-
15	Chaturaphak Phiman, Roi Et	15°49'59.77"N, 103°31'0.86"E	1	5	-
16	Kaset Wisai, Roi Et	15°39'13.70"N, 103°35'58.39"E	-	67	-
17	Huai Saneng Reservoir, Surin	14°47'14.70"N, 103°28'34.50"E	-	11	-

Table 1. Locality, co-ordination and sample size of each species used in the present study. Locality numbers refer to the localities shown in Figure 1.

(uncorrected-p) sequence distances were also calculated using MEGA 6.06 (Tamura et al. 2013).

Nucleotide sequences obtained in this study have been deposited in the GenBank database under the GenBank ID: KJ551848–KJ551855.

Results

All 435 examined specimens in this study were assigned as belonging to the genus *Hirudinaria* by the following distinct characters; male pore and female pore separated

by 5–7 annuli, sensillae large and elongated, salivary papillae present, and without vaginal stalk. From these identified characters, the specimens were determined to be three species: as *Hirudinaria javanica*, *Hirudinaria manillensis*, and an unidentified morphotype, *Hirudinaria* sp. (Figs 2 and 3).

Systematics

Family Hirudinidae Whitman, 1886 Subfamily Hirudininae

Genus Hirudinaria Whitman, 1886

Hirudinaria Whitman, 1886: 373. Moore 1927: 207.

Type species. Sanguisuga javanica Wahlberg, 1856, by original designation.

Hirudinaria javanica (Wahlberg, 1856)

Figs 2A, 3A, 4A, 5A-C

Sanguisuga javanica Wahlberg, 1856: 233. Type locality: Samarang, Java [Semarang, Central Java, Indonesia].
Hirudinaria javanica – Whitman 1886: 373–376, pl. 20, fig. 56.
Limnatis (Poecilobdella) javanica – Blanchard 1897: 349–351, text figure 7.
Limnatis javanica – Kaburaki 1921: 711.
Hirudinaria javanica – Moore 1927: 210–218, figs 50–52.

Material examined. Ban Donsala, Na Wa, Nakhon Phanom: CUMZ 3402 (17 specimens), 3404 (18 specimens; Figs 3A, 4A, 5A-C), 3429 (9 specimens). Ban Nongwang, Tao Ngoi, Sakon Nakhon: CUMZ 3413 (9 specimens). Ban Janpen, Tao Ngoi, Sakon Nakhon: CUMZ 3415 (16 specimens). Ban Nonghai, Khamchaee, Mukdahan: CUMZ 3422 (4 specimens), 3424 (9 specimens; Figs 2A, 6A–B). Chaturaphak Phiman, Roi Et: CUMZ 3419 (1specimen).

Description. In preserved specimens body length 41–184 mm, width 5–16 mm. In live specimens, dorsal side olive green, dark green or yellow brown. Middle dorsal line distinct, black, continuous, parallel with two series of black spots on both sides, two faint black stripes present on each side. Body margin yellow with one ordered series of black spots. Ventral side green without marker. Jaw trignathous, approximately 134 teeth. Number of salivary papillae, both small and large, is 43 glands (Fig. 5A–C). Gonopores separated by seven annuli. Male reproductive system located in middle of body between somites XI and XIII. Ejaculatory bulbs short and small. Ejaculatory



Figure 2. The color pattern of **A** *Hirudinaria javanica* CUMZ 3424 from Mukdahan **B** *Hirudinaria manillensis* CUMZ 3403 from Nakhon Phanom, and **C** *Hirudinaria* sp. CUMZ 3405 from Nakhon Phanom.

ducts long, connect with atrium side in somite XI. Atrium short, small, pear-shaped with unclear penis sheath. Vas deferens straight, runs along almost entire body, with 11 testisac pairs (12 pairs in some specimens). Nerve cord runs along body length on right side of atrium. Ovisacs stout, albumin gland not well developed, common oviduct short, opens into female bursa. Vagina caecum short, ovate in shape, no vaginal stalk (Fig. 4A).



Figure 3. The dorsal and ventral sides of **A** *Hirudinaria javanica* CUMZ 3404 from Nakhon Phanom **B** *Hirudinaria manillensis* CUMZ 3403 from Nakhon Phanom, and **C** *Hirudinaria* sp. CUMZ 3406 from Nakhon Phanom.

Hirudinaria manillensis (Lesson, 1842)

Figs 2B, 3B, 4B, 5D-F

Hirudo manillensis Lesson, 1842: 8. Type locality: Philippine Islands.
Hirudo sanguisorba Tennent, 1859: 305. Type locality: Ceylon. Tennent 1861: 483–484, with text figure. Type locality: Caylon [Sri Lanka].
Hirudo multistriata Schmarda, 1861: 3, Taf. 16, fig. 141. Type locality: Ceylon [Sri Lanka].
Hirudo luzoniae Kinberg, 1866: 356. Type locality: Manila [Philippines].
Hirudo maculosa Grube, 1868: 39–40, Taf. 4, fig. 6. Type locality: Singapore.
Hirudo maculata Baird, 1869: 315. Type locality: Siam [Thailand].
Limnatis (Poecilobdella) granulosa Blanchard, 1893: 28. Type locality: Java, Indonesia Blanchard 1897: 338–349, figs 3–6. Kaburaki 1921: 673–675.
Limnatis granulosa – Robertson 1909: 676–679, fig. 4.
Hirudo boyntoni Wharton, 1913: 369–371. Type locality: Philippines Islands.
Limnatis (Poecilobdella) manillensis – Moore 1924: 376.
Hirudinaria manillensis – Moore 1927: 218–226, fig. 53.

Material examined. Ban Donsala, Na Wa, Nakhon Phanom: CUMZ 3401 (21 specimens), 3403 (4 specimens; Figs 2B, 3B, 4B, 5D–F), 3430 (57 specimens). Ban Majang, Na Wa, Nakhon Phanom: CUMZ 3427 (51 specimens). Ban Nongwang, Tao Ngoi, Sakon Nakhon: CUMZ 3412 (4 specimens). Phang Khon, Sakon Nakhon: CUMZ 3428 (2 specimens). Mueang, Sakon Nakhon: CUMZ 3417 (2 specimens). Phu Phan, Sakon Nakhon: CUMZ 3416 (6 specimens). Ban Janpen, Tao Ngoi, Sakon Nakhon: CUMZ 3414 (1 specimen). Ban Nonghai, Khamchaee, Mukdahan: CUMZ 3423 (13 specimens). Khong Chai, Kalasin: CUMZ 3409 (28 specimens). Ban Thatoom, Mueang, Mahasarakham: CUMZ 3407 (4 specimens; Figs 6C–D). Huai E-pong, Phu Wiang, Khon Kaen: CUMZ 3425 (11 specimens). Bung, Mueang, Amnat Charoen: CUMZ 3410 (30 specimens). Pa Tio, Yasothon: CUMZ 3411 (3 specimens). Khemarat, Ubon Ratchathani: CUMZ 3408 (26 specimens). Chaturaphak Phiman, Roi Et: CUMZ 3418 (5 specimens). Kaset Wisai, Roi Et: CUMZ 3426 (67 specimens). Huai Saneng Reservoir, Surin: CUMZ 3420 (6 specimens), 3421 (5 specimens).

Description. In preserved specimens, body length 27–248 mm, width 3–30 mm. In live specimens, dorsal side dark green or brown. Middle dorsal line distinct, black, incontinuous, with two faint black stripes on each side. Body margin yellow with disrupted black spots. Ventral side brown without marker. Jaw trignathous, approximately 148 teeth. Number of salivary papillae, both small and large sizes, is 30 glands (Fig. 5D–F). Gonopores separated by five annuli. Male reproductive system located in middle of body between somites XI and XII. Ejaculatory bulbs long and large. Ejaculatory ducts short, connect with atrium side in somite XI. Atrium relatively long, large, elongated in shape with penis sheath. Vas deferens curved, runs along almost entire body, 11 pairs of testisac. Nerve cord runs along body length on left side of atrium. Ovisacs stout, albumin gland well developed, common oviduct short, opening into female bursa. Vagina caecum relatively long, ovate in shape, no vaginal stalk (Fig. 4B).



Figure 4. Illustrations of the reproductive system of **A** *Hirudinaria javanica* CUMZ 3404 from Nakhon Phanom, **B** *Hirudinaria manillensis* CUMZ 3403 from Nakhon Phanom, and **C** *Hirudinaria* sp. CUMZ 3405 from Nakhon Phanom. Abbreviations are: ag = albumin gland, at = atrium, cod = common oviduct, eb = ejaculatory bulb, ep = epididymis, g = ganglion, o = ovary, ps = penis sheath, vas = vas deferens, vc = vagina sac, vd = vagina duct.

Hirudinaria sp.

Figs 2C, 3C, 4C, 5G-I

Material examined. Ban Donsala, Na Wa, Nakhon Phanom: CUMZ 3405 (1 specimen; Figs 2C, 4C, 5G–I), 3431 (4 specimens). Ban Majang, Na Wa, Nakhon Phanom: CUMZ 3406 (1 specimen; Figs 3C, 6E–F).

Description. In preserved specimens body length 107–140 mm, width 11–16 mm. In live specimens, dorsal side dark green, brown and dark brown. Middle dorsal line not present. Two brown stripes present each side of mid-dorsal region. Body margin yellow or orange with one ordered series of short black lines.



Figure 5. SEM images of the jaws of **A–C** *Hirudinaria javanica* CUMZ 3404 from Nakhon Phanom **D–F** *Hirudinaria manillensis* CUMZ 3403 from Nakhon Phanom, and **G–I** *Hirudinaria* sp. CUMZ 3405 from Nakhon Phanom. (**A**, **D**, **G**) overall jaw, (**B**, **E**, **H**) each jaw characteristic, and (**C**, **F**, **I**) salivary papillae.

Ventral side brown or dark brown without marker. Jaw trignathous, approximately 167 teeth. Number of salivary papillae, both small and large sizes, is 25 glands (Fig. 5G–I). Gonopores separated by five annuli. Male reproductive system located in middle of body between somites XI and XII. Ejaculatory ducts short, connect with atrium side in somite XI. Atrium moderate sized, penis sheath curved, opening on ventral side. Vas deferens relatively smooth, runs along almost entire body, 11 pairs of testis sacs. Nerve cord runs along body length on right atrium side. Ovisacs somewhat long, albumin gland well developed, common oviduct long, opening into female bursa. Vagina caecum long, elongated in shape, no vaginal stalk (Fig. 4C).



Figure 6. Meiotic and mitotic metaphase chromosome spreads of **A**, **B** *Hirudinaria javanica* (n = 13, 2n = 26) CUMZ 3424 from Mukdahan **C**, **D** *Hirudinaria manillensis* (n = 12, 2n = 24) CUMZ 3407 from Mahasarakham, and **E**, **F** *Hirudinaria* sp. (n = 14, 2n = 28) CUMZ 3406 from Nakhon Phanom.



Figure 7. Karyotypes of A Hirudinaria javanica B Hirudinaria manillensis, and C Hirudinaria sp.

Karyotype results

The chromosomes were typically indistinct because of their small size. Nevertheless, all cleared metaphase arrangements could be observed and the spermatogonial meiotic and mitotic chromosome numbers could be confirmed for all the examined species (Fig. 6). Haploid and diploid numbers of the three species of *Hirudinaria* were found to differ, ranging from n = 12, 2n = 24 for *Hirudinaria manillensis*, n = 13, 2n = 26 for *Hirudinaria javanica*, and n = 14, 2n = 28 for *Hirudinaria* sp. (Figs 6 and 7) and did not differ within each species across their respective geographic populations (Table 3).

Chromosomal data of the three investigated *Hirudinaria* species obtained in the present study are summarized in Table 3 along with that for three other hirudinid species (all from the genus *Hirudo*) from the literature for comparison.

The karyotypes of all three species were asymmetric, and mostly telocentric, chromosomes. The distinct bi-armed chromosome marker varied among the three species, being found on the first pair in *Hirudinaria javanica*, on pairs 1, 2, 3 and 5 for *Hirudinaria manillensis* and on pairs 3 and 5 for *Hirudinaria* sp.

Phylogenetic analysis

The samples used for phylogenetic analysis and their collection locations are summarized in Table 4. A total of 3, 4 and 2 adult specimens of *Hirudinaria manillensis, Hirudinaria javanica* and *Hirudinaria* sp., respectively, were included. Fragments of the mitochondrial COI gene (DNA barcode region) containing 658 base pairs (bp) were used for the phylogenetic tree estimation. The final alignment data metric contained a total of 224 variable sites, 162 sites of which were parsimony informative. The nucleotide compositions of the gene fragments were A (28.32%), C (15.78%), G (15.51%) and T (40.39%). The phylogenetic tree showing the evolutionary relationships among *Hirudinaria* species and related taxa is shown in Fig. 8. Tree topology estimated by ML and BI analyses gave identical topologies with a high support for all major nodes (ML bootstrap values of 99.3–100% and a BI bipartition posterior probability of 1). The phylogenetic tree strongly supported the monophyly of the genus *Hirudinaria*. *Hirudinaria bpling* was basal to the *Hirudinaria javanica* and *Hirudinaria manillensis* clades. *Hirudinaria* sp. came out within the *Hirudinaria manillensis* clade.

The uncorrected p-distances between the members of the genus *Hirudinaria* are shown in Table 5. The highest value of 0.132 was between *Hirudinaria bpling* and *Hirudinaria* sp. (2n = 28) and the lowest value of 0.014 was between *Hirudinaria manillensis* (2n = 24) and *Hirudinaria* sp. (2n = 28).

Discussion

All 435 examined specimens in this study were found by morphological analysis to belong to three distinct species within the genus *Hirudinaria*, and were identified as *Hirudinaria javanica*, *Hirudinaria manillensis* and an unidentified morphospecies (*Hirudinaria* sp.). They all shared various diagnostic characters reported in other studies, such as: a medium to large body size; five pairs of large eyes with the third and fourth pairs separated by one annulus, and the fourth and fifth pairs separated by 5–7 annuli, and the absence of a vaginal stalk (Whitman 1886, Moore 1927, Klemm 1972, Nesemann and Sharma 2001, Lai and Chen 2010).



Figure 8. Phylogenetic relationships of the genus *Hirudinaria* and their related species, with chromosome number data. Tree topology was obtained from ML analysis based on a 658 bp fragment of the mitochondrial COI gene (DNA barcode region). Nodes with a 0.95 or higher bipartition posterior probability for BI and/or 70% or higher bootstrap value for ML were regarded as sufficiently resolved nodes, and are shown for the major clades (ML/BI). Numbers in parentheses refer to sampling localities in Figure 1 and the list in Table 1. Chromosome data of the related species were taken from Vitturi et al. (2002) and Utevsky et al. (2009).

The unidentified species (*Hirudinaria* sp.) was different from the other two (*Hirudinaria javanica* and *Hirudinaria manillensis*) in both its morphology and also in its chromosome number and karyotype. Morphologically, *Hirudinaria* sp. had fewer salivary papillae (25) than the other two species (43 and 30 for *Hirudinaria javanica* and *Hirudinaria manillensis*, respectively) and a higher estimated number of teeth per jaw (167 versus 134 and 148 for *Hirudinaria javanica* and *Hirudinaria manillensis*, respectively) (Fig. 5). Although previous studies have reported a higher number of teeth for *Hirudinaria javanica* and *Hirudinaria manillensis* at 150 and 145, respectively (Moore 1927, Phillips 2012), than found in this study, these were still lower than that found for *Hirudinaria* sp. in this study. Comparison of all the taxonomic characters (Table 2) revealed that *Hirudinaria* sp. was quite similar to *Hirudinaria manillensis* in terms of having the gonopores separated by five annuli, but it differed in color pattern (Figs 2 and 3). However, the phylogenetic analysis, based upon

		1	1	1
Characters	Hirudinaria bpling	Hirudinaria javanica	Hirudinaria manillensis	<i>Hirudinaria</i> sp.
Color	dark brown	dark green	dark brown/brown	dark green/brown
Distance (annuli) between male & female pores	5	7	5	5
Position of male and female organs	XI-XII	XI-XIII	XI-XII	XI-XII
Atrium	bulbous	short	long	relative long
Pairs of testisacs	-	12	11	11
Common oviduct	short	short	short	long
Vagina caecum	wide, long	small, ovate	small, ovate	large, elongate
References	Phillips (2012)	This study	This study	This study

Table 2. Comparative morphological characters among *Hirudinaria* species in this study.

Table 3. Comparison of chromosome numbers of the genera *Hirudo* and *Hirudinaria*.

Species	Locality no.1	No. ²	Haploid (n)	Diploid (2n)	Reference
Hirudo medicinalis	Kharkiv, Ukraine	5	14	28	Utevsky et al. (2009)
Hirudo verbana	Odesa and Kharkiv, Ukraine	6	13	26	Utevsky et al. (2009)
Hirudo orientalis	Lake Taskul, Kazakhstan	7	12	24	Utevsky et al. (2009)
Hirudinaria javanica	1	4	13	26	This study
	3	1	13	26	This study
	7	2	13	26	This study
	8	3	13	26	This study
	15	1	13	26	This study
Hirudinaria manillensis	1	5	12	24	This study
	2	3	12	24	This study
	3	1	12	24	This study
	4	1	12	24	This study
	5	1	12	24	This study
	6	2	12	24	This study
	8	2	12	24	This study
	9	2	12	24	This study
	10	2	12	24	This study
	11	2	12	24	This study
	12	3	12	24	This study
	13	2	12	24	This study
	14	4	12	24	This study
	16	4	12	24	This study
	17	4	12	24	This study
Hirudinaria sp.	1	5	14	28	This study
	2	1	14	28	This study

¹ Locality refers to the location where the sample was collected from, as coded in Table 1.

² No = Number of specimens examined.

the 658 bp of the mitochondrial COI gene sequence, placed *Hirudinaria* sp. in the same clade as *Hirudinaria manillensis*. Thus, they may represent recently sympatrically separated species. *Hirudinaria manillensis* was the most abundant and frequently found species in

Taxon	Locality no. ¹	Gen Bank accession nos.
<i>Hirudinaria javanica</i> (2n = 26)	1	KJ551852
	7	KJ551853, KJ551854
	8	KJ551851
	15	KJ551855
Hirudinaria manillensis (2n = 24)	1	KJ551850
	5	KJ551850
	17	KJ551850
<i>Hirudinaria</i> sp. (2n = 28)	1	KJ551848
	2	KJ551849
Hirudinaria bpling	Phang Nga, Thailand	JQ846012*
Haemopis sanguisuga	Sweden	AF462021*
Hirudo verbana	USA	GQ368752*
Hirudo orientalis	-	JN104645*
Hirudo medicinalis	Sweden	HQ333518*

Table 4. Taxa examined in the phylogenetic analysis, with collection localities and COI GenBank accession numbers.

¹ Locality refers to the location where the sample was collected from, as coded in Table 1.

* Sequences were obtained from GenBank.

Table 5. Average uncorrected p-distance for the 658 bp COI gene sequences of the genus Hirudinaria.

Speceis	1	2	3	4
1. Hirudinaria javanica (2n = 26)	-			
2. Hirudinaria manillensis (2n = 24)	0.101	-		
<i>3. Hirudinaria</i> sp. (2n = 28)	0.110	0.014	-	
4. Hirudinaria bpling	0.119	0.129	0.132	-

this study (346/435 or 79.5% of the collected specimens and found in all 17 sampled localities), compared to 83 (19%) specimens from five locations for *Hirudinaria javanica* and the seemingly rarer 6 samples (1.4%) from only two locations for unidentified *Hirudinaria* sp. Surprisingly, the northeastern Thailand population of *Hirudinaria manillensis* examined in this study showed a distinctly different internal morphology from that previously reported elsewhere. It contained a nerve cord running along on the left side of the atrium, instead of the right side as previously reported (Lai and Chen 2010), and also as found in *Hirudinaria javanica* and *Hirudinaria* sp. in this study.

With respect to the karyotypic analysis, the haploid and diploid chromosome numbers were similar to those reported previously in other genera of Hirudinidae (n = 14 in *Hirudo medicinalis*, n = 12 in *Hirudo orientalis* and n = 13 in *Hirudo verbena*) (Utevsky et al. 2009), but differed in chromosome structure and morphology. Moreover, distinctive karyotypic chromosome markers were presented, such as a distinct biarm chromosome that was only found on the first pair in *Hirudinaria javanica*, on pairs 1, 2, 3 and 5 in *Hirudinaria manillensis*, and on pairs 3 and 5 in *Hirudinaria* sp. That *Hirudinaria manillensis* showed the lowest chromosome number and the widest

distribution across northeastern Thailand of the sampled species is of interest since, in general, it is believed that the original or ancestor species has the lowest chromosome number and is often the most common species (Sumner 2003). The unidentified species (*Hirudinaria* sp.) in this study had the same haploid and diploid chromosome numbers as *Hirudo medicinalis*, but their karyotypes were different (Utevsky et al. 2009) and their phylogenetic placement was markedly different, being placed in well-supported distinct clades, confirming that they are indeed separate biological species.

Our current identification of these 435 samples to three morphospecies (two nominal species and one unidentified morphospecies) was quite clear because of the distinct appearance of their external and internal organs, and was supported by the distinct chromosome numbers and karyotypes of the analyzed samples of each species. However, given the apparent variation between that reported here for, for example, *Hirudinaria manillensis* and that reported for the same nominal species elsewhere, indicates a need for further comparative studies utilizing type specimens and additional molecular analysis of these and congener species, for species confirmation and prior to any further systematic discussion and taxonomic re-classification. In particular, the potential recent sympatric speciation of *Hirudinaria manillensis* and *Hirudinaria* sp. requires further confirmation.

Acknowledgements

This project was funded partly by a grant from the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). The main funding was from The TRF Senior Research Scholar, The Thailand Research Fund (RTA 5580001) to SP. We appreciated the critical reading the manuscript by Dr. Robert Butcher from the PCU Unit, Faculty of Science, Chulalongkorn University. We thank all members of the Animal Systematics Research Unit, Chulalongkorn University, and Kridsada Deein, Pramook Ruekaewma and Parinda Ratanadang from Fisheries Department, Ministry of Agriculture for assistance in collecting some material.

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RESEARCH ARTICLE



A new species of Atheroides Haliday (Hemiptera, Aphididae) native to North America

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http://zoobank.org/9DD1F878-50DF-4BA6-9735-43392FD02ABF

Citation: Miller GL, Jensen AS, Metz MA, Parmenter RR (2014) A new species of *Atheroides* Haliday (Hemiptera, Aphididae) native to North America. ZooKeys 452: 35–50. doi: 10.3897/zookeys.452.8089

Abstract

We report and describe the first species of *Atheroides* Haliday presumed to be native to North America, collected at the Valles Caldera National Preserve, New Mexico, USA. We hypothesize its placement among the Siphini based on morphological, phylogenetic analysis and extend the distribution of the genus to the Holoarctic. We expand the key of the known *Atheroides* to include the new species and discuss the current hypotheses of the geographic distribution of the type species, *Atheroides serrulatus* Haliday.

Keywords

Valles Caldera National Preserve, Nearctic, cladistics, new species, *Atheroides*, Aphididae, Chaitophorinae, Siphini

Introduction

The Jemez Mountains in northern New Mexico, USA, constitute a "sky island" at the southern end of the Rocky Mountains of North America. This area now serves as a Pleistocene biological refugium, supporting high-elevation ecosystems left behind by the last retreating Ice Age 20,000 years ago (Goff 2009). Increasing aridity during the last 10,000 years has created arid grassland valleys around the Jemez Mountains,



Figure 1. Map of the Valles Caldera National Preserve, New Mexico, USA, showing collection location.

isolating the montane forested and meadow ecosystems from similar habitats in adjacent mountain ranges. In the center of the Jemez Mountains lies a super-volcano's caldera, which today encompasses the Valles Caldera National Preserve (VCNP) under the U.S. Department of Agriculture. As part of an inventory of the Preserve's natural resources, VCNP scientists and entomologists from the USDA's Systematic Entomology Laboratory began an extensive survey of the forests and *valles*. While producing numerous new distribution records for the state of New Mexico, no new insect taxa endemic to this ecosystem had been found. This work constitutes one of the first published accounts of a new species of insect collected at VCNP and unknown from any other locality. Remarkably the species is in a genus of aphids, *Atheroides* Haliday, 1839, with no known native, New World species. Because *Atheroides* prior to this discovery appeared to have a Palaearctic distribution except for the North American adventive, *A. serrulatus*, this account may represent the first support for a geologic refuge.

Atheroides is part of the tribe Siphini Mordvilko, 1928, the most derived clade of the Chaitophorinae Mordvilko, 1909, and was treated extensively by Wieczorek (2009, 2010) and Wieczorek and Kajtoch (2011). The genus is considered to have a Palaearctic distribution. Although, the type species, *A. serrulatus* Haliday, 1839, has been collected in Canada, the remaining species are known only from the Palaearctic region. Because of this apparent disjunct distribution *A. serrulatus* was considered
an adventive, albeit non-invasive, species to the New World (Richards 1972, Foottit and Richards 1993, Foottit et al. 2006). Compared with other members of Siphini, *Atheroides* can be distinguished by their elongate, narrow body and a semicircular tergite VIII that covers the cauda (Wieczorek 2010). Species of *Atheroides* are known to feed on grasses and sedges. Their long, flat bodies seem ideal for positioning themselves between blades of grass, and the blunt, apical segment of the rostrum is diagnostic for grass-feeding aphids (Wieczorek 2009). Species in the genus also live singly or in small colonies (Wieczorek 2009), so considering their semi-reclusive, inconspicuous habits there may be more species awaiting discovery. In this paper we report and describe the first species presumed to be native to North America; hypothesize its placement among the Siphini based on morphological, phylogenetic analysis; and unquestioningly expand the distribution of the genus to Holoarctic.

Methods

We follow the recommendations of Thompson and Mathis (1980) for the names of Haliday first appearing in Curtis 1837 in terms of availability, validity, and priority. After DNA extractions were obtained, we mounted the specimens in Canada balsam (Favret 2005) and deposited them in the U.S. National Aphid collection, located at the Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland, USA.

We made observations with both stereo and compound microscopes at magnifications from 100-400×. We used a Visionary Digital BK Lab System[®] and SolMate[®] Trans-Illumination System to take digital photographs of slide-mounted specimens with a Canon[®] EOS 5D Mark II DSLR camera. We montaged Z-stacks with Helicon Focus Pro[®]. Illustrations were first hand-drawn using a Nikon[®] Eclipse E600 and a drawing tube. Final illustrations were rendered on Denril[®] multi-media vellum using Pigma[®] Micron[®] 005 and 01 technical pens. We performed digital image editing/enhancement/manipulation in the Gnu Image Manipulation Program (GIMP). Dimensions of structures in the description are reported in millimetres; for apterous viviparae the first measure is that of the holotype and the second that of the paratype; for oviparae ranges are followed by means in parentheses.

We approached the phylogenetic analysis with the assumptions that Siphini was monophyletic and part of the Chaitophorinae, that the species in question was undescribed, and that the new species belonged to Siphini. The phylogenetic question was the correct generic placement of the new species. We sampled taxa within Siphini so as to have all of the type species of the currently included genera (Wieczorek 2010; Wieczorek and Kajtoch 2011), and chose as the outgroup taxon the type species of Chaitophorinae. We included two species of each subgenus of *Sipha*, since support of a monophyletic *Sipha* requires further research (Wieczorek 2010; Wieczorek and Kajtoch 2011). Our entire taxon sample for testing the placement of the new species was: *Chaitophorus leucomelas* Koch, 1854 (type of Chaitophorinae); *Atheroides serrulatus* (type of *Atheroides*); *Laingia psammae* Theobald, 1922 (type of *Laingia* Theobald,

1922); Sipha (Rungsia) maydis Passerini, 1860 (type of the subgenus Rungsia Mimeur, 1933); Sipha (Rungsia) elegans Del Guercio, 1905; Chaetosiphella berlesei (Del Guercio, 1905) (Sipha) (type of Chaetosiphella Hille Ris Lambers, 1939); Sipha (Sipha) glyceriae (Kaltenbach, 1843) (Aphis L., 1758) (type of Sipha); Sipha (Sipha) flava (Forbes, 1884) (Chaitophorus Koch, 1854); Caricosipha paniculata Börner, 1939 (type of Caricosipha Börner, 1939). We included in the cladistic matrix characters used previously by Wieczorek (2010) and Wieczorek and Kajtoch (2011) (Characters 1, 3, 5, 8, 9, 10, and 11 in this analysis), but could not use those author's other characters because they were either uninformative among the current taxa sampled, were for characters of other life stages and/or sexes, or we could not score them unambiguously. We considered the coded states of their character 7 ("dorsal cuticle: (0) reticular or spinulose structures present; (1) smooth") to be not homologous, so separated those features into two separate characters (Characters 6 and 7 in this analysis.). We introduced characters 0, 2, and 4. As a part of character mining, we measured all body segments, leg segments, antennal segments and rostral segments on the exemplars of all taxa, and used these measures to calculate every combination of ratios among them. We plotted the distribution of the calculated ratios among all taxa on histograms to check for bi- or multimodal distributions. We removed all characters we could not score unambiguously or had overlap in quantitive measures/ratios. We weighted all characters equally and coded them as unordered. We preferred combinations of binary characters and nominal state coding over multistate characters. We performed preliminary phylogenetic analysis using the Parsimony Ratchet routine in Winona (5000 iterations per rep, 5 trees held per iteration, 1 character sampled, and remaining parameters set to default; Nixon 1999). We did not need multiple reiteration on a dataset of this size. We then conducted exhaustive searches (implicit enumeration) using TNT (Willi Hennig Society edition; Goloboff et al. 2008) to corroborate the preliminary tree hypothesis. We calculated Bremer values (Bremer 1988) in TNT from exhaustive searches of progressively longer suboptimal trees (increments of 1 step).

We stored the HT apterous adult female and two oviparous adults collected in Valles Caldera National Preserve in 95% ethanol at -20 °C, until the DNA extractions were performed. We extracted DNA non-destructively with Qiagen[®]'s Blood & Tissue kits (Valencia, CA) using a technique described by Favret (2005). The extractions followed the "Purification of Total DNA Animal Tissues" protocol with a few modifications (Qiagen 2006). Firstly, instead of the whole insect being pulverized, we pierced the integument of the aphid using a minuten insect pin. Secondly, we allowed the initial cell lysis step to continue for 24 hours instead of the recommended 1–3 hours. This extra time allowed the specimens to clear for microscope slide mounting. We amplified the barcoding region of COI using the forward primer: C1-J-1490: 59-ATTCAACCAATCATAAAGATATTGG-39 and reverse primer: C1-N-2198: 59-TAAACTTCTGGATGTCCAAAAAATCA-39 (Hajibabaei et al. 2006). We sequenced the fragment of COI in both directions using these primers with Applied Biosystems BigDye[®] kits, version 3.1, and read on an Applied Biosystems[®] sequencer.

Results

We were able to resolve a total of 12 binary morphological characters to address the question of generic placement of the new species among the genera of Siphini. Description of characters and character states included in the analysis follow:

0. Ratio of the Entire Body Length to the Body Width Measured Side to Side Across the Siphunculi

- 0 Body length less than $3 \times$ width of body
- 1 Body length at least 3.5× width of body

Wieczorek (2009) diagnosis *Atheroides* as "elongate, slender, nearly linear" (except *A. brevicornis* Laing, 1920). We used a ratio of length to width to quantify the elongation and narrowness of the taxa since the total length among the taxa investigated does not vary considerably. The length of the body was taken from the frons to the terminus of tergite VIII, and the width of the body was taken at the level of the siphunculi so as to make it objectively repeatable. The new species shares the derived state with *A. serrulatus* and *L. psammae*.

1. Number of Antennal Segments

- 0 Six
- 1 Five

A five-segmented antenna has long been considered a diagnostic feature of Siphini among aphid workers and was corroborated by Wieczorek (2010) through cladistic analysis. The new species shares the derived state with all the species of Siphini included in the analysis.

2. Ratio of Length of Dorsad Apical Seta of Antennal Segment III to Width of Antennal Segment III Measured at Middle

- 0 Seta length at least 1.3× width of antennal segment 3 at middle
- 1 Seta absent or seta length subequal or less than width of antennal segment 3

The new species is notable among Siphini in having short, sparse setae on the antenna, particularly antennal segment III. We used a ratio of the length of the most apical dorsal seta on antennal segment III to the width of antennal segment III at the middle of the segment since that width did not vary considerably among the taxa investigated. The new species shares the derived state with *A. serrulatus*.

3. Ratio of the Length of the Processus Terminalis to the Base of the Terminal Antennal Segment

- 0 Processus terminalis shorter or subequal in length to the base of the terminal antennal segment
- 1 Processus terminalis at least 1.5× longer than the base of the terminal segment

The ratio of the lengths of the base of the terminal antennal segment and the processus terminalis is used extensively among aphid taxa for genus and species level identification. This character is stable among taxa in the analysis – autopomorphic for *Chaetosiphella berlesei* – so is uninformative in this tree hypothesis, but is left in for the benefit of future work in the group.

4. Ratio of Hindfemur Length to Midfemur Length

- 0 Hindfemur length less than 1.5× length of midfemur
- 1 Hindfemur length at least 1.6× length of midfemur

The new species is notable among Siphini in having a short femur on the fore- and midlegs, particularly as compared to the femur of the hindlegs. The new species shares the derived state with *A. serrulatus*.

5. Shape of the Setae on the Dorsum of the Body

- 0 Dorsal setae almost exclusively acuminate
- 1 Dorsal setae scale-like, denticulate, and/or flabellate

The new species shares with other species of *Atheroides* the presence of non-acuminate setae throughout the body surface. The setae of the dorsum are characteristic of this diagnostic feature. The new species shares the derived state with *A. serrulatus*.

6. Denticulate or Spiculate Cuticle Covering Most of Body

- 0 Absent
- 1 Present

The integument between setal sockets may have raised, densely-distributed extensions that can be acuminate or blunt, as opposed to smooth cuticle. The derived state of this character does not support any monophyletic group resolved in the tree hypothesis.

7. Dorsal Cuticular Surface Wrinkles and Folds

- 0 Absent
- 1 Present

The new species shares with other species of *Atheroides* the presence of wrinkles and/or folds throughout the dorsal cuticle of the body reminiscent of the surface of the human brain. The new species shares the derived state with *A. serrulatus*.

- 8. Position of the Base of the Siphunculi
 - 0 On abominal segment VI
 - 1 On abdominal segment V

The primitive state among Aphididae is for the siphunculi to be positioned on tergite VI. Among most Siphini the position of the siphunculi is on tergite V. The tree hypothesis created from this analysis suggests that the derived state supports the monophyly of Siphini sans *Caricosipha paniculata* with a reversal in *Laingia psammae*.

9. Orifice of Siphunculus

- 0 Elevated above the surface of the abdomen on a tube
- 1 Flush with the surface of the abdomen or on a short mound of cuticle, not elevated by a tube

The primitive state among Aphididae is for the siphunculous to be in the form of a tube or cylinder such that the external orifice is elevated above the surface of the dorsum. The new species shares the derived state with all the species of Siphini included in the analysis except *Caricosipha paniculatae*.

10. Posterior Margin of Tergite VIII

- 0 Not expanded posteriorly, cauda visible in dorsal view
- 1 Expanded posteriorly hiding cauda mostly or completely from dorsal view

The new species shares with the derived state with *Atheroides serrulatus* and *Laingia psammae*.

11. Shape of cauda

- 0 Constricted basally or subbasally creating an apical knob
- 1 Broadly rounded, truncate, or emarginate

The new species shares with other species of *Atheroides* the shape of the cauda, being broadly rounded, truncate, or emarginate as opposed to having a posterior elongation or a constriction with an apical knob. The new species shares the derived state with all the species of Siphini included in the analysis except *Caricosipha paniculatae* and the two species of *Sipha* (*Sipha*).

There were no missing or inapplicable characters in the matrix. The complete matrix is given in Table 1. An exhaustive tree search resulted in a single, most parsimonious hypothesis of 14 steps (CI=85, RI=88) (Fig. 3). There is no character evidence to support relationships among the two species of *Sipha (Rungsia)* and *Chaetosiphella berlesei* so we collapsed these nodes (Fig. 3). The data support a monophyly including the new species and *A. serrulatus*, the type species of *Atheroides*, (Bremer support of 4; Fig. 3).

We amplified and sequenced a 652 bp DNA fragment containing the barcoding region of the mitochondrial COI gene from the HT and two oviparous vouchers. Since all individuals produced sequences that were identical, only one sequence was submitted to GenBank (accession number KJ737374). We searched for matching sequences in Gen-Bank and found multiple matches for Aphididae, but there were no sequences available for the taxa sampled in the phylogenetic analysis with enough overlap to warrant inclusion.

						Char	acter					
Taxon	0	1	2	3	4	5	6	7	8	9	10	11
Chaitophorus leucomelas Koch, 1854	0	0	0	0	0	0	0	0	0	0	0	0
Sipha (Rungsia) elegans Del Guercio, 1905	0	1	0	0	0	0	1	0	1	1	0	1
Sipha (Rungsia) maydis Passerini, 1860	0	1	0	0	0	0	1	0	1	1	0	1
Sipha (Sipha) flava (Forbes, 1885)	0	1	0	0	0	0	1	0	1	1	0	0
Sipha (Sipha) glyceriae (Kaltenbach, 1843)	0	1	0	0	0	0	0	0	1	1	0	0
Caricosipha paniculatae Börner, 1939	0	1	0	0	0	0	0	0	0	0	0	0
Laingia psammae Theobald, 1922	1	1	0	0	0	0	0	0	0	1	1	1
Chaetosiphella berlesei (Del Guercio, 1905)	0	1	0	1	0	0	1	0	1	1	0	1
Atheroides vallescaldera sp. n.	1	1	1	0	1	1	1	1	1	1	1	1
Atheroides serrulatus Haliday, 1839	1	1	1	0	1	1	1	1	1	1	1	1

Table I.Characters matrix.

Atheroides Haliday, 1839

Atheroides Haliday 1837: 218 (Nomen nudum)

Atheroides Haliday, 1839: 189. Type species Atheroides serrulatus Haliday, 1839: 189 by subsequent designation (Kirkaldy 1906: 10) (Note: Laing (1920) incorrectly designates A. serrulatus Haliday as the type species again subsequent to Kirkaldy). Apteroides Mordvilko, 1929: 91. (Subsequent misspelling)

Corealachnus Paik, 1971: 3. Type species *Corealachnus suwonensis* Paik, 1971: 4 by original designation.

Atheroides vallescaldera Miller & Jensen, sp. n. http://zoobank.org/DBF1845E-8DBC-4B1B-920B-4AD9F9197FF6

Diagnosis. This new species can be distinguished from other species in the genus by the following combination of characters: setae on the dorsum flabellate and dentate, and arranged in rows; dorsum sclerotized with rugose sculpturing; marginal setae of abdominal tergites I-VI easily visible, longer than width of hindfemur at middle, acuminate; empodial setae flat, but with base and apex of equal width, not spatulate.

Description (slide-mounted specimens). *Apterous vivipara* (Figs 4–11, Table 2) (n = 2): Body at least 3 times longer than wide, dorsum rugose, dorsal setae on segments I-VII 0.018–0.058, mostly dentate and flabellate with some acuminate, arranged in rows, marginal setae acuminate, longer than width of hindfemur at middle, present on all abdominal segments. Head (Fig. 6) rectangular, flattened dorsoventrally and frons flat with bluntly pointed projections, one medial projection more prominent, rugose; setae acuminate on front and sides and denticulate (Fig. 8) dorsally; antennal tubercle undeveloped, basal antennal articulation flush with side of head; eyes slightly inset, eye outer margin almost flush with head margin, sometimes partially obscured

	A. vallesca	<i>ldera</i> sp. n.	<i>A. serrulatus</i> (from Wieczorek 2009, 2010)		
	Oviparae	Viviparae	Oviparae	Viviparae	
	N=8	N=2	N=6	N=17	
Body [mm]	2.08-2.30	2.07-2.21	2.00-2.35	1.70-2.20	
Antenna [mm]	0.54-0.58	0.53-0.55	-	-	
Antenna / Body [times]	0.24-0.27	0.25-0.26	0.21	0.19	
Ant. segm. III [mm]	0.17-0.19	0.17-0.18	-	-	
Ant. segm. IV [mm]	0.06-0.08	0.07-0.07	-	-	
Ant. segm. V base [mm]	0.07-0.09	0.08-0.08	-	-	
Ant. Segm. V base / Ant segm. III [times]	0.41-0.53	0.44-0.49	0.50-0.55	0.50-0.70	
Ant. segm. V processus terminalis [mm]	0.07-0.09	0.08-0.08	-	-	
Ant. segm. V processus terminalis / Ant. segm. III [times]	0.39–0.53	0.43-0.45	-	-	
Ant. segm. V processus terminalis / base [times]	0.77-1.29	0.89–1.03	1.00-1.05	0.77-0.91	
Ant. Segm. V / Ant. Segm. III [times]	0.82-1.00	0.89-0.92	1.00-1.10	1.10-1.30	
Ant. Segm. V / Ant. Segm. IV [times]	2.07-2.58	2.27-2.33	3.00-3.25	2.20-3.50	
Ultimate rostral segm. [mm]	0.12-0.13	0.12-0.13	0.07-0.10	0.07-0.10	
Ultimate rostral segm. / its basal width [times]	1.77-2.36	2.10-2.15	-	-	
Ultimate rostral segm. / Ant. segm. V base [times]	1.43-1.79	1.50-1.53	-	-	
Ultimate rostral segm. / Ant. segm. III [times]	0.66-0.76	0.67-0.73	0.69	0.69	
Ultimate rostral segm. / Hind tarsus, 2nd segm. [times]	0.77-1.00	0.83-0.98	0.75	0.75	
Hind femur [mm]	0.33-0.38	0.37-0.37	-	-	
Hind tibia [mm]	0.55-0.65	0.62-0.62	-	-	
Hind tibia / Body [times]	0.26-0.29	0.28-0.30	-	-	
Hind tarsus, 2nd segm. [mm]	0.13-0.14	0.13-0.14	-	-	
Siphunculus width [mm]	0.02-0.03	0.02-0.03	-	-	

Table 2. Morphometric data for apterous viviparae and oviparae of Atheroides vallescaldera sp. n.

dorsally, triommatidium outer margin projecting only as far as eye outer margin. Antenna (Fig. 5) 5-segmented, not reaching hind margin of prothorax, without secondary sensoria, antennal setae length usually subequal to width of corresponding antennal segment or less at middle of segment. Ultimate rostral segment (Fig. 7) with 2 pairs of primary setae and 1 pair of secondary setae. Basitarsi with 1 stout spine and 4 acuminate setae, one longer than basitarsus, the rest subequal to basitarsus; empodial setae flat, but base and apex of equal width, not spatulate (Fig. 10). Siphunculus flush with the surface of tergite V, without any elevation above the surface of the cuticle, orifice surrounded by a thickened band of cuticle. Tergite VIII (Fig. 9) broadly rounded, extending posteriorly so that it covers cauda, with robust, acuminate marginal setae. Cauda indistinct, obscured by setae of tergite VIII. Anal plate slightly emarginate, genital slightly emarginate (Fig. 11) with numerous irregularly arranged setae. Morphometric data are in Table 2.



Figure 2. Second author in the habitat of the type locality for the new species along Santa Rosa Creek.

DNA barcode (COI) for the holotype and one paratype contains 652 nucleotides (GenBank # KJ737374):

Alate vivipara and male: unknown.



Figure 3. Single, most parsimonious tree of 14 steps (CI=85, RI=88) resulting from an exhaustive search in TNT. Closed circles indicate unique forward changes. Open circles indicate either forward changes with homoplasy or reversals. Numbers on nodes in squares indicate Bremer support values for that node.

Ovipara (n = 8): Dorsal abdominal setae on segments I-VII 0.015–0.070. With 18–27 circular pseudosensoria on each hindtibia predominantly organized in pairs that are 8-shaped, others are single circles or triplets (conjoined pairs or triplets are still counted as single pseudosensoria). Otherwise similar to apterous vivipara. Morphometric data are in Table 2.

Etymology of specific epithet. The specific epithet, *vallescaldera*, is derived from the locality in which the specimens were collected, the Valles Caldera National Preserve, and should be considered a compound noun in apposition.

Specimens examined. Type-locality. USA: NEW MEXICO: Sandoval Co., near unit 12, lower Santa Rosa Creek watershed, a perennial stream tributary of the Rio San Antonio, 35.951; -106.521, VCNP# 144, 23.ix.2010, 2,595m, A. Jensen coll., ex grass in open meadow next to Santa Rosa Creek, dominated by sedges, grasses, rushes and a variety of forbs (Fig. 2).

Holotype apterous vivipara (Figs 4–11): Slide-mounted in balsam USNMENT 00826485.

Original label: "New Mexico: Valles Caldera Nat'l Preserve Near Unit 12 22 Sept. 2010 ex: grass in open meadow A. Jensen coll. SEL VCNP#144 Balsam"

Paratypes: same data as holotype (1 apterous vivipara USNMENT 00826486, 8 oviparae USNMENT 00826487-89, USNMENT 00826480-84).

Host plants and habitat. Unknown bunch grass. All other known *Atheroides* spp. feed on a variety of grasses (Poaceae), sedges (Cyperaceae) and rushes (Juncaceae) (Wieczorek 2009). On the VCNP, there are 88 taxa of grasses, 31 taxa of sedges, and



Figures 4–11. *Atheroides vallescaldera* n. sp. **4** holotype habitus (Left side focal planes from dorsum to middle. Right side focal planes from middle to ventral surface.) **5** antenna of apterous vivipara **6** head (left half dorsal, right half ventral) **7** ultimate rostral segments **8** examples of dorsal abdominal setae variation **9** tergite VIII (dorsal) **10** empodial setae **11** anal and genital plates. (Scale indicated by 0.20 mm measure bars and corresponding figure number.).

11 taxa of rushes (includes subspecies and varieties). Among these plant taxa at the collection locale are common host plants of other *Atheroides* spp., including *Deschampsia caespitosa*, *Phleum* spp., *Festuca* spp., *Carex* spp., and *Juncus* spp.

Key to the known species of *Atheroides* (apterous viviparae) (modified from Wieczorek 2009)

1	Setae on the dorsum of the body exclusively acuminate
1	Setae on the dorsum of the body acuminate, forked, dentate, and/or flabel- late
2 (1)	Spinal setae very long, as long as or longer than marginal ones. Cauda covered by abdominal tergite VIII. On <i>Deschampsia caespitosa</i>
	doncasteri Ossiannilsson, 1955
2'	Marginal setae very long, longer than spinal ones. Cauda not covered by ab-
	dominal tergite VIII. On various grasses hirtellus Haliday, 1839
3 (2')	Dorsum partially sclerotic without visible sculpture. Antennal segment III
	with 4–8 long setae. On <i>Festuca ovina</i> and <i>Stipa splendens</i>
	karakumi Mordvilko, 1948
3'	Dorsum sclerotic with visible, rugose sculpture. Antennal segment III with
	0–4 short setae. On various grasses
4 (3')	Dorsal setae arranged in visible rows
4'	Dorsal setae not arranged in visible rows
5 (4)	Marginal setae of abdominal tergites I–VI short, hardly visible, rarely as long
	as width of hindfemur, dentate. Empodial setae spatulate, flat and broadened
	at apex
5'	Marginal setae of abdominal tergites I–VI easily visible, longer than width of
	hind femur at middle, acuminate (Fig. 4). Empodial setae flat, but with base
	and apex of equal width, not spatulate (Fig. 10) vallescaldera sp. n.
6 (4')	Body elongate, oval, 1.50–2.40 mm long. Antennae 4- or 5-segmented,
. ,	0.12–0.15 times body length. Antennal segment I with 2 pointed and 1 den-
	tate seta brevicornis Laing, 1920
6'	Body elongate, slender, nearly linear, 1.55–1.72 mm long. Antennae 5 seg-
	mented, 0.18–0.25 times body length. Antennal segment I with 1 erect fan-
	shaped setapersianus Wieczorek, 2009

Key to the North American species of *Atheroides* (oviparae) (modified from Wieczorek 2010)

1	Hind tibiae with more than 30 pseudosensoria. Marginal setae of abdominal
	tergites 1- v I short, narchy visible, rarchy as long as which of mind remut, den-
	tate. Empodial setae spatulate, flat and broadened at apex
	serrulatus Haliday, 1839
1'	Hind tibiae with less than 30 pseudosensoria. Marginal setae of abdominal
	tergites I-VI easily visible, longer than width of hindfemur at middle, acumi-
	nate (Fig. 4). Empodial setae flat, with base and apex of equal width, not
	spatulate (Fig. 10)vallescaldera sp. n.

Discussion

We have shown that *A. vallescaldera* is morphologically most similar to *A. serrulatus* among described *Atheroides*, yet there are clear morphological differences between the species. The facts that it is found in such an unusual geological feature as the Valles Caldera, and far from the known populations of *A. serrulatus* in Canada, suggest that *A. vallescaldera* is native to North America. This is a simpler explanation than the notion that two species of *Atheroides* have invaded North America, with one being unknown in the Palearctic yet establishing in an isolated and unique habitat in North America. Further, we suggest that it is possible that *A. serrulatus* is naturally Holarctic as opposed to adventive in North America.

The discovery of a new *Atheroides* species in the course of general aphid collecting using a beating tray technique (i.e. not specifically targeting cryptic grass feeders such as *Atheroides*) suggests that directed searching for *Atheroides* in North America may lead to discovery of additional native species. Future field work should include accurate species identification of host plants, searching a range of habitats including extremes of altitude, latitude, and precipitation, and detailed notes on microhabitats.

Acknowledgments

The authors thank A. Carmichael, Systematic Entomology Laboratory, Beltsville, MD, for assistance in DNA extraction and sequencing; and the Valles Caldera National Preserve for logistical and field collection support. We thank K. Wieczorek for examining specimens of *A. vallescaldera* and commenting on their identity prior to publication. We also thank the ZooKeys editor and two anonymous reviewers for their helpful comments and suggestions. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer.

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RESEARCH ARTICLE



The Stenopodainae (Hemiptera, Heteroptera) of Argentina

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Academic editor: T. Henry	Received 29 October 2013	Accepted 16 July 2014	Published 5 November 2014
	http://zoobank.org/C00B076F-3E;	7E-4B2C-8E54-59A0F78ACF1	B9

Citation: Diez F, Coscarón MC (2014) The Stenopodainae (Hemiptera, Heteroptera) of Argentina. ZooKeys 452: 51–77. doi: 10.3897/zookeys.452.6519

Abstract

In Argentina, 10 genera and 33 species of Stenopodainae (Hemiptera: Reduviidae) have been recorded. Diagnoses of the genera, subgenera and species are given, and an illustrated key to genera is provided. Six species are new records for Argentina and an additional seven species represent new records for provinces.

Keywords

Reduviidae, Stenopodainae, key, distribution, new record, Argentina

Introduction

The Stenopodainae are characterized by the presence of a large cell, usually pentagonal or hexagonal, in the venation of the hemelytra, formed by the cubital and postcubital veins and the apical and posterior cubital and postcubital crossveins (Barber 1930; Weirauch and Munro 2009). The antenniferous tubercles and juga (mandibular plates) are usually strongly produced anteriorly. The elongate and incrassate scapus is also an important subfamily character (Barber 1930; Schuh and Slater 1995).

This subfamily contains 113 genera with 713 species worldwide (Maldonado Capriles 1990). A total of 10 genera with 27 species have been recorded in Argentina (Coscarón

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in press). The Stenopodainae subfamily is monophyletic (Weirauch 2008, Weirauch and Munro 2009, Hwang and Weirauch 2012). This subfamily is phylogenetically closely related to the subfamily Triatominae and the genera *Zelurus* Burmeister and *Opisthacidius* Berg of the subfamily Reduviinae (Hwang and Weirauch 2012). Eggs are laid singly and loosely inside soil exposing their apices (Ambrose 1999); some species are nocturnal and can be captured by light traps (Villiers 1948 and personal observation).

Argentina – the geographical area considered in this report – lies in the Neotropical faunal region. The country covers an area of 2,791,810 km² and is bordered by Uruguay, Brazil, Paraguay, Bolivia, and Chile. Approximately 75% of the country is occupied by arid and semiarid areas, but some places, such as the Yungas and Paranaense regions, are covered by rainforest.

The objective of this report is to provide an illustrated key of the genera of Stenopodainae from Argentina, including new diagnoses, geographical distribution records, and lists of species for each genus.

Material and methods

This study is based on material provided by the Museo Argentino de Ciencias Naturales (MACN) and the Museo de La Plata (MLP) (http://heteroptera.myspecies.info), Argentina. We have followed the terminology of Barber (1930) and Giacchi (1970, 1974). Distance from the anterior margin of the eyes to the apex of the antenniferous tubercles is the anteocular region. Distance from the posterior margin of the eyes to the pronotal collar is the postocular region.

Images were taken with a digital camera (PANASONIC DMC-S3) and a Wild M-stereomicroscope. The material was compared with photographs of type from the Naturhistoriska Riksmuseet of Stockholm, Sweden (http://www.nrm.se) and the American Museum of Natural History of New York (http://www.amnh.org). The distributions we list for Argentina were taken from Coscarón (in press). We used the program DIVA-GIS 7.1.7 (http://www.diva-gis.org) and the distribution of those specimens for which global positioning system data were available to construct the maps.

Results

Key to the genera of Stenopodainae for Argentina modified from Wygodzinsky and Giacchi (1994)

1a	Scapus produced beyond insertion of the basiflagellomere (Fig. 1)
1b	Scapus not produced beyond insertion of basiflagellomere
2a	First labial segment approximately twice as long as the second and third seg-
	ments combined (Fig. 2) Pygolampis Germar (Fig. 32)



Figures 1–8. Generic characters. I Head *Pnirontis stali* 2 Head *Pygolampis spurca* 3 Pronotum lateral view *Ocrioessa cornutulus* 4 Pronotum lateral view *Stenopoda guaranitica* 5 Pronotum dorsal view *Ocrioessa cornutulus* 6 Scutellum lateral view *Seridentus maculosus* 7 Head and pronotum lateral view *Seridentus maculosus* 8 Pronotum lateral view *Ctenotrachelus* sp. (Ad pu: adpressed pubescence; Ap scu: apex of scutellum; Pr tu: pronotal tubercles; Sc exp: expansions of scapus; I: first labial segment; II: second labial segment).

2b	First labial segment equal to or shorter than second and third segments com-
	bined (Fig. 3)
3a	Prosternum behind coxae as long as or longer than coxae (Fig. 3)4
3b	Prosternum behind coxae shorter than coxae, or coxae inserted at hind mar-
	gin of prosternum (Fig. 4)7
4a	Disc of anterior lobe of pronotum with 1+1 distinct tubercles (Figs 3, 5). First
	labial segment about as long as second segment (Fig. 3). Anterolateral angles
	of collar angles acutely spinous (Fig. 5). Fore coxae elongate cylindrical, about



Figures 9–16. Generic characters. 9 Head dorsal view *Stenopoda guaranitica* 10 Head dorsal view *Gnathobleda toba* 11 Head dorsal view *Oncocephalus validispinis* 12 Tibiae ventral view *Oncocephalus validispinis* 13 Head and pronotum dorsal view *Diaditus latulus* 14 Tibiae dorsal view *Diaditus latulus* 15 Head dorsal view *Narvesus carolinensis* 16 Tibiae dorsal view *Narvesus carolinensis.* (Ad pu: adpressed pubescence; Jug: juga).

	twice as long as wide (Fig. 3), hemelytral apical cubital and postcubital cross-
	vein obsolete (Fig. 26) Ocrioessa Bergroth (Fig. 26)
4b	Disc of fore lobe of pronotum without 1+1 distinct spine-like tubercles 5
5a	Apex of scutellum angularly raised or vertical (Fig. 6). Anteocular region
	as long as or slightly shorter than postocular region (Fig. 33). Two lines of



Figures 17–36. Dorsal view. 17 Ctenotrachelus minor Barber 18 Ctenotrachelus striatus Barber 19 Ctenotrachelus testaceus Barber 20 Diaditus pilosicornis Bergroth 21 Diaditus latulus Barber. 22 Gnathobleda toba Giacchi 23 Gnathobleda litigiosa Stål 24 Narvesus carolinensis Stål 25 Narvesus minor Barber 26 Ocrioessa cornutulus (Berg) 27 Oncocephalus validispinis Reuter 28 Pnirontis edentula (Berg) 29 Pnirontis infirma Stål 30 Pnirontis scorpiona (Berg) 31 Pnirontis stali (Mayr) 32 Pygolampis spurca Stål 33 Seridentus maculosus (Haviland) 34 Stenopoda cana Stål 35 Stenopoda guaranitica Giacchi 36 Stenopoda subinermis Stål.

	spines on ventral side of head slightly surpassing the anterior and posterior
	margins of eyes, ventral spine about half or as long as posteroventral spines
	behind eyes (Fig. 7)
5b	Apex of scutellum horizontal (Fig. 8). Anteocular region twice as long as
	postocular region. Spine on ventral side of head much smaller than the vent-
	rolateral one behind eyes (Fig. 7) <i>Ctenotrachelus</i> Stål (Figs 17–19)
6a	Body and appendages with dense, adpressed pubescence and numerous tiny,
	erect bristles (Figs 4, 9, 34–36). Margins of head nearly parallel-sided, in
	dorsal view, abruptly constricted at neck (Fig. 9). Foretibiae with elongate
	fossula spongiosa
6b	Body glabrous or variously pubescent but not as above
7a	Anteocular and postocular regions of equal length (Fig. 10). Body elongate
	fusiform, often five times or over five times as long as maximum width (Figs 22,
	23). Male genitalia with cuplike posterior extension of pygophore completely
	covering parameres. Female genital area narrowly pointed posteriorly
	<i>Gnathobleda</i> Stål (Figs 22, 23)
7b	Anteocular region longer than postocular region (Fig. 11). Body not elongate
	fusiform, broader, always less than five times as long as maximum width (Fig.
	27). Male genitalia with parameres not covered, clearly visible. Female genital
	area not narrowly pointed posteriorly
8a	Forefemora strongly incrassate, at least twice as thick as middle and hind
	femora (Fig. 27). Foretibia without distinct fossula spongiosa, or the latter
	not longer than diameter of tibia (Fig. 12) Oncocephalus Klug (Fig. 27)
8b	Forefemora slender, less than twice as thick as mid and hind femora (Fig. 20)9
9a	Jugum subcylindrical, parallel, round apically, projecting well beyond apex
	of head (Fig. 13). Tibiae of hind legs with setae of a length less than twice the
	diameter of the tibia (Fig. 14) Diaditus Stål (Figs 20, 21)
9b	Jugum triangular bifurcated, apically sharp, not projecting beyond apex of
	head (Fig. 15). Tibae of hind legs with setae of length equal to four or five
	times the diameter of the tibia (Fig. 16)

Taxonomy

Ctenotrachelus Stål

Ctenotrachelus Stål, 1868: 127.

Type species. Ctenotrachelus macilentus Stål, 1872, subsequent monotypy.

Diagnosis. (After Barber 1930, Giacchi 1985, Maldonado Capriles 1994a) Anteocular region twice as long as postocular region. Setigerous tubercle on ventral side of head much smaller than the ventrolateral tubercle behind eyes. Pronotum longer than



Figures 37–41. Geographical distributions of species of Stenopodainae in Argentina: 37 *Ctenotrachelus* Stål 38 *Diaditus* Stål 39 *Gnathobleda* Stål 40 *Narvesus* Stål 41 *Ocrioessa* Bergroth.

wide, with the anterior lobe much longer than posterior one. Scutellar spine horizontal, metascutellar spine small. Fore femora sligthly incrassate. Anterior legs with third tarsal segment longer than first and second together.

Ctenotrachelus minor Barber

Ctenotrachelus minor Barber, 1930: 188, 200.

Diagnosis. (After Barber 1930, Maldonado Capriles 1995) Scapus three times as long as anteocular margin. Pronotum less than twice as long as head. Prefemur strongly incrassate.

Material examined. Corrientes: 1♂ (MLP) Colonia Carlos Pellegrini (28°31'54.0984"S, 57°9'49.8204"W), Coscarón M. coll.

Observation. New record for Argentina.

Ctenotrachelus striatus Barber

Ctenotrachelus striatus Barber, 1930: 197; Giacchi 1985: 67; Coscarón 2003: 361; Melo et al. 2004: 61.

Diagnosis. (After Barber 1930) Preocular region of head one third longer than postocular one. Lateral margins of pronotum unarmed. First two ventral abdominal segments carinate.

Material examined. Corrientes: 13 (MLP) Colonia Carlos Pellegrini, Coscarón M. coll.

Distribution in Argentina. Corrientes: Colonia Carlos Pellegrini (28°31'54.0984"S, 57°9'49.8204"W), Ituzaingó (27°40'30.8742"S, 56°48'13.9428"W).

Ctenotrachelus testaceus Barber

Ctenotrachelus testaceus Barber, 1930: 189.

Diagnosis. (After Barber 1930) Postocular and preocular regions of head nearly equal or postocular region shorter than preocular one. Head behind eyes armed with three simple spines. Lateral margins of pronotum unarmed. First four segments of ventral abdominal segments carinate.

Material examined. Corrientes: 1♂ (MLP) Ituzaingó (27°40'30.8742"S, 56°48'13.9428"W), Coscarón M. col.

Observation. New record for Argentina.

Diaditus Stål

Diaditus Stål, 1859: 383.

Type species. *Diaditus semicolon* Stål 1859.

Diagnosis. (After Barber 1930, Giacchi 1973) Preocular region longer than postocular region. Juga long, robust and blunt apex, well extended beyond apices of antenniferous tubercles. Scapus shorter than head. First labial segment nearly equal to the second and third segments together. Hind tibiae with short setae, never reaching twice the diameter of the tibia. Anterior femora scarcely incrassate. Abdomen in ventral view with a median longitudinal carina, extending from sternum II to VI.



Figures 42–46. Geographical distributions of species of Stenopodainae in Argentina: 42 Oncocephalus Klug 43 Pnirontis Stål 44 Pygolampis Germar 45 Seridentus Osborn 46 Stenopoda Laporte.

Diaditus latulus Barber

Diaditus latulus Barber, 1930: 221; Wygodzinsky 1949: 66; Dispons 1971: 274; Giacchi 1982: 26; Maldonado Capriles 1990: 501; Martin-Park and Coscarón 2011: 56.

Diagnosis. (After Barber 1930, Giacchi 1982) Head short, less than twice as long as wide. Head shorter than pronotum. Males with setae in the ventral and lateral internal face of Pedicellus, seta length equal to twice the diameter of Pedicellus. Juga short, robust, subparallel, not reaching 1/4 of scapus in males, but reaching almost half in females. Collar angles blunt.

Material examined. La Pampa: 1♂ (MLP) Santa Rosa (36°36'56.8902"S, 64°17'49.7106"W), Diez F. Col.; Córdoba: 3♂ (MACN) Departamento Calamuchita: El Sauce (31°6'0.3312"S, 64°19'0.0084"W).

Daguerre (34°39'17.4636"S, Distribution in Argentina. Buenos Aires: 58°28'53.2878"W). 58°34'10.1598"W), Delta (34°14'12.4188"S, Dolores (36°18'53.2044"S, 57°40'47.7798"W), Hurlingham (W34°35'52.4004"S58°38'8.7"), Baradero(W33°48'30.4704"S59°30'19.6986"),Rosas(35°57'56.7714"S,58°56'24.1944"W), San Miguel (34°32'39.4152"S, 58°42'59.457"W), Wilde (34°42'15.7752"S. 58°19'13.623"W); Córdoba: Sierras (31°26'20.4678"S, 64°50'4.0992"W); Entre Ríos: Villaguay (31°51'53.0244"S, 59°2'8.5956"W); Mendoza; Salta; San Juan; Santa Fe: Bridarolli (31°37'56.5998"S, 60°41'58.0518"W).

Observation. First record for La Pampa province.

Diaditus pilosicornis Bergroth

Diaditus pilosicornis Bergroth, 1907: 50; Melo et al. 2011.

Diagnosis. (After Barber 1930, Giacchi 1982) Males with setae on ventral and lateral internal face of Pedicellus, seta length three times the diameter of Pedicellus. Juga reaching more than 1/3 of scapus in males and more than half in females. Prosternum glabrous, if tubercles or setae are present, these are scarce and conspiscuous. Collar angle obtuse. Fore femora in the ventral surface, basally with one spiniferous tubercle, the height is twice or more than setigerous tubercles of the trochanter.

Material examined. Chaco: 1^{\bigcirc} (MLP) Chaco National Park.

Distribution in Argentina. Chaco: Chaco National Park (26°48'24.9984"S, 59°26'36.4986"W).

Diaditus semicolon Stål

http://heteroptera.myspecies.info/taxonomy/term/1828 http://www2.nrm.se/en/het_nrm/s/diaditus_semicolon.html

Diaditus semicolon Stål, 1859: 383; Berg 1879: 278; Lethierry and Severin 1896: 86; Pennington 1921: 22; Barber 1930: 220; Giacchi 1982: 22; Maldonado Capriles 1990: 501; Nanni et al. 2011: 34; Dellapé and Carpintero 2012: 130.

Diaditus annulipes Berg, 1883: 112; Lethierry and Severin 1896: 86; Pennington 1921: 22; Coscarón 1998: 2.

Diagnosis. (After Barber 1930, Giacchi 1982, Blinn 2009) Males with setae on ventral and lateral internal face of pedicellus, seta length three times the diameter of pedicellus. Juga reaching 1/5 of scapus in males and 1/3 in females. Fore femora with one or two setigerous tubercles, not larger than setigerous tubercles of the trochanter.

Material examined. 1 \bigcirc (MLP) Typus *Diaditus annulipes* Berg synonymized by Wygodzinsky 1949, 1:66, 67. (Geographic origin not given). Salta: 3 \bigcirc , Embarcación (23°12'42.0798"S, 64°6'4.9026"W), 2 \bigcirc (MLP) City (24°47'6"S, 65°24'32.9904"W).

Mendoza $\stackrel{\bigcirc}{\rightarrow}$ (MLP) Typus *Diaditus annulipes* Berg synonymized by Wygodzinsky 1949, 1:66–67.

Distribution in Argentina. Buenos Aires: Baradero (33°48'30.4704"S, 59°30'19.6986"W), Chacabuco (34°38'22.4304"S, 60°28'9.9726"W), Partido de Campana: Delta del Paraná (34°9'9.8166"S, 58°58'11.136"W); Córdoba; Catamarca; Chaco: San Bernardo (27°17'18.6072"S, 60°42'45.6516"W), Tandil; Corrientes; Entre Ríos; Formosa; Jujuy; La Pampa; La Rioja; Mendoza; Misiones; Neuquén; Salta; San Juan; San Luis; Santa Fe: Colonia Mascías (30°48'7.6032"S, 60°0'19.6266"W), Departamento General Obligado, Lanteri (28°50'27.765"S, 59°38'9.981"W); Santiago del Estero; Tucumán.

Observation. First record for Salta province.

Remarks. The species currently assigned to the taxon is listed in Coscarón et al. (2014).

Gnathobleda Stål

Gnathobleda Stål, 1859: 380.

Type speccies. Gnathobleda fraudulenta Stål 1859.

Diagnosis. (After Wygodzinsky and Giacchi 1986) Sericeous pilosity. Anteocular and postocular portions of equal length. Large, pointed, laterally compressed juga. Presence of 1+1 conspicuous tubercles on the pronotum. More or less developed posterior projections on the connexival segments.

Note. Wygodzinsky and Giacchi (1986) synonymised *Pnohirmus* Stål, with *Gna-thobleda* Stål. Latter, Wygodzinsky and Giacchi (1994) in the key to the genera of the Stenopodainae of the new world they included the subgenera *Ganthobleda* (*Gnathoble-da*) and *Gnathobleda* (*Pnohirmus*). In this article they did not mentioned the species for each subgenera. We do not use the subgenera of *Gnathobleda* due to this confusion.

Gnathobleda fraudulenta Stål

Gnathobleda fraudulenta Stål, 1859; Nanni et al. 2011: 34.

Diagnosis. (After Wygodzinsky and Giacchi 1986) Head with a simple setigerous tubercle. Juga triangular. Prosternal processes conspicuous, spinelike. Undersurface of fore femora with two rows of processes.

Distribution in Argentina. Buenos Aires: Partido de Campana: Delta del Paraná (34°9'9.8166"S, 58°58'11.136"W).

Gnathobleda litigiosa Stål

http://www2.nrm.se/en/het_nrm/l/gnathobleda_litigiosa.html

Gnathobleda litigiosa Stål, 1862: 442.

Diagnosis. (After Wygodzinsky and Giacchi 1986) Length less than 14 mm. Genae conspicuously projecting beyond base of rostrum. Connexival segments light-colored with apical portion dark. Undersurface of fore femora with two series of processes, one setigerous, one spiniferous.

Material examined. 2^{\uparrow}_{\circ} (MLP) between Corrientes and Formosa (unspecified locality). **Observation.** New record for Argentina.

Gnathobleda toba Giacchi

Gnathobleda toba Giacchi, 1970: 126; Maldonado Capriles 1990: 503; Melo et al. 2004: 61.

Diagnosis. (After Wygodzinsky and Giacchi 1986) Total length 14 mm or more. Some of the sublateral setigerous spines of the postocular region of the head bifurcate. Genae conspicuously projecting beyond base of rostrum, connexival segments concolorous. Undersurface of fore femora with one series of spiniferous processes.

Material examined. Buenos Aires: 13° (MLP) La Plata. Corrientes: $23^{\circ} 29^{\circ}$ (MLP) Bella Vista (28°30'27.8274"S, 59°2'39.6492"W), 13° (MLP) between Corrientes and Formosa (unspecified locality). Santa Fe: 13° (MLP) Rosario.

Distribution in Argentina. Buenos Aires: Buenos Aires City (34°36'13.5102"S, 58°22'53.4678"W), La Plata (34°55'8.9616"S, 57°57'21.495"W); Chaco: General Vedia (26°55'58.728"S, 58°39'41.3958"W), Río de Oro (26°56'6.0858"S, 58°40'19.5414"W); Corrientes: Bella Vista, Colonia Carlos Pellegrini (28°32'5.4312"S, 57°10'27.5196"W).

Observation. First record for Santa Fe.

Gnathobleda violenta (Stål)

Pnohirmus violentus Stål, 1859: 384; Giacchi 1985: 66; Coscarón 2003: 361. *Gnathobleda violenta* Wygodzinsky and Giacchi, 1986: 141.

Diagnosis. (After Wygodzinsky and Giacchi 1986) Sublateral setigerous spines of postocular region of the head absent, simple or at most fused at base. Genae not conspicuously projecting beyond base of rostrum. Head without setigerous spines. Juga imperceptible in lateral view. Prosternal processes small, rounded, underside of femora with one row of spiniferous processes.

Distribution in Argentina. Buenos Aires: Delta (34°14'12.4188"S, 58°34'10.1598"W); Chaco: General Vedia (26°55'59.1234"S, 58°39'42.015"W), Río de Oro (26°56'6.0792"S, 58°40'19.5564"W); Corrientes: Manantiales (27°55'17.2878"S, 58°6'0.2874"W), Apóstol; Entre Ríos: Primero de Mayo (32°15'24.21"S, 58°25'22.5588"W); Santa Fe: Bridarolli, Piquete (31°34'19.6932"S, 60°43'19.023"W), Rosario (32°57'30.276"S, 60°39'32.688"W).

Narvesus Stål

Narvesus Stål, 1859: 384.

Type species. Narvesus carolinensis Stål, 1859.

Diagnosis. (After Barber 1930, Giacchi 1973, Giacchi 1974) Juga acute at the tip and divergent, never extending beyond the length of tylus. Scapus shorter than the head. Hind legs with very long setae on the tibia, four or five times the diameter of the tibia.

Narvesus carolinensis Stål

Narvesus carolinensis Stål, 1859: 385; Diez and Coscarón 2014: 290.

Diagnosis. (After Barber 1930, Giacchi 1974) Anterior and middle tibia bifasciate. Fore femora without a row of spiniform tubercles on ventral face.

Material examined. Buenos Aires: 1♀ (MLP) Olivos; Chaco: 1♂ (MACN) Río Oro. Distribution in Argentina. Buenos Aires: Olivos (34°30'39.1356"S, 58°29'44.7354"W); Chaco: Río Oro (26°56'6.0792"S, 58°40'19.5564"W).

Narvesus minor (Barber)

Narvesus minor Barber, 1930: 224; Giacchi 1974: 62; Maldonado Capriles 1990: 508; Carpintero 2009: 299; Diez and Coscarón 2014: 294.

Diagnosis. (After Barber 1930, Giacchi 1974) Anterior and mid tibiae trifasciate. Fore femora with a row of spiniform tubercles on ventral face.

Material examined. Santa Fe: 13 (MACN) Colonia Mascías; Neuquén: 13 (MLP) (unspecified locality).

Distribution in Argentina. Buenos Aires: Parque Costero del Sur (35°16'22.6266"S, 57°15'50.724"W); Misiones: Bocceti, Montecarlo (26°34'30.0648"S, 54°45'33.4542"W), Zaimán (27°25'6.801"S, 55°53'40.47"W); Neuquén; Río Negro: Lamarque (39°25'12.2982"S, 65°42'0.9324"W). Santa Fe: Colonia Mascías (30°48'1.9362"S, 60°0'48.6138"W).

Ocrioessa Bergroth

Ocrioessa Bergroth, 1918: 312.

Type species. Reduvius (Oncocephalus) notatus Klug 1830.

Diagnosis. (After Barber 1930, Giacchi 1985) First labial segment about as long as second segment. Posterior ocular region shorter than anteocular region. Pronotum longer than wide, with deep transverse groove before the half. Disc of fore lobe of pronotum with 1 +1 distinct tubercles. Apical angles of segments II to VI terminated in triangular lobes, apical angles of segment VII ending in two acute lobes directed posteriorly.

Ocrioessa cornutulus (Berg)

http://heteroptera.myspecies.info/taxonomy/term/2052

Rhyparoclopius cornutulus Berg, 1879: 277; Lethierry and Severin 1896: 85; Pennington 1921: 22; Coscarón 1998: 509.

Ocrioessa cornutulus Giacchi 1985: 68; Maldonado Capriles 1990: 509.

Diagnosis. (After Barber 1930, Giacchi 1985) Diameter of the gula much wider, being about twice as wide as the diameter of base of second labial segment. Scapus about twice as long as the preocular region. Discal spines of anterior lobe situated before the constriction with tubercles reduced.

Material examined. Misiones: 1 \bigcirc (MLP) Montecarlo.

Distribution in Argentina. Chaco; Misiones: Montecarlo (26°34'23.4294"S, 54°45'29.7462"W).

Remarks. The species currently assigned to the taxon is listed in Coscarón et al. (2014).

Oncocephalus Klug

Reduvius (Oncocephalus) Klug, 1830: 2. Type species: Reduvius (Oncocephalus) notatus Klug, 1830.

Oncocephalus Fieber, 1860: 42.

Diagnosis. (After Giacchi, 1984) Body oval. Eyes of male large, eyes of female smaller, with several rather distinct setigerous tubercles behind eyes. Antennae and tibiae with long setae, particularly in males. Basal segment of rostrum shorter than the two apical segments together. Fore femora strongly incrassate and distinctly spinose (with one or two row(s) of teeth on the ventral side).

Oncocephalus hirsutus Giacchi

Oncocephalus hirsutus Giacchi, 1984: 57; Maldonado Capriles 1990: 514.

Diagnosis. (After Giacchi 1984, Barber 1930) Pedicellus with long setae, more than three times the diameter of segment in males. The lateral tubercles of pronotum cov-

ered with stiff setae. Fore femora with seven spines ventrally and dorsally covered by conspicuous setigerous tubercles.

Distribution in Argentina. Misiones: Loreto (27°18'59.925"S, 55°31'58.8462"W).

Oncocephalus maiusculus Giacchi

Oncocephalus maiusculus Giacchi, 1984: 58; Maldonado Capriles 1990: 515; Martin-Park and Coscarón 2011: 57.

Diagnosis. (After Barber 1930, Giacchi 1984) Scapus, in the male, (in dorsal internal lateral view) with setae on the distal half or basal third. Setae length equal to half the diameter of scapus. Posterior lobe of pronotum brown and smooth.

Distribution in Argentina. Catamarca: Los Alamitos (28°28'59.4372"S, 65°13'8.2698"W).

Oncocephalus validispinis Reuter

Oncocephalus validispinis Reuter, 1882: 714; Giacchi 1984: 55; Maldonado Capriles 1990: 521; Martin-Park and Coscarón 2011: 57; Melo et al. 2011.
Oncocephalus mazzai Costa Lima, 1941: 342; Wygodzinsky 1949: 67.

Diagnosis. (After Barber 1930, Giacchi 1984) Scapus (in dorsal internal lateral view) with three setae shorter than the diameter of scapus in male and two in females. Setae shorter than the diameter of scapus. Posterior lobe of pronotum with medial longitudinal lines and carina. Two light brown bands on either side of carina.

Material examined. Chaco: $1 \circlearrowleft$ (MLP) Chaco National Park.

Distribution in Argentina. Buenos Aires: Delta (34°14'12.4188"S, 58°34'10.1598"W), Haedo (34°38'39.714"S, 58°35'43.6272"W), Hurlingham (34°35'52.4004"S, 58°38'8.7"W), Morón (34°39'21.0996"S, 58°37'0.195"W), San Miguel (34°32'34.9614"S, 58°42'43.0812"W), Villa Ballester (34°32'57.231"S, 58°33'31.6902"W), Ciudad Universitaria (34°34'46.5018"S, 58°24'17.2218"W); Chaco: Chaco National Park (26°48'24.9984"S, 59°26'36.4986"W); Córdoba: Sierras (31°26'20.4678"S, 64°50'4.0992"W); Entre Ríos: Colón (32°13'30"S, 58°8'40.1922"W), El Palmar (31°52'2.5932"S, 58°12'31.953"W); Santa Fe: Piquete (31°34'17.9826"S, 60°42'32.6736"W); Santiago del Estero.

Pnirontis Stål, 1859

Pnirontis Stål, 1859: 381.

Type species. Pnirotis scutellaris Stål 1859; subsequent designation by Van Duzee 1916.

Diagnosis. (After Barber 1930, Giacchi 1985, Giacchi 1988a) Body elongate longitudinally, fusiform and depressed. First labial segment almost three times longer than the second and third together, the second almost twice as long as the third. Scapus strongly incrassate, extended in an apical process that extends beyond the insertion of the second segment.

Pnirontis (Centromelus) Stål, 1868

Diagnosis. (After Wygodzinsky and Giacchi 1994) Antenniferous tubercles unarmed, or provided with minute spines. First segment of mid and hind tarsi shorter than second. Posterior angles of connexival segments varied.

Type species. *Pnirontis (Centromelus) spinosissimus* Stål, 1859; subsequent designation by Van Duzee (1916).

Pnirontis acuminata Barber

Pnirontis acuminata Barber, 1930: 156; Giacchi 1985: 64; Giacchi 1988a: 6. *Pnirontis (Centromelus) acuminata* Melo et al. 2004: 61.

Diagnosis. (After Barber 1930, Giacchi 1985, Maldonado Capriles 1986, 1994b) Head longer than pronotum. Scapus equal to length of preocular margin of head. Spines of fore femora long, two or three times as long as diameter of femur. Connexivum marked with fuscous at incisures. Male unknown.

Distribution in Argentina. Buenos Aires: Delta (58°17'37.0644"S, 58°17'37.0644"W); Chaco: General Vedia (26°56'0.153"S, 58°39'42.015"W), Río Oro (26°56'6.0792"S, 58°40'19.5564"W); Corrientes: Colonia Carlos Pellegrini (28°32'5.4312"S, 57°10'27.5196"W).

Pnirontis edentula (Berg)

Centromelus edentulus Berg, 1879: 275; Coscarón 1998: 4.

Pnirontis edentula Lethierry & Severin, 1896: 81; Barber 1930: 171; Wygodzinsky 1949: 68; Maldonado Capriles 1990: 525.

Pnirontes (Centromelus) edentulus Pennington, 1921: 22.

Diagnosis. (After Barber 1930) Scapus shorter than pronotum and about twice as long as preocular margin of head. Antenniferous tubercles long, about 1/4 longer than eye. Pronotum longer than wide.

Material examined. Buenos Aires: 1° (MLP) (unspecified locality). Corrientes: 1° (MLP) San Roque (28°34'31.1736"S, 58°42'31.032"W). Between Corrientes and

Formosa provinces: 1♂ 1♀ (MLP) (unspecified locality). Formosa: 2♂ (MLP) Laguna Oca (26°14'0.0234"S, 58°11'59.9742"W).

Distribution in Argentina. Argentina: Buenos Aires.

Observation. First record for Corrientes and Formosa provinces.

Pnirontis infirma Stål

Pnirontis infirma Stål, 1859: 382.

Diagnosis. (After Barber 1930) Scapus shorter than pronotum and about twice as long as preocular margin of head. Pronotum almost as wide as long. Antenniferous tubercles shorter, about equal to length of eyes.

Material examined. Chaco: 1 (MLP) Resistencia (27°27'23.3742"S, 58°58'55.776"W); Jujuy: $2 \stackrel{\frown}{_{\sim}} 2 \stackrel{\bigcirc}{_{\sim}}$ Reyes (MLP) (unspecified locality); Santa Fe: $1 \stackrel{\frown}{_{\sim}}$ (MLP) Colonia Mascías (30°47'55.8348"S, 60°0'52.3218"W); Santiago del Estero: $1 \stackrel{\frown}{_{\sim}}$ (MLP) Beltrán (27°49'43.6506"S, 64°3'35.5068"W).

Observation. New record for Argentina.

Pnirontis scorpiona (Berg)

Centromelus scorpionius Berg, 1879: 276; Coscarón 1998: 6.
Pnirontes (Centromelus) scorpionius Pennington, 1921: 22.
Pnirontis corpionia Barber, 1930: 161; Wygodzinsky 1949: 68; Giacchi 1985: 65; Maldonado Capriles 1990: 526; Coscarón 2003: 361.
Pnirontis scorpionica Lethierry & Severin, 1896: 81.
Pnirontis (Centromelus) scorpioni Giacchi, 1988a: 6.
Pnirontis scorpionia Carpintero & De Biase, 2011: 35

Diagnosis. (After Barber 1930, Giacchi 1985) Female head with tylus produced into a single process. Juga minute. Scapus longer than head. Genae well extended beyond apex of antenniferous tubercles. Anterior trochanters armed with a spine. Foretibiae with two series of spines, an inner series of 7–8 spines and an outer series of 4 spines. Corium and connexivum immaculate.

Material examined. Buenos Aires: 1 \bigcirc (MLP) Buenos Aires City. Chaco: 1 \bigcirc (MLP) Resistencia. Formosa: 1 \bigcirc (MLP) (unspecified locality). Santiago del Estero: 1 \bigcirc (MLP) (unspecified locality).

Distribution in Argentina. Buenos Aires: Baradero (33°48'30.4704"S, 59°30'19.6986"W), Buenos Aires City (34°36'13.5102"S, 58°22'53.4678"W), Isla Martín García (34°10'53.6154"S, 58°15'5.6592"W); Chaco: Resistencia (27°27'23.3742"S, 58°58'55.776"W); Córdoba: Sierras (31°26'20.4678"S, 64°50'4.0992"W); Corrientes: Estación Puerto Valle (29°2'0.225"S, 59°11'31.113"W),

Ituzaingó (27°40'30.8742"S, 56°48'13.9428"W), San Cayetano (27°34'14.9988"S, 58°41'40.9986"W); Entre Ríos: Victoria (32°37'18.9048"S, 60°9'27.3312"W); Santa Fe: San Cristóbal (30°18'30.2142"S, 61°14'19.9176"W).

Pnirontis stali (Mayr)

Pnirontis (Centromelus) stali Mayr, 1865: 437; Melo et al. 2004: 61. Centromelus stali Berg, 1879: 295. Pnirontes (Centromelus) stali Pennington, 1921: 22.

Diagnosis. Translated from Mayer (1865): Genae slightly longer than antenniferous tubercles. Scapus spiny underneath and almost 1/3 longer than the head. Pale yellow, in part dark, abdominal margin with small dark spots.

Material examined. 1 $\stackrel{\bigcirc}{\downarrow}$ (MLP), 3 $\stackrel{\frown}{\supset}$ (MLP) Geographic origin not given.

Distribution in Argentina. Buenos Aires: Chacabuco (34°38'22.4304"S, 60°28'9.9726"W); Corrientes: Colonia Carlos Pellegrini (28°32'5.4312"S, 57°10'27.5196"W); Misiones.

Pnirontis tabida Stål

Pnirontis tabida Stål, 1859: 381. *Pnirontis (Centromelus) tabida* Melo et al., 2004: 61.

Diagnosis. (After Barber 1930) Female tylus extending into a single stout process beyond apex of antenniferous tubercles. Juga very short, scarcely visible. Scapus, including long apical spine, 1/4 longer than head. Genae short, extending but little beyond apex of antenniferous tubercles. Foretibiae armed only with an inner series of spines and with preapical spur; corium and connexivum immaculate.

Distribution in Argentina. Argentina: Corrientes: Colonia Carlos Pellegrini (28°32'5.4312"S, 57°10'27.5196W).

Pygolampis Germar

Pygolampis Germar, 1817: 286.

Type species. Acanthia denticulata Rossi, Junior synonym of Cimex bidentatus Goeze, 1778.

Diagnosis. (After Barber 1930) Scapus not produced beyond insertion of basiflagellomere. First labial segment approximately twice as long as second and third segments. Scapus unarmed beneath. Head dorsally armed with two prominent tubercles. *Reduvius pectoralis* Say, 1832: 11. *Pygolampis pectoralis* Pennington, 1921: 22.

Diagnosis. (After Barber 1930) Scapus little if any longer than head. Head just behind eyes armed with a large ramose spine, followed by one or two smaller ones.

Distribution in Argentina. Misiones.

Pygolampis spurca Stål

Pygolampis spurca Stål, 1859: 379.

Diagnosis. (After Barber 1930) Scapus twice or as long as head. Basiflagellomere finely pilose with setae longer than diameter of the segment.

Material examined. Catamarca: 1 (MLP) Catamarca City (28°28'8.367"S, 65°46'44.2986"W), 1 (MLP) (unspecified locality); Corrientes: 1 (MLP) Santo Tomé (28°33'0.6696"S, 56°2'56.8062"W), 1 (MACN) Manantiales (27°55'28.0704"S, 58°6'9.7914"W); Formosa: 1 (MLP) (unspecified locality); Misiones: 1 (MACN) (unspecified locality); Santiago del Estero: 1 (MLP) Río Salado (unspecified locality).

Observation. New record for Argentina

Seridentus Osborn

Seridentus Osborn, 1904: 195.

Type species. Seridentus denticulatus Osborn, 1904.

Diagnosis. (After Maldonado Capriles 1994a) Anteocular space as long as or slightly shorter than postocular space. Two lines of setigerous tubercles on ventral side of head slightly surpassing the anterior and posterior margins of eyes. Spines about half as long as posteroventral setigerous tubercles behind eyes. Scutellar spine angulate, raised or vertical. Profemur moderately incrassate. Anterior legs with third tarsal segment twice as long as first and second combined.

Seridentus latissimus Giacchi

Seridentus latissimus Giacchi, 1998: 31.

Diagnosis. (After Giacchi 1998) Scapus three times as long as anteocular region of head.

Lateral margins of pronotum with a row of small to setigerous tubercles. Pronotum less.

Pronotum less than twice as long as head. Juga and scutellar spines nearly porrect. **Distribution in Argentina.** Misiones: Iguazú (25°57'2.289"S, 54°12'43.329"W).

Seridentus maculosus (Haviland)

Seridentus maculosus Haviland, 1931: 136. Seridentus maculosus: Wygodzinsky, 1949: 69.

Diagnosis. (After Maldonado Capriles 1994a) Scapus twice as long as anteocular region of head. Lateral margins of pronotum with a row of small setigerous tubrecles. Pronotum less than twice as long as head. Juga and scutellar spines nearly porrect. Clavus, corium and membrane sparsely spotted with brown.

Material examined. Misiones: 1♀ (MACN) Iguazú (25°57'2.289"S, 54°12'43.329"W).

Observation. New record for Argentina.

Stenopoda Laporte

Stenopoda Laporte, 1832: 26.

Type species. Stenopoda cinerea Laporte, 1832.

Diagnosis. (After Barber 1930, Giacchi 1969, Giacchi 1988b) First labial segment shorter than the second and third segments combined. Postocular region shorter than preocular one. Body and appendages with dense, adpressed pubescence and numerous tiny, erect bristles. Margins of head nearly parallel-sided in dorsal view, abruptly constricted at neck. Two median dorsal carinae (1+1) more or less elevated.

Stenopoda (Megastenopoda) Giacchi

Stenopoda (Megastenopoda) Giacchi, 1988b: 48.

Type species. *Stenopoda subinermis* Stål, 1859: 384.

Diagnosis. (After Giacchi 1988b) Total length of 23 to 35 mm. Fossula spongiosa of 1/3 to 1/2 the length of the foretibiae.

Stenopoda cana Stål

Stenopoda cana Stål, 1859: 384; Giacchi 1969: 11; Maldonado Capriles 1990: 540.

Diagnosis. (After Giacchi 1988b) Abdominal segments 1–5 divergent, the rest convergent. Pronotal setae longer than tubercles height. Tubercles conical and thick.

Material examined. Santiago del Estero: 1∂ (MLP) (unspecified locality).

Distribution in Argentina. Misiones: Puerto Iguazú (25°35'50.895"S, 54°34'42.873"W).

Observation. First record for Santiago del Estero province.

Stenopoda lativentris Giacchi

Stenopoda lativentris Giacchi, 1969: 13; Bachmann 1999: 215, 224.

Diagnosis. (After Giacchi 1988b) Abdominal segments 1–5 divergent, the rest convergent. Pronotal setae two times longer than tubercles height. Subcylindrical tubercles, longer than wide.

Distribution in Argentina. Misiones: Pindapoy (27°45'2.592"S, 55°47'28.4856"W), Puerto Iguazú (25°35'50.6862"S, 54°34'43.4922"W).

Stenopoda pallida Giacchi

http://research.amnh.org/iz/types_db/images/Stenopoda_pallida.jpg

Stenopoda pallida Giacchi, 1969: 13; Giacchi 1988b: 56.

Diagnosis. (After Giacchi 1988b) Side of the abdomen parallel. Pronotal setae curved and shorter than height of tubercle. Tubercles semispherical. Fossula spongiosa of 1/5 or 1/6 foretibia length.

Distribution in Argentina. Misiones: Eldorado (26°25'1.506"S, 54°36'41.3706"W); Río Negro: El Bolsón (41°58'10.9236"S, 71°32'14.3694"W).

Stenopoda subinermis Stål

Stenopoda subinermis Stål, 1859: 384; Melo et al. 2011: 7.

Diagnosis. (After Giacchi 1988b). Sides of the abdomen parallel. Pronotal setae shorter than tubercles height. Semispherical tubercles. Foretibiae with fossula spongiosa of equal length to half the length of the tibia.

Material examined. Chaco: $1 \stackrel{?}{\bigcirc}$ (MLP) Chaco National Park.

Distribution in Argentina. Chaco: Chaco National Park (26°48'24.9984"S, 59°26'36.4986"W).

Stenopoda (Stenopoda) Giacchi, 1988

Stenopoda (Stenopoda) Giacchi, 1988b: 48.

Type species. Stenopoda cinerea Laporte, 1832.

Diagnosis. (After Giacchi 1988b) Total length of 18 to 26 mm. Fossula spongiosa of 1/7 to 1/4 the length of the foretibiae.

Stenopoda cinerea Laporte

Stenopoda cinerea Laporte, 1832: 26; Barber 1930: 203; Quintanilla et al. 1976: 129; Froeschner 1988: 648.

Diagnosis. (After Giacchi 1988b) Spots of the connexival segments dark brown, ellipsoidal. Fossula spongiosa of 1/5 to 1/4 the length of the foretibiae.

Material examined. Chaco: 1 \bigcirc (MLP) Fontana (27°25'1.0452"S, 59°1'54.6882"W); Santiago del Estero: 1 \bigcirc (MLP) (unspecified locality).

Distribution in Argentina. Chaco: Fontana; Córdoba: as south as Córdoba; Corrientes: Departamento Monte Caseros (30°15'9.4212"S, 57°37'20.604"W), Departamento San Luis del Palmar (27°30'40.464"S, 58°33'30.4518"W).

Observation. First record for Chaco and Santiago del Estero provinces.

Stenopoda guaranitica Giacchi

Stenopoda guaranitica Giacchi, 1969: 19; Giacchi 1988b: 52; Maldonado Capriles 1990: 541; Bachmann 1999: 214; Coscarón 2003: 361.

Diagnosis. (After Giacchi 1988b) Total length between 18 and 26 mm. Pronotal setae one and a half times longer than tubercles height. Fossula spongiosa of 1/7 to 1/5 the length of the foretibiae.

Material examined. Río Negro: 13° (MLP) Pemona (39°29'9.2142"S, 65°36'33.5592"W); Formosa: 23° (MLP) Isla Oca (26°15'13.6722"S, 58°11'15.846"W), 19° (MLP) Río Paraj. 19° (unspecified province and locality), 33° (unspecified locality).

Distribution in Argentina. Chaco: Apóstol, Resistencia (27°26'37.0356"S, 58°58'7.8924"W), Río de Oro (58°40'19.5564"S, 58°40'19.5564"W); Córdoba; Corrientes: Garruchos (28°10'23.3076"S, 55°39'18"W), Ituzaingó (27°35'41.532"S, 56°41'56.022"W), Santo Tomé (28°32'51.507"S, 56°2'14.3232"W); Entre Ríos: El Palmar (31°51'51.5808"S, 58°12'30.5346"W); Formosa: El Coatí (25°43'59.8794"S, 59°37'59.8794"W), Palo Santo (25°33'49.7304"S, 59°20'10.5252"W); Jujuy: Calilegua (23°46'28.221"S, 64°46'16.575"W); Mendoza; Misiones: Arroyo Uruguaí (25°53'32.157"S, 54°35'58.1136"W), Eldorado (26°25'1.506"S, 54°36'41.3706"W),
Iguazú (25°36'40.0062"S, 54°35'14.067"W), Montecarlo (26°34'21.5646"S, 54°46'1.8042"W), Posadas (27°22'50.1918"S, 55°54'51.8472"W), Zaimán (27°25'6.801"S. 55°53'40.47"W), Departamento Concepción: Panembí (27°43'36.48"S, 54°54'54.5394"W), Pindapov (27°45'2.592"S, 55°47'28.4856"W); Santa Fe: Departamento De Garay: Colonia Mascías (30°47'55.8348"S, 60°0'52.3218"W); Salta: Departamento Anta: La Forestal (24°55'0.0114"S, 64°28'0.0012"W), Metán (25°29'47.4318"S 64°58'19.3044"W), Salta City (W 24°47'47.5902"S 65°23'33.666"), Las Delicias (W23°56'1.0428"S, 63°19'51.096"), Urundel (23°33'28.8288"S, 64°23'50.9994"W), Departamento Orán: Tablillas (22°38'0.0306"S, 63°51'0.1038"W), La Candelaria (26°6'4.554"S, 65°5'59.0814"W); Santiago del Estero: Colonia Mackinlay (30°22'0.9546"S, 62°7'0.8754"W); Tucumán: San Pedro de Colalao (26°14'4.2504"S, 65°29'19.9674"W).

Observation. First record for Río Negro province.

Stenopoda wygodzinskyi Giacchi

http://research.amnh.org/iz/types_db/images/Stenopoda_wygodzinskyi.jpg

Stenopoda wygodzinskyi Giacchi, 1969: 19; Maldonado Capriles, 1990: 541; Coscarón 2003: 61.

Diagnosis. (After Giacchi 1988b) Setae of scapus of length equal to its diameter. Pronotal setae three times longer than tubercles height. Fossula spongiosa of 1/7 to 1/6 the length of the foretibiae.

Distribution in Argentina. Corrientes: Santo Tomé (W28°33'6.6378"S, 56°2'43.52").

Acknowledgments

Special thanks to D. L. Carpintero (Museo Argentino de Ciencias Naturales "Bernardino Rivadavia") for lending specimens. We thank Dr. T. Henry (Systematic Entomology Laboratory, USDA, c/o National Museum of Natural History, Smithsonian Institution) and anonymous reviewer for revising and improving the manuscript. This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnologicas (CONICET) and Agencia Nacional de Promoción Científica y Tecnológica of Argentina.

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RESEARCH ARTICLE



Macrolepidoptera biodiversity in Wooster, Ohio from 2001 through 2009

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Academic editor: D. Lafontaine Received 2 June 2014 Accepted 3 October 2014 Published 5 November 2014

Citation: Downer RA, Ebert TA (2014) Macrolepidoptera biodiversity in Wooster, Ohio from 2001 through 2009. ZooKeys 452: 79–105. doi: 10.3897/zooKeys.452.8009

Abstract

A Skinner mercury vapor light trap was operated from 2001 through 2009 in a residential backyard to document biodiversity within the moth families Thyatiridae, Drepanidae, Geometridae, Mimallonidae, Apatelodidae, Lasiocampidae, Saturniidae, Sphingidae, Erebidae (including Lymantriinae and Arctiinae), Euteliidae, Nolidae, and Noctuidae. When making comparisons to older literature, we recalculated our results to conform to the older classification of the Noctuoidea. Moths were released after identification. There were 501 species documented in 77581 captures from 1290 sampling dates. There was a perceived risk that released moths would fly back into the trap the following evening. This should result in an abnormal number of rare moths that are caught multiple times. The number of species caught twice versus the number caught once was no different than a similar ratio for surveys that used more traditional sampling methods. Therefore this concern does not seem to be valid for these data. These data are provided in a supplementary file available for download.

There were three previous surveys conducted in nearby natural areas. They documented fewer species than were documented here. To understand this better, we examined several specialized groups of moths that tend to use host plants not typically found in an urban residential yard. More species in *Schinia* Hübner, *Catocala* Schrank, *Acronicta* Ochsenheimer, and Herminiinae Leech were found in this survey than the other local surveys. Only in the *Papaipema* Smith did we recover fewer species, though it was still above 70% of what was expected. This diversity could be a result of sampling effort, but it shows that this urban location has a very diverse moth fauna. We suggest that this diversity is partly due to the planting of native plant species in the area about the light trap. Therefore we would concur with others that urban landscapes can be planned to increase biodiversity relevant to more natural ecosystems.

In this study we looked at the ratio of the number of species of Geometridae divided by the number of species of Noctuidae as one approach to evaluating the level of disturbance in the moth assemblage. Although the yearly average was nearly constant, the seasonal ratio ranged from 0.09 to 0.91 depending on the sampling date. We also calculated alpha diversity and found that seasonal change in alpha diversity greatly exceeded yearly differences. This strong seasonal component means that a comparison between two studies requires a correction for seasonality and similar sampling intervals. In this study, a shift of two weeks would be sufficient to result in a significant difference in alpha diversity. This is the equivalent of increasing temperature by 1.53 °C. Seasonal shifts limit the usefulness of this methodology for environmental assessment because the within season change exceeds the between season change. This problem is compounded when sampling designs interact with this seasonality.

In describing our data, we made use of a growing degree day (GDD) model. This approach corrects for simple temperature dependent shifts in moth biology. Consequently, some of the variability in the data was removed, which should improve the power of statistical tests involving survey data. If sampling protocols were based on growing degree days rather than calendar dates, the bias caused by temperature induced shifts in seasonal cycles could be reduced.

Keywords

Organismal biological diversity, survey, seasonality, phenology, moth

Introduction

Moths play an important role in ecosystems. Adults pollinate flowers, and their larvae play a variety of roles as herbivores, detritivores, omnivores, or carnivores (Triplehorn and Johnson 2005). Moths are an important food resource for a variety of animals including lizards, small mammals (Kronfeld-Schor and Dayan 1999), birds (Schwenk et al. 2010; Visser et al. 2006), bats (Dodd 2006; Dodd and Lacki 2007; Dodd et al. 2008), and other insects (Howell and Davis 1972). Because of their pivotal role in ecosystem function, moths are sometimes used for assessing the effects of environmental change (Gimesi et al. 2012), habitat restoration (Highland and Jones 2014), or environmental impact assessment (Chaundy-Smart et al. 2012; Kitching et al. 2000; Slade et al. 2013).

The largest family of moths is the Noctuidae (Borror et al. 1976). However, the classification of the Noctuidae and closely related families has been extensively revised in recent years (Lafontaine and Schmidt 2010). Such revision improves our understanding of the biology of this diverse group of moths, and we will use the new classification when describing our results when there are no comparisons to older literature. However, we will use the older classification for the Noctuoidea when comparing our results to the older literature. If we cite a manuscript we will use the classification scheme that was used in the cited article. The old families Lymantriidae and Arctiidae are now two subfamilies of the Erebidae, and the old family Noctuidae now consists of the families Erebidae, Euteliidae, Nolidae, and Noctuidae (Lafontaine and Schmidt 2010).

Urbanization results in a large number of environmental changes. Physical changes from urbanization include elevated pollution levels in air and soil, elevated temperatures, increased soil compaction, and increased soil alkalinity (McKinney 2002). Biotic changes include biological deserts (roads, parking lots, and buildings), loss of native

host plants, reduction in patch sizes of suitable habitat, and the introduction of weedy species and ornamentals (McKinney 2002). This might result in a taxonomic homogenization through loss of specialists and an over-representation of generalists (Marie-Hélène et al. 2011). Thus urbanization is a biotic filter that favors a few generalists and excludes many species adapted to specific native environments (McKinney 2006; Niell et al. 2007). However, a decrease in biodiversity with increasing urbanization is not always observed (Krauss et al. 2003). Furthermore, sometimes elevated biodiversity is observed somewhere between the natural areas at the periphery of human habitation and the urban core. One explanation for this is the intermediate disturbance hypothesis where human disturbance creates more biotic boundaries and increased environmental heterogeneity. It is also possible to have greater diversity at the urban core relative to closely adjoining areas because new development in the adjoining areas tends to remove most of the existing vegetation, increases soil compaction, and removes topsoil (McKinney 2002). Such unnatural increases in biodiversity can be misleading when discussing biodiversity loss due to urbanization. Urbanization destroys key habitats that harbor specialists, and a simple count of the number of species may obscure loss of native biodiversity if an urban area is invaded by a diverse assemblage of generalists that can better utilize the exotic vegetation (McKinney 2006). These ideas have been tested through habitat manipulation. Replacing non-native vegetation with native species can quadruple insect abundance and triple biodiversity (Burghardt et al. 2008). Improved biodiversity in urban settings from habitat manipulation that favored native species was also observed in Finland (Valtonen et al. 2007). Thus, although urbanization can result in biodiversity losses, even small plantings of native species within an urban setting can mitigate these effects in localized areas (Tallamy and Shropshire 2009).

Biodiversity is one measure of the effect of environmental impact, but it can be distorted by an influx of new generalist species better adapted to disturbed environments. It has been suggested that the ratio of the number of geometrid moths to the number of noctuid moths is a better measure of environmental disturbance (Kitching et al. 2000). The idea was that noctuids tend to be larger moths capable of greater dispersal and they generally have a broader host range than the geometrids. The influence of body size on dispersal was examined quantitatively by measuring moth migration between small islands (Nieminen 1996), but dispersal ability does not always equate to migration rates (Slade et al. 2013). Kitching showed that uncleared remnants had a Geometrid:Noctuid ratio of 0.987, cleared remnants 0.682, and scramberland remnants 0.186 (scramberland sites are covered by Lantana camara L., Solanum mauritianum Scop., and a variety of other weeds with a high proportion of exotic species. Isolated rain forest shrubs and trees emerged from this understory). This ratio was proposed as a first approximation, and a more restricted list of moths in specific subfamilies within the Geometridae and Noctuidae were detailed as a more refined approach. Others have proposed similar indicators, though typically selecting specific groups within these and other families (Summerville et al. 2004).

Moth surveys are often justified as tools to document ecological processes like climate change (Fox et al. 2011), environmental impacts (Summerville 2011; Taki et al.

Cite	State	Location	No.	Spp. ¹	Noct.	Geo.
А	OR	Blue Mtns	20322	383	212	93
В	WV	Cooper's Rock State Forest	29983	400	220	102
С	WV	Turkey Run and Great Falls National Pks ²	Unk	480	278 ³	107
C1	WV	Camp Dawson Collective Training Area	3666	235	101	73
C2	WV	Southern West Virginia ²	Unk	751	418	191
D	FL	Blue Spring State Park ²	Unk	275	171	67
E	NJ	Hutcheson Memorial Forest	22880	410	253	98
F	LA	West Feliciana Parish	3155	314	122	68
G	LA	Long-leaf pine Savanna	1182	208	84	42
Н	IN	Morgan-Monroe State Forest	14537	324	110	72
Ι	IA	Neal Smith National Wildlife Refuge	9416	508	136	69
J	OH	Wilderness Center ²	Unk	413	233	94
K	OH	Funk Bottoms ²	Unk	262	159	46
L	OH	Atwood Lake State Park ²	Unk	376	221	93
	OH	Wooster (current study)	77581	501	3144	104
М	TN,NC	Great Smoky Mountains National Park ²	Unk	914	528	225
N	AR	Ozark mtns	8720	314	57 ⁵	33 ⁵
0	Hungry	Aggtelek National Park	127035	994	512	326
Р	Canada	Ministik Hills, Alberta	24578	264	151	66
Q	Canada	Acadia Research Forest, New Brunswick	31634	539	270	169
R	ME	Orono	43435	337	258	27

Table 1. Overview of moth surveys including number of moths sampled (no.), number of species recorded (spp.), and the number of species of Noctuidae (Noct.) and Geometridae (Geo.). The main focus was surveys from the United States.

Citations: A (Grimble et al. 1992) B (Butler and Kondo 1991) C (Steury et al. 2007) C1 (Butler et al. 2002) C2 (Albu and Metzler 2004) D (Profant 1989) E (Moulding and Madenjian 1979) F (Landau and Prowell 1999b) G (Landau and Prowell 1999a) H (Summerville et al. 2008) I (Lewis et al. 2005) J (Rings et al. 1987) K (Williams et al. 1977) L (Rings and Metzler 1988) M (Scholtens and Wagner 2007) N (Dodd et al. 2008) O (Szabo et al. 2007) P (Schmidt and Roland 2006) Q (Thomas 2001) R (Dirks 1937)¹) The published species counts often included families that were not part of this research. Therefore the number of species were recounted and species from families not part of this study were removed.²) Survey only, no abundance data presented.

³) Nolidae was separated in this list, and these were added back into the Noctuidae to get this number.

⁴) using older classification (Hodges 1983). Revised values for Noctuidae are 208, giving a ratio of 0.5.

⁵) These are minimums, some material not identified to species.

2010), and habitat restoration (Bucheli et al. 2006; Summerville 2008). In trying to integrate our results with these other studies, there are well-known problems associated with trapping methodology: type of light trap (Fayle et al. 2007; Leinonen et al. 1998; von Langevelde et al. 2011), number of trapping nights, number of traps, and environmental factors like moon phase (Sanyal et al. 2013), or artificial lighting (Schweitzer et al. 2011). However, seasonal variability, or more precisely incomplete seasonal coverage in most surveys, can result in major systemic errors (Summerville 2008), and this effect makes study-to-study comparisons problematic.

To put this survey in perspective, we compiled a table of several moth surveys from the last 70 years (Table 1). These surveys were from a wide variety of habitats, and not all collections were restricted to black light trapping of macrolepidoptera. The ratio of number of species of Geometridae divided by Noctuidae was very consistent with a ratio of 0.46 and a standard deviation of 0.14. The extreme values were from the Maine survey that was 2.5 standard deviations below this value whereas the West Virginia survey from Camp Dawson Collective Training Area was 1.9 standard deviations above.

There have been three Lepidoptera surveys in our local area. These took place at Funk Bottoms, The Wilderness Center, and Atwood Lake Park. Funk Bottoms Wildlife Area consists of periodically flooded moist meadows, bottomland hardwoods, and 80 ha of permanent marsh. However, thousands of hectares may be flooded for up to several months each year (Williams et al. 1977). This site was about 13 km SW of our light trap. Black light trapping was done at two locations from April through November in 1995 for a total of 30 trapping nights. The Wilderness Center features about 40.5 ha of virgin forest and a stream. Management programs have created a pond, a lake, and about 2 ha of thicket (Rings et al. 1987). The Wilderness center is about 25 km SE of our light trap. Collecting was done from 1977 through 1985. In 1984 and 1985, trapping was done at five sites by black light trap and sugaring. Light traps were run twice per week from May through October 1984 (24 sample nights), and March through June 1985 (16 sample nights). Atwood Lake Reservoir was constructed in 1937 on Indian Fork Creek. It had a natural oak-hickory and beech-maple woodlands that underwent a reforestation effort using pine and Liriodendron tulipifera L. (Rings and Metzler 1988). The Wildlife Area Atwood Lake Park was about 58 km SE of our light trap. Trapping was done at four locations by black light and sugaring on no more than 21 nights in 1985 and 14 nights in 1986. The primary repository of specimens from the study at the Wilderness Center was the Wilderness Center collection. Additional specimens were deposited in the reference collection at the Ohio Agricultural Research and Development Center (OARDC) (1680 Madison Ave, Wooster, Ohio). Specimens from the other studies were deposited in the OARDC reference collection. Subsequently, many of the OARDC specimens were relocated to the Museum of Biological Diversity at The Ohio State University.

Temperature plays a critical role in biological processes. A "growing degree day" (GDD) model is typically used where accumulated thermal units are explanatory variables for the biological process of interest (Forrest and Thomson 2011; Harrell et al. 2011; Kimball et al. 2012; Smitchger et al. 2012; Spear-O'Mara and Allen 2007). Therefore we used a growing degree day model to change calendar date into a variable more relevant to insect biology and examined biodiversity in this light. Using a growing degree day approach also allowed a more natural grouping of multi-year data because it corrects for yearly shifts in accumulated heat units (Kimball et al. 2012). Therefore we would expect that a GDD approach would result in less variability in the data relative to the mean response. This should improve the sensitivity of statistical tests in a variety of applications that use biodiversity estimates to assess environmental conditions. This approach can also distinguish between thermally induced shifts in life cycles versus a disruption of those life cycles.

It would be useful to define the area being sampled when conducting any sampling activity. Defining the sampling radius about a light trap is not simple in part because it is a probability function where the probability of capture decreases exponentially with increasing distance. The probability of capture also declines rapidly if the moth starts its movement outside the radius where the light is strong enough to be attractive. Anything that affects background light levels (moon phase, light pollution, cloud cover) will alter capture probabilities (Steinbauer et al. 2012; von Langevelde et al. 2011; Yela and Holyoak 1997). Estimates of attraction radii range from 3m to 800 m. Attraction radii are also species specific (Baker and Sadovy 1978; Beck and Linsenmair 2006; Truxa and Fiedler 2012). In recapture experiments, less than half of the moths released 5 m or less from the light were recaptured, and less than 20% were recaptured at 25 m (Truxa and Fiedler 2012). Other studies have estimated attraction radii of between 200 m at full moon to 520 m at no moon (Bowden 1982). Exact distances vary by trap type (wavelength, power, design), trap height, species, and environmental factors influencing the contrast between ambient light and trap light (Fayle et al. 2007; Hollingsworth and Hartstack 1972; von Langevelde et al. 2011; Yela and Holyoak 1997).

The above paragraph contains considerable uncertainty about the exact attraction radius. This is caused by differences in the methodology of the cited works. We provide two cases to illustrate the point. Baker and Sadovy (1978) used a 125W mercury vapor lamp using mark-recapture methods, and 5000 individuals of Noctua pronuba (L.) and Agrotis exclamationis (L.). Multiple traps were placed about a release point using two configurations. A sharp decline in the number of recaptures was observed starting at 5 meters if the light traps were dispersed about the release point. The other approach used two light traps, one closer to the release point than the other. In this case the further light trap ceased to capture any moths if it was more than 7 meters from the release point. In contrast, Truxa and Fielder (2012) used a mark recapture method, but traps with two 15W black lights were used. They used these traps to capture 2331 moths from 166 different species for subsequent marking and releasing. Two experiments were run, the first in a deciduous tree forest at University of Bayreuth with tree heights from 5 to 8 m. Moths were trapped, identified, marked and released 24h after capture. A single light trap was placed along a gravel path and moths were released at 13 distances from 2 to 40 m distant. The second experiment was done in a deciduous tree forest at the Donau-Auen National park along a straight forest road. The same type of trap was used, but there were 12 release points from 5 to 100 m distant. In the first experiment 20% of the moths released at 35 m were recaptured, but none of the moths released at 40 m were recovered. In the second experiment, no moths released past 80 m were ever recovered. Baker and Sadovy used two species but moths were allowed to go in any direction. Truxa and Fielder used many species but the cleared forest path forms a tunnel that could funnel moths towards the trap. None of the cited experiments are flawless, but they all indicate that the attraction radii of most traps will be fairly limited. Elevated traps may have larger attraction radii (Baker and Sadovy 1978), but the attraction radii of elevated traps is not relevant to this study. From another perspective, anyone who has held and released a moth will point out that

many of these moths have the ability to fly much further than a few hundred meters. However, that is not the point. This is about the probability of capturing a moth that starts its flight activity some distance from the light. That probability declines rapidly with increasing distance. The cited studies suggest that the probability is very low past a few hundred meters.

Our goals were to; 1) Document biodiversity in an urban setting to compare to three previous surveys in natural settings. 2) A quantification of the effect of seasonal changes in moth diversity. 3) Document the utility of a phenological model in understanding biological survey results.

Materials and methods

The trap was located in an urban (as defined by US Census Bureau (Anonymous 2010)) setting in Wooster, Ohio, USA (40.80917°N by 81.93722°W), population 26,000 (www.city-data.com viewed 21/7/2010). The residential back yard was 0.16 ha of lawn on the Killbuck-glaciated Pittsburgh Plateau (http://www.dnr.state.oh.us/ portals/10/pdf/physio.pdf) at 353 m elevation (http://www.usgs.gov). The acreage was determined using the Wayne County Auditor's assessment of lot size less the auditor's measure of the size of the house (Waynecountyauditor.org viewed Nov 2010). Neighboring parcels were smaller than this one with an average parcel size (including the house) of 0.127 ha (standard deviation 0.059). The neighborhood contained mature trees and shrubs including oaks, ash, locust, cherry, conifers, maples, blueberries, lilacs, and dogwoods. Much of the neighborhood was dominated by turf grass and associated weeds (Cheng et al. 2008). The yard with the trap had a variety of native and nonnative annuals and perennials, and a small (about 2 meter diameter) artificial pond/ marsh area. The garden was developed gradually beginning in 1993, and one goal in selecting plants for this garden was to provide nectar and larval food resources for a variety of native pollinator species. Such activities are known to increase biodiversity in urban landscapes even on small 0.13 ha parcels (Burghardt et al. 2008), though the biodiversity benefit of specific activities can sometimes be variable (Gaston et al. 2005) despite a general observation that plant biodiversity increases insect biodiversity in natural habitats (Schaffers et al. 2008).

Moths were collected using a Skinner mercury vapor light trap with a 125 Watt mercury vapor bulb (model 7591 from Watkins and Doncaster (www.watdon.co.uk)) with the filament 33 cm above ground level. The performance of this trap relative to others was recently evaluated (Fayle et al. 2007). The trap was run most nights when temperatures were above freezing and there was no rain. Moths were identified and most were released on the other side of the house on the morning after the trap was run, about 20 m distant. There were street lights on the eastern side of the house where moths were released. Voucher specimens for the new county records were retained and deposited with the Museum of Biological Diversity, The Ohio State University, 1315 Kinnear Rd. Columbus, OH, USA 43212. These records were additions to earlier work

on the moths of Ohio (Rings and Downer 2001) see http://www.oardc.ohio-state. edu/rb1192/default.asp (accessed 6, September 2009). Additional vouchered records for most Ohio Noctuidae and Erebidae were published earlier (Rings et al. 1992). Although physical specimens don't exist for the remaining identifications, photographic documentation for many specimens can be found at www.butterfliesandmoths.org. Below the banner click on regional checklists. Then select the region United States/ Ohio/Wayne, click apply. From the checklist for Wayne County, click on the species of interest and proceed to another page. Scroll down and click on Sightings Table where all the sightings for the species are listed. Scroll through these to find the records for submitter "rogerdowner".

We suggest using the GPS coordinates provided earlier and Google Earth® (http:// www.google.com/earth/index.html) for a detailed view of the environment about the moth trap. Botanical composition of nearby parks (1 km distant) is largely irrelevant due to the short attraction radii of black light trapping methods (<520 m). Furthermore, the light was close to the ground, so buildings, trees, and tall shrubs all block light and serve to further restrict this radius.

Moths were identified and catalogued using an older classification system (Hodges 1983), that was subsequently updated (Lafontaine and Schmidt 2010). In a few cases this required personal communication with Dr. Lafontaine to correct our species list. The older system was retained when making comparisons to the older literature. In this system Arctiidae and Lymantriidae are separate families. New results utilize the newer classification where the Arctiidae and Lymantriidae become subfamilies in the Erebidae, and the old Noctuidae is divided into the Erebidae, Euteliidae, Nolidae, and Noctuidae.

Phenology

A lower developmental threshold of 10 °C was used to estimate growing degree days (GDD) (Pruess 1983). Weather data were measured at a weather station located at the OARDC about 8 kilometers south of the trapping site. The method used to calculate GDD was a modified sign wave method (Allen 1976; Pruess 1983) as outlined at http://www.oardc.ohio-state.edu/gdd/glossary.htm (viewed Jan 2009) and see also (Cardina et al. 2007). We recognize that many of the moths may have developmental thresholds different from 10 °C, but for consistency, we retain the base temperature of 10 °C even for those few species where sufficient research exists to justify a different base. The calculation for GDD in the OARDC site was based on English units, which were converted to metric using GDD in °C = -0.00013+0.555639* GDD in °F (F=57017608; df 1,363; P<0.0001). We used the single triangulation method in cases where we needed to recalculate GDD (Lindsey and Newman 1956), and note that there tends to be close agreement between the various sine and triangulation methods (Roltsch et al. 1999). The use of a fixed threshold temperature for different species has been used to model voltinism changes in Finnish moth species (Poyry et al. 2011).

Analysis

We used the various approaches to estimating species richness implemented in EstimateS (Colwell 2013) set to run 1000 randomizations without replacement. We calculated species richness using both individual sampling dates and yearly pooled samples. However the difference between the estimates was less than the estimated standard deviation for either method. Therefore we only present results using individual sampling dates.

We used the proportion of species represented by a single capture as an indication of the effectiveness of the sampling protocol (Carlton et al. 2004; Williams et al. 2007). This approach assumes that no viable moth population can be represented by a lone individual, so the capturing of only a single individual indicates that the method missed some individuals. Although some singletons are indicative of an ineffective sampling methodology, e.g., moth species that do not come readily to light, some singletons should be expected since they could come from migrating individuals that have little interest in the trap or its environment.

The study site had bats, birds, and wasps that preyed on moths attracted to the light. There may also have been other vertebrate and invertebrate predators. Moths were released in different locations in the yard to reduce such predation. However, we could not quantify the level of predation or the effectiveness of any effort at reducing predation. Sometimes moths were too worn to be properly identified, and these individuals were ignored.

Results and discussion

Potential problem

We expect that three traps run six times per year for one year (Summerville and Crist 2003) would have less impact on the local ecosystem than would one trap run at the same location 115 to 215 times per year for nine years (this study). Long term intensive sampling has shown the potential to negatively impact moth populations (Vaisanen and Hublin 1983). Consequently moths were released after identification. This methodology may inflate abundance estimates, though it would not affect the number of observed species. So we address the issue of multiple captures internally using the frequency of doubletons, and externally by comparing with the published literature.

Quantitative assessment of the effect of multiple captures was made by examining the number of moth species captured once per year versus the number represented by two captures per year. A methodology that increased the probability of recapturing moths should have a disproportionate number of rare species captured twice. The average doubleton+singleton ratio for each year was 0.574 (standard deviation [SD] of 0.211). We also look at this ratio for each sampling date because in this case doubletons cannot be recaptures of the same individual. The doubleton+singleton ratio for each night where there were both singletons and doubletons was 0.382 (SD 0.336). The yearly average was not significantly greater than the daily average (F=2.91; df=1, 980; P=0.09). Obviously, a failure to detect a significant difference is not the same as proving that there was no effect. Summerville et al. (2003) reported a ratio of 0.568 (SD of 0.054), whereas Summerville et al. (2004) found a ratio of 0.472 (SD of 0.092) (these numbers based on data provided by Dr Summerville from research published in cited literature). Lower values have been observed in other studies, 0.311 [Vancouver, Canada] (deWaard et al. 2009), 0.552 (SD of 0.247) [Rothamsted insect survey site 336, United Kingdom] (Harrington and Woiwod 2007), as well as higher values 0.932 [Blue Mts, Oregon] (Grimble et al. 1992), 0.618 [Birch Mts., Alberta, Canada] (Macaulay and Pohl 2005). A collection from Inverness Ridge in California had a value of 0.362 (data provided courtesy of Jerry Powell). A collection from Annville, Pennsylvania had a value of 0.222 (data provided courtesy the Pennsylvania Natural Heritage Program) (Ferster et al. 2008). The most comparable study would be the 31 years of data from Rothamsted because the data were yearly counts over multiple years from one locality. We conclude that our result of 0.574 is not unusual compared to these studies, and therefore the possibility of capturing the same individual twice doesn't seem to result in an excessive bias in this study. However, we don't know if we got lucky, or if this is a typical result.

Raw data

The raw data are included as supplemental data. The data file is in Excel format. We recommend that users read the "Introduction", which is the first page (left-to-right) in the file. The next page to the right in the file includes the weather data. Farther to the right are nine pages with yearly capture data. These pages include the number of growing degree days accumulated by each collection date. Cells are blank if no individuals of a given species were captured on a specific date. Next is a page "Condensed List" that contains total number of each species, and the number of years each species was collected. This page contains the species as they were identified and the equivalent under the system by Lafontaine and Schmidt (2010). It also lists the range in capture date, and range in growing degree days. Then follows total captures per year, and a list of known host plants. Next there is a list of the 13 new county records and their collection date. Next is a page with a list of univoltine and bivoltine species selected based on abundance and environmental fidelity. Next is a listing of the 20 pest species and their yearly abundance. This was extracted from the main list to facilitate access. Lastly is a page with the moon phases. We did not find this of any use, but it may prove useful to someone else.

Diversity and abundance

In 1290 sampling dates from 1 January 2001 through 31 December 2009, a total of 77,581 moths were captured and identified. This averages to 60 moths/night. However

Family	Individuals captured	dualsNumber ofNumber ofTotal percentageuredgeneraspeciessingletons		Average percentage singletons	
Thyatiridae	16	3	3	33	50
Drepanidae	31	2	2	0	43
Geometridae	8578	70	104	13	20
Mimallonidae	3	1	1	0	100
Apatelodidae 8		2	2	0	63
Lasiocampidae	229	3	5	0	3
Saturniidae	42	8	8	25	50
Sphingidae 184		9	13	7	41
Notodontidae 2755		18	32	16	22
Erebidae	17197	11	112	15	23
Euteliidae	112	3	5	0	34
Nolidae	340	3	6	0	21
Noctuidae 48086 122		122	208	11	22

Table 2. Genera, species, and abundance compositions for 12 Families of macrolepidoptera in Wooster Ohio. Total percentage singletons is the number of species represented by a single capture in the nine years of the survey divided by the number of species. Average percentage singletons is the average of the number of singletons caught each year divided by the number of species caught that year.

this number has little value because it includes early and late season samples that have few moths. In 2001 the range was from 1 to 496 moths per night with an average of 96. Within this nine year sampling effort were 501 species, of which 122 were found in all nine years.

The numbers of species within a family that were represented by a single capture has been used as a metric for evaluating the effectiveness of a sampling methodology. Carlton et al. (2004) reported that singletons accounted for 38 to 43% of their sample, and this was considered indicative of sufficient sampling effort. Using this criterion, the average singleton rate per year for the Thyatiridae, Drepanidae, Mimallonidae, Apatelodidae, and Saturniidae all indicate that the sampling strategy might be ineffective. Either the moths do not respond well to black light traps, or we may be sampling transients. The Noctuidae and Geometridae accounted for the majority of singletons (Table 2). However, relative to the number of species in these families, the number of singletons in these families was low, thereby indicating sufficient sampling effort. The Lasiocampidae, Sphingidae, Erebidae, Euteliidae, and Nolidae also had singleton percentages that were with acceptable limits. The estimated total number of species was between 533 for the Bootstrap method and 599 for the Jacknife 2 method (Table 3).

The number of species only present in a single year was greatest in 2001 (Table 4). At the other extreme, 2005 and 2006 had an unusually small number of species that were only captured in that year. For the first six years, the number of species never before captured declined (Table 4). Eventually it should converge to the speciation rate plus the immigration rate of new species. However, 2007 and 2009 were unusual years in that more new species were added than one would expect from the initial pattern.

Statistic	Mean	Lower 95% CI	Upper 95% CI
Chao 1 Mean	553.69	529.61	598.78
Chao 2 Mean	560.43	533.43	609.52
		Standard Deviation	
Jacknife 1	568.95	8.33	
Jacknife 2	598.97	1.18	
Bootstrap	533.27	0.49	

Table 3. Diversity statistics¹: Estimates of the number of species.

1) (Colwell 2013)

Table 4. Summary by year, and over the nine year study period for macrolepidoptera in Wooster Ohio. We list the number of days sampled (Days), number of individuals captured (Captured), number of genera (Genera), number of species (Species), the species that had never been captured prior to that year (Never Before), the species captured only in the given year (Only Once), percentage of species represented by only one capture (Only One), Fisher's alpha (Alpha), and the standard deviation of Fishers alpha (SD).

Year	Days	Captured	Genera	Species	Never before	Only once	Only one	Alpha	SD
2001	133	12,819	219	339	339	19	87(26%)	64.12	1.54
2002	115	6,688	176	257	37	6	68(26%)	53.05	1.56
2003	146	8,094	193	288	36	6	63(22%)	58.29	1.60
2004	121	6,754	175	278	29	13	66(24%)	58.42	1.67
2005	127	6,950	182	274	14	5	39(14%)	56.93	1.63
2006	126	7,067	192	278	11	5	64(23%)	57.73	1.64
2007	164	9,837	199	317	15	8	79(25%)	60.17	1.67
2008	142	7,476	172	263	5	4	60(23%)	53.11	1.52
2009	216	11,892	209	333	15	15	74(22%)	63.59	1.56
All	1290	77,581	2934	501			64(13%)	71.86	1.17

These two years therefore have a large influence on the estimated total number of species. There was no obvious pattern in the new species for 2007 and 2009. None of the species were pests. In 2007, four of the 15 species fed on oak, maple, or walnut, whereas six of the 15 species from 2009 had these hosts. Seven of the 15 species in 2007 were captured again in either 2008 or 2009. In 2003 there was an F2 tornado that went through the city. Another F2 was within a few miles of the city in 2009. Smaller tornados occur in Wayne County nearly every year (http://www.tornadohistoryproject.com/ tornado/Ohio/2003/map). Another environmental disturbance happens twice per year as the city applies insecticide for mosquito control using a truck mounted fogger. None of these events seem related to patterns in our survey.

Given that we documented 501 species at this one location, one might suggest that this urban environment had greater macrolepidopteran diversity than 15 of the 19 North American sites in Table 1. However, this comparison is problematic. Sampling effort both within season and the number of seasons affect the number of species collected (Gotelli and Colwell 2001). The other studies used multiple traps in a

Location	Arct	Geo	Noc	Noto	Sat	Sphing
Funk Bottoms						
In Common	14	40	100	18	6	4
Unique to cited	2	6	25	1	1	1
Unique to ours	6	64	161	15	2	9
Wilderness Center						
In Common	16	64	191	28	6	10
Unique to cited	3	32	51	8	0	4
Unique to ours	3	40	117	5	2	3
Atwood Lake Park						
In Common	14	74	135	22	5	9
Unique to cited	1	19	37	5	2	2
Unique to ours	6	30	126	11	3	4

Table 5. Similarity between our results and those from other surveys in Ohio in numbers of species in each family or subfamily. Arct = Arctiinae, Geo = Geometridae, Noc = Noctuidae, Noto = Notodontinae, Sat = Saturniidae, Sphing = Sphingidae.

variety of habitats, but usually did so over a shorter time span both in terms of the number of years sampled and in terms of the number of trapping nights per year. The type of black light trap may also have an influence (Schweitzer et al. 2011; Truxa and Fiedler 2012). Furthermore, many of the sampled habitats in the other studies might be more homogeneous than an urban landscape with corresponding influence on biodiversity (Fox et al. 1997; Krauss et al. 2003; McKinney 2002; Southwood et al. 1979), especially considering that small plantings of native vegetation augment botanical diversity and concomitant increases in moth biodiversity (Burghardt et al. 2008). Considering the large number of species found in this survey, we would agree with the idea that it should be possible for urban planning committees to design urban landscapes that support an abundant and diverse macrolepidopteran fauna (Ockinger et al. 2009; Valtonen et al. 2007), which might also improve the habitat for birds and other wildlife.

There have been three moth surveys near this survey. The Wilderness Center had the fewest number of shared species with our study (Table 5). The greatest similarity was in the Geometridae where 15% of the combined species were in common. The Atwood Lake Park survey and the Funk Bottoms survey were much more like our survey with a 28% or better overlap in species lists (average overlap 52%). If urbanization at the Wooster site had elevated diversity due to habitat fragmentation and colonization by a diverse assemblage of generalists, then one would expect that most of the species unique to the Wooster survey would be in the Noctuidae. However, this was the case only for comparisons with Atwood Lake Park where 46% of the Noctuid moths captured at Wooster were unique to Wooster, while only 29% of the Geometrids were. This is in contrast to Funk Bottoms where 58% of the Noctuids were unique to Wooster while 62% of the Geometrids were unique, and the Wilderness Center had 35% and 38% respectively. This outcome is inconsistent with the hypothesis that ur-

Source	Schinia	Catocala	Acronicta	Papaipema	Psaphidini	Herminiinae
А	3	26	23	10	2	25
В	1	16	14	12	2	18
С	2	19	19	8	2	11
D	2	11	5	13	2	11
Е	2	12	19	6	2	31
F	0	15	20	6	2	25

Table 6. Number of species collected from specific groups for several faunal surveys. These groups contain a large proportion of specialists that could be adversely impacted by urbanization.

A) Current Study B) Atwood Lake (Rings and Metzler 1988) C) Wilderness Center (Rings et al. 1987)D) Funk Bottoms (Williams et al. 1977) E) Turkey Run (Steury et al. 2007) F) Coopers Rock (Butler and Kondo 1991).

banization at this location has increased diversity by attracting more generalists at the expense of naturally occurring moths.

An alternative strategy to assess the value of this urban moth assemblage is to examine specific genera within the Noctuoidea that are not associated with typical urban vegetation. Larvae from moths in the Noctuid genus Schinia are mostly associated with plants in the Asteraceae and Fabaceae. Species in the genus Catocala are specialists on plants in the Fabaceae, Fagaceae, Rosaceae, Juglandaceae, Myricaceae, and Salicaceae. The genus Acronicta larvae feed on woody shrubs and trees, some are specialists. Larvae of moths in the genus Papaipema are borers in stems of plants in the Asteraceae and other weedy species. The tribe Psaphidini primarily feed on members of the Juglandaceae and Fagaceae with the exception of Copivaleria grotei (Morrison), which feeds on ash. The subfamily Herminiinae is a member of the family Erebidae, with larvae that primarily feed on senescent plant material (http://www.eeb.uconn.edu/people/ wagner/USDA Noctuid Guide Most Current.doc). Table 6 compares the number of species within these groups collected at the various locations. There doesn't appear to be any pattern. For example, in the Papaipema there were six species not recovered in this survey but present in one or more of the other surveys: P. lysamachiae Bird, P. rigida (Grote), P. rutila (Guenée), P. unimoda (Smith), P. marginidens (Guenée), P. nelita (Strecker), and P. birdi (Dyar). In the case of Turkey Run (E in Table 6) and Coopers Rock (F in Table 6), half of the species in each case were in common with this survey, and only two species were in common between the two cited works. No species of Papaipema was common to all locations, though P. inquaesita (Grote & Robinson) was only missing from the Funk Bottoms survey (D in Table 6). Finally, the Papaipema in this study are moderately abundant with records of 2 to 90 specimens in total over the nine year survey. The agricultural pest P. nebris (Guenée) ranked fifth in abundance within this group. The point is that there does not seem to be a pattern that would indicate that the Wooster moth fauna are lacking in species associated with non-urban environments. It is possible that the abundance of these species is lower in Wooster than in more natural settings. However, the other local surveys did not record abundance data.

Temporal distribution

A Whittaker plot showed no obvious difference in ranked abundance between any of the years (Fig. 1). A Whittaker plot for each month showed that September and October had the most even distribution, and evenness decreased on either side (Fig. 1). Alpha diversity was greatest in early August (Fig. 2). Abundance of all macrolepidoptera had three peaks (Fig. 3). The first and last peaks were caused by the emergence of abundant bivoltine moth species, whereas the central peak was caused by abundant univoltine species. The first peak was between 250 and 583 GDD, the second between 750 and 1111 GDD, and the third between 1334 and 1611 GDD. This corresponds to late June, early August, and late September.

We calculated the number of species that went missing from one year to the next expressed as a proportion of the number of species originally present (e.g., 100* number of species in 2001 not collected in 2002 divided by the total number of species collected in 2001). We also calculated the number of immigrants expressed as a proportion of the number of species present in the year of collection (e.g., 100* number of species in 2002 not collected in 2001 divided by the total number of species in 2002). The missing rate averaged 24.0% (standard deviation 0.0701) while the immigration rate averaged 23.9% (standard deviation 0.0632) from 2001 through 2009. This would suggest that the biodiversity in this area was relatively stable over this nine year period.

The Noctuidae had three peak abundances in the year, with the first peak ending at about 722 GDD (late June), a second peak from 722 to 1056 (early August), and the third peak from 1056 GDD onwards (Fig. 4). The first and third peaks were the most substantial, with the third peak containing about 1/3rd more individuals than the first peak. In contrast, Geometrid abundance gradually increased through July, and declined thereafter. The Geometridae lacked the sharp peaks seen in the Noctuidae (Fig. 4).

The presence of seasonal patterns has been documented previously, though the specific pattern may be unique to a specific location (Szabo et al. 2007). Because diversity in both Noctuidae and Geometridae had a seasonal component, there was also a seasonal component to the ratio of these two groups (Fig. 5). Although the yearly average was nearly constant, the seasonal ratio ranged from 0.09 to 0.91. Thus a small mismatch in season could result in finding significant differences that are an artifact of seasonality interacting with an experimental design. Seasonal changes in biodiversity could introduce a potentially large inadvertent bias into biodiversity research that was based on a sampling a few dates each year (Summerville 2008). Our data provided a concrete example of this bias. We noted the peak in alpha diversity was in early August (Fig. 2). Yearly alpha diversity (Table 4) was less than peak seasonal alpha diversity (Fig. 2), but yearly alpha diversity would overestimate seasonal diversity through most of a season. For this reason one needs to know where sampling has taken place relative to the seasonal shift in alpha diversity if one is to make valid comparisons with similar studies. Otherwise one does not know if differences between studies represent ecological differences or a mismatch in seasonality. We note in Fig. 2, that if one is sampling at



Figure 1. Whittaker plots for both year (where month is ignored), and month (where year is ignored). The number in parentheses is the total number of sample days and total number of captures in that month from 2001 through 2009.



Figure 2. Seasonal and yearly change in a diversity. Bars show the 95% confidence interval. The top date for season was the average date for the midpoint, while the bottom dates give the range in month/ day format.

peak alpha diversity, then a shift of only two weeks could result in a significant difference. What might be required to cause such a shift? The current total GDD achieved by August 4 could be achieved by July 25 if every hourly observation was raised by 1.53 °C, or if both minimum and maximum daily temperatures were increased by 1.50 °C. Roughly, this is the equivalent of changing elevation by 153 to 416 m based on an environmental lapse rate of 3.6 to 9.8 °C/1000 m (Sheridan et al. 2010; Varmghani 2012). Alternatively, one could drive about 217 km closer to the equator, assuming a change of 6.9 °C/1000 km (Colwell et al. 2008; Jump et al. 2009). In the current



Figure 3. Seasonal abundance by growing degree days and Julian Date.

context, this also means that we cannot determine how much of the difference between our results and the three previous surveys was due to the collection dates versus biological differences. Furthermore, the simple approach of first selecting only sampling dates that our study has in common with the other studies will not work to overcome this problem, although it would solve the problem of unequal sampling effort.

A long term trapping effort is managed as the Hungarian Plant Protection and Forestry Light Trap Network (Szentkiralyi 2002; Szontagh 1975). Results from 55+ traps per year sampled from 1962 through 2006 were recently published (Gimesi et al. 2012). They showed three peaks in the number of captured individuals, although in their case the central peak was much larger than the other two. The Hungarian data had a broad peak in Fisher's alpha corresponding to warmer summer months, and there was a strong relationship between mean daily temperature and biodiversity. This pattern was present in our data (Fig. 2), but in our results alpha had a distinct peak in early August. Seasonal shifts, multiple traps over a broad geographic range, and av-



Figure 4. Seasonal abundance based on growing degree days for Noctuidae, and Geometridae.

eraging results over longer time spans would tend to smooth out seasonal trends into a much broader peak in Fisher's alpha. The Hungarian data showed distinct losses in both abundance and diversity over this period, but one could find nine year spans in their data where abundance and diversity increased (Szentkiralyi 2002). Furthermore, the Hungarian data showed that seasons have gotten earlier by about 2 to 3 weeks over a 44 year span (Gimesi et al. 2012). Based on these results, our inability to detect a similar trend in our data could be due to having only nine years of data.

We were interested in the difference between using a growing degree day model versus a calendar date. We selected 37 individuals with 350 or more captures in the nine year study, and calculated the average day of capture. For each species we divided the mean by the standard deviation, and used a paired t-test for a significant difference between using Julian day versus GDD (df 36; t=7.12; Pr>|t|<0.001). On average there was a 57% reduction in this ratio for GDD relative to using Julian Day (95% CI: 51.7 to 61.3%). Therefore, the GDD approach should significantly increase the statistical power of tests for treatment differences relative to using calendar date.

Looking at the number of *Catocola, Acronicta*, and species in the Herminiinae that we collected relative to surveys from less disturbed environments, we would conclude that our sample from an urban environment was not inflated by a large number of generalists attracted to the mix of exotics in the urban landscape. Therefore we would concur with others that urban landscapes can be planned to increase biodiversity relevant to more natural ecosystems (Ockinger et al. 2009; Valtonen et al. 2007). Our survey showed that there were three peaks in moth abundance, whereas biodiversity had a single peak late in the year. We also showed that moth biodiversity was relatively stable with nearly equal missing and immigrating species from year to year. There was also a regular progression of species throughout the year. The sequential gain and loss of species each month resulted in seasonal shifts in the Geometrid:Noctuid ratio such that it is unlikely to be generally useful as a single number describing habitat distur-



Figure 5. Seasonal and yearly fluctuation in the geometrid/noctuid ratio. Bars are one standard deviation from the mean.

bance. Furthermore, the seasonality demonstrated in these data would suggest that any species ratio would need careful validation prior to use. In describing our data, we made use of a growing degree day (GDD) model. This has the effect of rescaling the data (see Fig. 3). It also corrects for temperature dependent shifts in moth biology. Consequently, some of the variability in the data was removed and this should improve the power of statistical tests involving survey data.

There is no end point to general surveys. No matter how many years of sampling, there will always be an additional species that can be added to the list if sufficient effort is expended. One reason for making such lists is that they provide quantifiable justification for maintaining a natural area to preserve biodiversity. In some cases a threatened local population is being preserved, and those individuals may be locally abundant. More commonly we are preserving rare species associated with a specific habitat. In this case, there is no end to the survey because it is not possible to identify all the species present at an instant in time nor is it possible to identify all the potential species that could live in that habitat. Partly this is a function of forces like climate change, but there are also changes in the spatial distribution of all plant communities. An end point might be reached if the survey goal is to identify those species visitors to the park are likely to encounter and ask "what is this?" In this survey there were 122 species encountered every year. Five species stop flying in May. Five only start flying in August, and three start flying in September. So one could ask how many years it would take to get all 122 species by sampling once per month from May through October. Sampling the first day of these six months will result in recovering an average of 63.2 of these 122 species in any one year. This sampling protocol will only recover 117 of these species in the nine years sampling took place. How does this answer change if we took two or three samples each month? What if we shifted the sampling dates by a few days? Another simple option is to choose the date with the most number of species for the year. In this study that date would fall between 24 July and September 1. The maximum number of species recovered on a single night averaged 51.1. Thus a simple sampling design has difficulty recovering species that we know are present every year. The required sampling effort increases greatly if one desires to go beyond a species list to an understanding of the underlying relationships between these ecologically important organisms.

Acknowledgements

We thank J. Donald Lafontaine for help in updating our species list to the modern classification of the Noctuoidea. We thank Ian Woiwod for access to the data from trap 336 from the Rothamsted Insect Survey. We thank the Pennsylvania Natural Heritage Program for access to data from Annville, Pennsylvania. We also thank Jerry Powell for data from Inverness Ridge in California, and Keith Summerville for data from his researches on moth biodiversity in other parts of Ohio. We thank two anonymous reviewers whose comments greatly improved this manuscript. We thank Michael Rogers from University of Florida for helping with page charges.

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Supplementary material I

Nightly moth captures in Wooster, Ohio and summaries of these data

Authors: Roger A. Downer, Timothy A. Ebert

Data type: Excel workbook with multiple worksheets

- Explanation note: The first worksheet on the left is the "Introduction". The next page to the right in the file includes the weather data. Then there are nine pages with yearly capture data. These pages include the number of growing degree days accumulated by each collection date. Cells are blank if no individuals of a given species were captured on a specific date. Next is a "Condensed List" that contains total number of each species, and the number of years each species was collected. This page contains the species as they were identified and the equivalent under the system by Lafontaine and Schmidt (2010). It also lists the range in capture date, and range in growing degree days. Then follows total captures per year, and a list of known host plants. The next worksheet is a list of the 13 county records and their collection date. Next is a page with a list of univoltine and bivoltine species selected based on abundance and environmental fidelity. Next is a listing of the 20 pest species and their yearly abundance. This was extracted from the main list to facilitate access. Lastly is a page with the moon phases. We did not find the moon phase data of any use, but it may prove useful to someone else.
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REVIEW ARTICLE



Taxonomic studies of the Lygephila lubrica (Freyer, 1842) species complex with notes on other species in the genus (Lepidoptera, Erebidae, Toxocampinae)

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Academic editor: Alberto Zilli Received 22 June 2014 Accepted 13 October 2014 Published 5 November 2014
http://zoobank.org/F7F5E4C3-2D76-4ACA-A4C3-5B8DE5D0F90C

Citation: Pekarsky O (2014) Taxonomic studies of the *Lygephila lubrica* (Freyer, 1842) species complex with notes on other species in the genus (Lepidoptera, Erebidae, Toxocampinae). ZooKeys 452: 107–129. doi: 10.3897/zookeys.452.8152

Abstract

The taxa of the *Lygephila lubrica* (Freyer, 1846) species complex are revised. The genital features of all known taxa are described and illustrated, with special reference to the structure of the vesica. Genitalia of *L. lubrica* from different places in Russia, Central Asia and China are studied, illustrated and compared with different Mongolian populations. *L. kazachkaratavika*, described as a subspecies, is raised to a species level, **stat. n**. Neotypes of *Lygephila lubrosa* (Staudinger, 1901), *L. lubrosa kazachkaratavika* Stshetkin YuL & Stshetkin YuYu, 1994 [1997] and *L. lubrosa orbonaria* Stshetkin YuL & Stshetkin YuYu, 1994 [1997] are designated. The female genitalia of the type of *L. lupina* (Graeser, 1890) is described and illustrated for the first time, and *L. mirabilis* (Bryk, 1948) treated here as a junior subjective synonym, **syn. n**.

Keywords

Lepidoptera, Erebidae, Toxocampinae, Lygephila lubrica species complex, L. mirabilis, L. lupina, vesica structure

Introduction

This paper is dedicated to clarify the taxonomic status of the taxa in the *L. lubrica* species group, which is proved to contain more than a single species. Special attention was paid to revising the poorly-known taxa described from Central Asia and the identity

of the historical names that have been used confusingly in the literature. The examined material is considered as representative for the entire area of the species complex, including all available types preserved in the collections of Püngeler, Staudinger, Bang-Haas, and Stshetkin. Neotypes are designated when required by the taxonomic results.

Materials and methods

Male and female genitalia were dissected and mounted in Euparal on glass sides. Photos of genitalia were made by Svitlana Pekarska using a Nikon SMZ745T microscope and Moticam 2500 camera. Photos of imagines where taken by the author using a Nikon D3000/Sigma 105, f/2.8 camera.

Abbreviations: HNHM = Hungarian Natural History Museum Budapest (Hungary); IZIP = Institute of Zoology and Parasitology, Tajik Academy of Sciences Dushanbe (Tajikistan); MA = Matov Alexey, St. Petersburg (Russia); MNHU = Museum für Naturkunde der Humboldt-Universität zu Berlin (Germany); NHMW = Naturhistorisches Museum Wien (Vienna, Austria); ZISP = Zoological Institute, Russian Academy of Sciences St. Petersburg (Russia); ZFMK = Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn; ZSM = Zoologische Staatssammlung München; AV = Anton Volynkin (Barnaul, Russia); GB = Gottfried Behounek (Grafing, Germany); JB = János Babics (Budapest, Hungary); OP = Oleg Pekarsky (Budapest, Hungary); LR = László Ronkay (Budapest, Hungary); WB = Wiltshire Berlin (slide made by Edward P. Wiltshire in the collection of MNHU).

Systematic accounts

Description of the Lygephila lubrica species complex

Head and body brownish grey with frons and collar chocolate brown. Forewing broad, apex less pointed than in the *L. lusoria* group (Babics and Ronkay 2009, Pekarsky 2013), ground colour brownish grey or unicolorous grey with traceable crosslines; orbicular stigma as a small white dot, reniform stigma large, approximately triangular, dark brown; hindwing ground color varies from brown and greyish brown to yellowish or intensive ochreous, discal spot visible only on underside, transverse line distinct, marginal band conspicuously dark. Male genitalia of *L. lubrica* nearly symmetrical; clasping apparatus of other species slightly asymmetrical (right valva narrower with longer ampulla), uncus well developed, long, strong, its distal half broadened with acute tip; valva margins more or less parallel with rounded apex; clasper long, sclerotized, digitiform, located subapically; aedeagus cylindrical, long, straight; vesica globular, membranous, multidiverticulate (six or seven diverticula various in shape and size), terminal tube long, membranous; ostium bursae large; antrum sclerotized, funnel shaped with considerable cleft in middle of posterior margin; ductus bursae small, corpus bursae membranous, elliptical or ovoid.
Lygephila lubrica lubrica (Freyer, 1846) Figs 1–10

Ophiusa lubrica Freyer, 1846, Neuere Beiträge zur Schmetterlingskunde mit Abbildungen nach der Natur. 6: 7, Tab. 483, fig. 4. (TL: not given)

Synonymy: Lygephila lubrica sublubrica (Staudinger, 1896);

Toxocampa lubrica var. *sublubrica* Staudinger, 1896, Deutsche Entomologische Zeitschrift Iris 8: 271. (TL: [Mongolia, Uliastai], Uliassutai)

Type material examined. *Lygephila lubrica sublubrica* (Staudinger, 1896), **Type** ∂, [Mongolia, Uliastai], Uliassataj, slide No. WB12 (coll. MNHU).

Additional material examined. 2 \bigcirc & 1 \bigcirc , Russia, C Tuva, W of Ujukskyi Mts, Kamennyi riv. valley, h=800-1000 m, 11-20.07.2003, leg. S. Vaschenko, slide Nos: OP1955m, OP2438m, OP1956f (coll. O. Pekarsky); 1 👌, Russia, Altai Mts, 700 m, Kupchegen, 23-25.VII.2002, leg. Hácz & Juhász, slide No. OP1962m (coll. O. Pekarsky); 1 3, Russia, NW Altai Mts, Tigireksky ridge, slide No. AV0907 (coll. A. Volynkin); 1 🗟 Russia, Altai rep., Aktash, 1400 m, 12–14.VIII.2010, leg. R. Yakovlev, slide No. OP2439m (coll. O. Pekarsky); 1 \mathcal{Q} , Russia, SE Altai Mts, Aktash vill., slide No. AV0906 (coll. A. Volynkin); 1 3, Mongolia, Central aim., Nr. 1148, leg. Z. Kaszab, slide No. LR1401m (coll. HNHM); 1 👌, Mongolia, Chövsgöl aimak, Nr. 1128, leg. Z. Kaszab, slide No. LR1402m (coll. HNHM); 1 9, Mongolia, Central aimak, 26 km O von Somon Lun, 1180 m, Nr. 260, 3.VIII.1964, leg. Z. Kaszab, slide No. OP2010f (coll. HNHM); 1 ♂ & 2 ♀♀, Mongolia, Selenga aimak, Orhon v., Sir Orhon, 715 m, N49°08'956", E105°15'099", 3-4.07.2004, leg. K. Gaskó, slide Nos: OP2296m, OP2295f, OP2297f (coll. P. Gyulai); 1 👌 [Kazakhstan], Russia, Uralsk, 1937.VII., ex coll. Velez, slide No. LR1403m, (coll. HNHM); 1 ♂ & 1 ♀, [Kazakhstan], Uralsk, slide Nos: Hacker2536m/ZSM2510m, Hacker2334f/ZSM2508f (coll. ZSM); 1 🖧 & 1 🍳, Russia, S Ural, Orenburg reg., Donskoe vill., Verbljushka Mt., 25– 29.6.2009, leg. L. Srnka, slide Nos: OP2124m, OP2125f (coll. O. Pekarsky); 4 승승 & 1 Q, Russia, Bashkortostan, Yantysh vill., 29–31.VII.2011 slide Nos: OP2005m, OP2007m, OP2440m, OP2441m, OP2006f (coll. O. Pekarsky); 1 ♂ & 1 ♀, Russia, Kabardino-Balkaria, C Caucasus Mts, Bydyk, 1250m, 18.7.2012, leg. L. Srnka, slide Nos: OP2151m, OP2152f (coll. O. Pekarsky); 1 ♀, Kasakhstan, 40 km W Ust Kamenogorsk, Kalbinski Altai, Monastyri, 600 m, 06.08.1994, leg. Lukhtanov, slide No. OP2013f (coll. P. Gyulai); 1 3, Kazakhstan, Boro-Khoro Mts, 30km N of Panfilov, (20 km from Chinese border), N44°29'765" E80°03'848", 1830 m, 30.06.2010, leg. S.K. Korb, slide No. OP2083m (coll. O. Pekarsky); 1 3, Kirgizstan, Inner Tjan-Shan, Min-Kush circ., 2300 m, 2.08.2000, leg. I. Pljushtch, slide No. OP2004m (coll. O. Pekarsky); 1 $\cancel{3}$ & 1 $\cancel{9}$, Kyrgyzstan, Naryn reg., Kekemeren river, n., Sarykamysh, 1400 m, 6-8.07.1996, leg. V.A. Lukhtanov, slide Nos: OP2015m, OP2016f (coll. P. Gyulai); 1 3, [Kyrhyzstan], Issykkul, Tianschan, 949, ex coll. Kotzsch, slide No. OP2426m (coll. ZFMK); $2 \ QQ$, China, Xinyiang [Xinjiang] – Uygur, Boro Horo Shan, Balguntay city, 2000 m, 13.7.1996, leg. Nykl, slide Nos: OP2011f, OP2012f



Figures 1–10. Adults. *Lygephila lubrica* $[\mathcal{C}]$, Russia, Orenburg $2 \mathcal{Q}$, Russia, Orenburg $3 \mathcal{C}$, Kazakhstan, Boro-Khoro Mts $4 \mathcal{Q}$, Kabardino-Balkaria, Bydyk $5 \mathcal{Q}$, Kyrgyzstan, Naryn reg. $6 \mathcal{Q}$, China, Xinyiang – Uygur $7 \mathcal{C}$, Russia, Altai, Kupchegen $8 \mathcal{Q}$, Russia, Tuva $9 \mathcal{C}$, SW Mongolia, Hovd aimak $10 \mathcal{Q}$, SW Mongolia, Hovd aimak.

(coll. P. Gyulai); 1 3, China, Boro Boro shan, Balguntay city, 2000 m, 13.7.1996, slide No. OP2289m (coll. P. Gyulai); 1 Q, Aksu Bakalik, Anf. VI.1912, ex coll. Rückbeil, slide No. OP2339f (coll. ZSM); 1 ♂, Aksu Bakalik, Anf. VI.1912, ex coll. Rückbeil, slide No. OP2338m (coll. ZSM); 1 🖧 [China], Aksu, [19]11, 225, ZFMK76/64 Boppard, slide No. OP2427m (coll. ZFMK); 1 Å, Mongolia, Uliasutai, slide No. 0326Matov (coll. ZISP); 1 3, [Mongolia], Uliassatai, 946, ex coll. Kotzsch, 8/57, slide No. OP2428m (coll. ZFMK); 1 ♂ & 1 ♀, SW Mongolia, Hovd aimak, Bodonchijn-Gol basin, Hundij-Gol riv. valley, 1600 m, 46°06'N; 92°30'E, 3.vii.2010, leg. E. Guskova & R. Yakovlev, slide Nos: OP1957m, OP1958f (coll. O. Pekarsky); 2 づご, W Mongolia, Hovd aimak, near Erdene-Buren somon, h=1 400 m, 04.07.2007, leg. Yakovlev R.V. & Guskova E.V., slide Nos: AV0283, AV0285 (coll. A. Volynkin); 1 🖧 & 1 Q, Mongolia, Hovd Aimak, Altaj Mts, 10 km NE of Dott, 2000 m, 10.08.1996, leg. S. Farkas & I.Zs. Tóth, slide No. OP2290m, OP2291f (coll. P. Gyulai); $2 \sqrt[3]{3} \otimes 1$ W Mongolia, Hovd aimak, near Erdene-Buren-Somon, 1400 m, 1.07.2010, 2500-2850 m, leg. R. Yakovlev, E. Guskova, slide Nos: OP2350m, OP2351m, OP2352f (coll. O. Pekarsky); 1 3, Mongolia, Bulgan aimak, 54 km W of Erdenecant, 1260 m, 104°05'E 47°05'N, 22.07.1987, leg. L. Peregovits, M. Hreblay & T. Stéger, slide No. OP2008m (coll. HNHM); 1 &, Mongolia, [Khentii] Chentaj aimak, Tsenkher-Mandal, Modoto, 1600-1800 m, 9-14.07.1984, leg. K. Cerny, slide No. GB2550m, (coll. G. Behounek); 1 3, [Russia], Yakovlevka Spas. u., Ussur. kr., 12.VIII.[1]926, [leg.] D'iakonv Filip'ev (in russian), slide No. 0330Matov, (coll. ZISP); $1 \, \bigcirc$, Russia, Primorsky ter., Lesozavodsk reg., Innokentievka, 26–30.VIII.[19]94, slide No. OP2298f (coll. P. Gyulai); $1 \, \bigcirc$, [China], Mien-shan (Prov. Shansi), Obere Höhe ca. 2000 m, 2.8.1937. [leg.] H. Höne, slide No. OP2423f, 2 3 3, 9.8.1937, slide Nos: OP2421m, OP2425m, 1 Å, 13.8.1937, slide No. OP2422m (coll. ZFMK); 1 Q, [China], Tapaishan im Tsinling, Sued-Shensi, Ca. 3000 m, 17.6.1936, [leg.] H. Höne, slide No. OP2424f (coll. ZFMK).

Taxonomy. Lygephila lubrica was described in 1846 by Freyer in the genus Ophiusa. The exact type locality was not given in the original paper and also there was no information about the types. In 1896, Staudinger supposed during the description of Toxocampa lubrica var. sublubrica, that Ophiusa lubrica was described by Freyer from Altai: «Freyer sagt von seiner Lubrica nur, dass er sie von Kindermann erhielt; es muss sicher die von diesem Sammler im Altai gefundene Art sein, von der ich drei Stücke aus Lederer's Sammlung besitze». Based on this assumption the type locality of L. lubrica is most probably "Russian Altai" near Ust-Bukhtarminsk settlement (not existing now), which was located near the junction of the Bukhtarma and Irtysh rivers in the modern territory of Kazakhstan. Staudinger & Wocke (1871) placed this species in the genus Toxocampa, and later Staudinger (1896) described a variation named as sublubrica from Uliastai on the western edge of Khangai Mountains in the western part of Mongolia. The type specimen of sublubrica was not found in the collection of MNHU in Berlin however the genitalia slide made by Edward Wiltshire is in the museum (genitalia slide collection, Figs 27, 28). The current combination – Lygephila lubrica - occurs first in Sheljuzhko (1967) and later in Ronkay (1983). The taxon

sublubrica is considered as a subspecies of *L. lubrica* in these two works. Poole (1989) incorrectly treated *Lygephila lubrica* (Freyer, 1842) as a new combination, and listed *Toxocampa lubrica* var. *sublubrica* Staudinger, 1896 and *Toxocampa lubrica* var. *lubrosa* Staudinger, 1901, and have been listed as such in subsequent works (e.g., Goater et al. 2003; Kononenko 2010).

Diagnosis. The main external distinctive feature of the species is the brownishgrey ground colour of forewings and hindwings. *Lygephila lubrica* differs from the externally somewhat similar *L. lubrosa* by its characteristic brownish-grey ground color of the forewings; from *L. kazachkaratavika* by more unicolorous forewings with a lessdeveloped pattern; and from both related species by its brownish hindwings, which are generally ochreous in the two latter species. The differences in the genitalia structures among the three similar species are easily recognisable in both sexes. In males, the uncus dilation in *L. lubrica* is wider than in *L. lubrosa*, but narrower than in *L. kazachkaratavika*, and the ampulla is more proximal, closer to the middle of the valve, than in the two other species; in the females, the cleft on the posterior margin of the antrum is U-shaped or V-shaped in *L. lubrica*, whereas in *L. lubrosa* it is evenly concave; in *L. kazachkaratavika* the ostium cleft is deep, narrow, slit-like.

Description. Wingspan 37–50 mm, on average 42–48 mm. Head and body brownish grey; collar dark chocolate brown. Forewing brownish grey, sometimes dark brown; subbasal line indistinct; antemedial line arched, consisting of two elongated patches; medial fascia diffuse, wide and waved, with two costal patches; reniform stigma approximately triangular, dark brown, sometimes with sharp extension at inner corner and with satellite streak-like spots on outer margin; orbicular stigma as small white dot; postmedial line distinct; subterminal line with light fascia; terminal line a black sinuous stripe. Hindwing varies from brown to greyish brown; transverse line distinct; narrow discal spot present on underside; outer dark third with defuse inner margin; fringes as ground color.

Male genitalia (Figs 21–32, 39–41). Uncus with short stem and dilated distal two thirds, apex with fine tip, anal tube membranous with characteristic oval hardening of tissue - scaphial crown on scaphium and sclerotized plate on subscaphium; valva elongated, relatively wide with parallel margins, valval apex rounded; clasper digitiform, slightly curved towards costa, situated rather far from apex. Aedeagus straight, long, tubular. Vesica globular, multidiverticulate, membranous; 1st subbasal diverticulum small, adjacent to 2nd terminal diverticulum; medial diverticulum large, tapering, with medium-large oblong chamber at base; 1st terminal diverticulum large, more or less wedge shaped with one part densely scobinated and membranous, cauliflowerlike, opposite part bears numerous small pockets; 2nd terminal diverticulum tubular, scobinated on top; 3rd terminal diverticulum irregular shaped with large rectangular scobinated basal part and membranous cylindrical extension; 4th terminal diverticulum medium sized, situated between 1st and 3rd medial diverticulum; 2nd subbasal diverticulum small, tubular, sometimes chili-pepper-like (Fig. 41), terminal tube membranous, as long as aedeagus, opening point of terminal tube located subbasally near carina.

Female genitalia (Figs 46–57). Ovipositor relatively large, broad, papillae anales hairy with long setae on apical edges. Apophyses anteriores stout, apophyses posteriores thin, longer than apophyses anteriores. Ostium broad, antrum tapering, funnel shaped, posterior margin incised producing large U-shaped cleft; ductus bursae small, inflated with ventral sclerotized ribbon; appendix bursae small; corpus bursae membranous, ovoid.

Distribution. Siberian. Distributed from Zaporozhie region of Ukraine to Rostov, Samara, Povolzhie regions to Ural of Russia through Kazakhstan, Russian Altai and northern Mongolia.

Lygephila lubrosa lubrosa (Staudinger, 1901)

Figs 17, 18

Toxocampa lubrica var. *lubrosa* Staudinger, 1901, Catalog der Lepidopteren des Palaearctischen Faunengebietes. I: 252. (TL: [Kazakhstan], Ili, [Kyrgyzstan, Issyk Kul], "Iss. K.")

Type material examined. Neotype (here designated) male, Kazakhstan, Ili river valley near bridge 23,4 km asimut 222 from Koktal, 600 m, N43°58'004", E79°35'905", 04.07.2010, leg. S.K. Korb, slide No. OP2082m (coll. O. Pekarsky, deposited in HNHM Budapest).

Additional material examined. 1 \Diamond , with same data as neotype; 1 \Diamond & 1 \heartsuit , Kazakhstan, Ili river valley near Koktal, 506 m, N43°57'57.50", E79°36'1.06", 03.07.2010, leg. S.K. Korb, slide No. OP2489f (coll. O. Pekarsky); 1 \heartsuit , [Kazakhstan], Syr-Daria, Baigacum, Koshantschikoff, 23.6.1913, 4/7, ex. coll. Püngeler, slide No. OP1979f (coll. MNHU); 1 \Diamond , [Kazakhstan], Aj-Darle, Syr-Darja, 25.V.1909, leg. Koshantshikoff, slide No. 0325Matov (coll. ZISP).

Taxonomy. Described by Staudinger in 1901 as a variation of *L. lubrica*; with the type locality mentioned as [Kazakhstan], Ili [river] and [Kyrgyzstan], Issyk Kul [lake]. The original description stated that the forewings are pale grey ("cinereo-griseis") without dark outer part, and that the hindwings are ochreous with broad marginal fascia. This description corresponds exactly with the external appearance of the moths from Ili river in Kazakhstan, therefore the neotype is designated from this area. Moths from Issyk Kul show, however, marked differences in habitus, especially the brown coloration of most parts of the forewings. These two taxa are different in genital structures of both sexes, which are discussed in detail under *L. kazachkaratavika*. Starting from the 1980's, Stshetkin YuL treated *L. lubrosa* in his publications as a distinct species (Stshetkin et al. 1988, Stshetkin 1991). The explanation of this act was given only in 1994 [1997] by Stshetkin YuL & Stshetkin YuYu. Their argumentation was based only on the original description of *L. lubrosa*, but neither the type material nor the genitalia of the syntypes were studied. Unfortunately, the authors evidently failed in their taxonomic interpretation of the species complex. They were correct to suppose

L. lubrosa Staudinger, 1901 is a separate taxon different from *L. lubrica*, but they failed to define this taxon, and did not recognize that the yellowish hindwinged populations include two different species.

The main fault of the Stshetkins' work is the lack of definition of L. lubrosa Staudinger, 1901. In their article they provided the following description of the genitalia of L. lubrosa: "Гениталии самца симметричные. Ункус слабо изогнутый, расширенный в средней части, заостренный. Вальвы удлинённые с немного выпуклыми дорзальными и вентральными краями. Вершинный отросток класпера пальцеобразный, длиннее, чем у L. lubrica; его конец находится близ дорзального края вальвы (у L. lubrica он далеко не достигает края). Конец вальвы от основания этого отростка до его заднего конца заметно короче, чем у L. Інbrica. В оральной трети длины вальвы продольная хитинизированная складка класпера, направляясь орально, плавно прогибается несколько к вентральному краю вальвы и при этом не образует резкого угла с бугорком-гарпой, имеющегося у L. lubrica Frr. Нижняя фультура под эдеагусом без особого изгиба прямо идет в сторону саккуса, как у L. lusoria L." The translation of this text is as follows: "The male genitalia are symmetrical. Uncus slightly curved, dilated in the middle part, pointed. Valva elongated with slightly convex dorsal and ventral edges. Apex of clasper digitiform, longer than that of *L. lubrica*; its end close to the dorsal margin of the valva (as for *L. lubrica*, the latter is far from reaching the margin). The end of the valva from the base of the clasper till its back end is noticeably longer than that of L. lubrica. In the oral [basal] third of the valva, the longitudinal chitinized fold of the clasper is directed orally [basally] and is slightly curved towards the ventral margin of the valva without forming an abrupt angle with the hump-harpe, which is typical for *L. lubrica* Frr. Lower fultura [juxta] under aedeagus almost straight and directed towards the saccus as L. lusoria L." This description is contradictory as it includes characteristics of both yellow hindwinged species occurring in Central Asia. To be precise, "Uncus dilated in the middle part" is only typical for the moth (L. kazachkaratavika) from Kyrgyzstan (lake Issyk Kul) and Kazakhstan (city of Kizilorda and station Baigacum on the river Syr Darja) (Figs 37, 38); "Apex of clasper digitiform and longer than that of L. lubrica; its end close to the dorsal margin of the valva" is only typical for the moths (L. lubrosa) from Kazakhstan (river Ili) and Tajikistan (river Pianj) (Figs 33–36). The female genitalia are described as follows: "В гениталиях самки копулятивная сумка мешковидная и вместе с едва заметным бугорком-буллой вся перепончатая (у L. lubrica булла конусовидная, хитинизированная, как и весь проток и часть сумки). Проток сумки значительно короче, его оральная часть перепончатая". The translation is as follows: "In the female genitalia the copulative pouch [corpus bursae] is saccular and all membranous along with a barely noticeable bulla (while the bulla of L. lubrica is conical and chitinized as well as the whole antrum and part of the pouch [corpus bursae]). The antrum is significantly shorter with membranous oral [basal] part." The characteristics mentioned as "the antrum is significantly shorter than that of *L. lubrica*" partially corresponds to the moths from the Ili region. However, it is not diagnostic because in many specimens of L. lubrica the antrum has the same length. The antrum of the moths

(*L. kazachkaratavika*) from the Issyk Kul region and the river Syr-Darja is one and a half times longer than that of *L. lubrica* and two times longer than that of the moths from the valley of the river Ili (*L. lubrosa*). The other characteristics mentioned by the authors are general, non-autapomorphic and unsuitable for determination.

In the same work the authors described two subspecies of *L. lubrosa* on the basis of external characteristics, admitting that the two subspecies do not differ in genitalia structure from the nominotypical subspecies. However, the moths from the Kazakh Karatau, station Balamurum collected by V. Kozhantshikov in 1909 (*L. kazachkarata-vika*) differ significantly in their genitalia structure from the moths from the valleys of the river Ili (*L. lubrosa lubrosa*) and the river Pianj (*L. lubrosa orbonaria*). All above-mentioned data prove that the authors did not consider the subject of their research, which caused unsatisfactory results and added further difficulties for the clarification of this species-complex. A further difficulty is that the authors did not define holotypes or paratypes (or simply type series) for the newly described taxa. According to the information from the museum curators of ZISP and IZIP, they do not possess the aforesaid type specimens with the corresponding type labels.

In order to correctly identify the taxa of this species complex, in view of complexity of the current taxonomic situation, and to give an accurate definition of *L. lubrosa*, it is necessary to designate a neotype of this taxon.

Diagnosis. Easily distinguishable from all other members of the species group by its unicolorous grey forewings. Comparing the genital structures of the taxa of the group, it differs from all related species by the narrow uncus without a real dilatation (only some slight thickening is present), the wide valva, and the subapically located, strong clasper with its tip reaching the valval edge (males); and by the funnel-shaped antrum bent dorsally at 45 degrees, being a unique female character for the whole genus (Fig. 60).

Description. Wingspan 42–46 mm. Head and body grey with some brownish scales; collar chocolate brown. Forewing almost unicolorous, wing pattern poorly developed; subbasal line hardly traceable, represented by groups of dark scales on veins; antemedial line semicircular; medial shade not expressed; reniform stigma small, indistinct, with one or two black dots basad; orbicular stigma small dot-like; postmedial and subterminal lines distinct; terminal line a row of black dots on veins. Hindwing pale ochreous; transverse line not discernible; outer dark third with sharply defined inner margin; fringes ochreous.

Male genitalia (Figs 33, 34, 44, 45). Uncus long, stout, slightly thickened medially with skewed fine tip, anal tube membranous with oval hardening of tissue scaphial crown on scaphium and sclerotized plate on subscaphium; valva elongated, wide, with parallel margins in the middle, valval apex rounded; clasper digitiform, strong, thickened with wide base, placed subapically, asymmetrical, left one shorter than the right one, both almost reach valval costa. Aedeagus tubular with narrow, long, acute sclerotised lamina on ventral side of carina. Vesica globular, multidiverticulate, membranous; basal diverticulum small; medial diverticulum large with small lateral hemispherical bulging; 1st terminal diverticulum large, more or less wedge shaped, membranous with cauliflower-like part bearing numerous small pockets; 2nd terminal diverticulum large, cone shaped, scobinated on top; 3rd terminal diverticulum medium-sized, bifurcated, Y-shaped; 4th terminal diverticulum large, bilobate, located oppositely to the 3rd medial diverticulum; terminal tube membranous as long as aedeagus, opening point of terminal tube located near to carina.

Female genitalia (Figs 58–61). Ovipositor relatively large, broad, papillae anales hairy with long setae on apical edges. Apophyses anteriores stout, apophyses posteriores thin, longer than apophyses anteriores. Antrum funnel shaped, bent dorsally at 45 degrees, boomerang shaped from lateral view; ostium bursae broad, posterior margin U-shaped; ductus bursae medium sized; appendix bursae small; corpus bursae membranous, bevelled oval.

Destribution. Kazakhstan, valley of the river Ili.

Lygephila lubrosa orbonaria Stshetkin YuL & Stshetkin YuYu, 1994 [1997]

(TL: SW Tajikistan, "Tigrovaya balka" reserve) Figs 19, 20

Type material examined. Neotype (here designated) male, Tajikistan, down stream of Planj river, "Tigrovaya Balka" reserve, 1–5.08.2006, leg. V. Gurko, slide No. JB1218m (coll. P. Gyulai, will be deposited in HNHM Budapest).

Additional material examined. 1 ♂, S. Tajikistan, down stream of Pianj riv., "Tigrovaya Balka" reserve, 1–5.08.2006, V. Gurko lgt., slide No. OP2268m (coll. M. Dvořák).

Taxonomy. Described as a subspecies of *L. lubrosa*. The original description does not contain any information about the genitalia structures. However, the male genitalia show some recognisable differences compared with those of the nominate subspecies.

There is no trustworthy information about the holotype and paratypes of this taxon. According to the information from the Lepidoptera collection of IZIP, Stshetkins's collection was totally destroyed sometime after the end of the 1990's. Also, there are no corresponding type labels in institute's collection. To ensure the stability and identification of the taxon it is necessary to designate a neotype of *Lygephila lubrosa orbonaria*.

Diagnosis. Differs from *L. lubrosa lubrosa* by its smaller size and better marked reniform stigma. In the male genitalia, ssp. *orbonaria* differs from ssp. *lubrosa* by its narrower uncus without a medial thickening, and the narrower upper part of valva with more expressed asymmetry.

Description. Wingspan 34–43 mm. The external features, with the exception of the smaller size and somewhat roundish forewing, match those of the nominate subspecies.

Male genitalia (Figs 35, 36). Uncus long, stout, sabre-like, anal tube membranous with oval hardening of tissue - scaphial crown on scaphium and sclerotized plate on subscaphium; valva elongated, wide, with parallel margins in the middle, tapering to apex; clasper digitiform, strong, thickened with wide base, placed subapically, somewhat asymmetrical, left one short, right one longer, almost reaches costa. Aedeagus

tubular with narrow, long, acute sclerotised lamina on ventral side of carina. Vesica globular, multidiverticulate, membranous; basal diverticulum small; medial diverticulum large with small lateral hemispherical bulging; 1st terminal diverticulum large, more or less wedge shaped, membranous with cauliflower-like part bearing numerous small pockets; 2nd terminal diverticulum large, cone shaped, scobinated on top; 3rd terminal diverticulum medial sized, bifurcated, Y-shaped; 4th terminal diverticulum large, bilobate, located opposite to 3rd medial diverticulum; terminal tube membranous, as long as aedeagus, opening point of terminal tube located near carina.

Female genitalia. Unknown.

Distribution. SW Tajikistan, Pianj river valley.

Lygephila kazachkaratavika Stshetkin YuL & Stshetkin YuYu, 1994 [1997], stat. n. (TL: Kazakhstan, Balamurum) Figs 11–16

Lygephila lubrosa kazachkaratavika Stshetkin YuL & Stshetkin YuYu, 1994 [1997]

Type material examined. Neotype (here designated) male (Fig. 11), 1 \mathcal{E} , [Kazakhstan], Balamurum, Kara-tau, 1909.VI.21, leg. Koshantshikoff [Kozhantshikov], ex coll. John, slide No. OP2009m (coll. HNHM Budapest).

Additional material examined. 1 \bigcirc , label1: [Kyrgyzstan], Asia Centr., (Issykul), 1896, revers label1: Toxocampa, von R. Tancré, 5.98, ex. coll. Püngeler, slide No. OP1981f (coll. MNHU); 1 \bigcirc , [Kyrgyzstan], Issi-Kul, slide No. OP2067m (coll. NHMW); 1 \bigcirc , label1: [Kazakhstan], Syr-Daria, Baigacum, Koshantschikoff, revers label1: 20.6.13, label2: 21/6, 1913, 3/7; 1 \bigcirc , label1: [Kazakhstan], Syr-Daria, Baigacum, Koshantschikoff, revers label1: 21.6.13, label2: 21/6, 1913, 4/7; 1 \bigcirc , label1: [Kazakhstan], Syr-Daria, Baigacum, Koshantschikoff, revers label1: 22.6.13, label2: 22/6, 1913, 5/7, ex. coll. Püngeler, slide No. OP1932f (coll. MNHU); 1 \bigcirc , label1: [Kazakhstan], Syr-Daria, Baigacum, Koshantschikoff, revers label1: 23.VI.13, label2: 23/6, 1913, 6/7, ex. coll. Püngeler, slide No. OP1980f (coll. MNHU); 1 \bigcirc , Kazakhstan, Taldy-Kurgan reg., Ili riv., Boroghudsir, 450m, 7–19.06.1996, slide No. OP2017m (coll. P. Gyulai).

Taxonomy. Described as subspecies of *L. lubrosa*. It is known that the author did not visit the museum collection of ZIN (ZISP) before writing his article on *Lygephila* and did not designate a holotype (personal comment of A. Matov). Also, potential type material of *Lygephila lubrosa kazachkaratavika* has not been found in any of the private collections where Stchetkin YuL's material was purchased. So, the holotype most likely was never designated. To ensure stability of nomenclature and identification of the taxon it is necessary to designate neotype. A specimen from Kozhantshikov's material preserved in the HNHM Budapest with the same label data as published in original description is hereby designated as neotype.

Diagnosis. Easily separable from *L. lubrica* and *L. lubrosa* by the very contrasting, well-developed pattern on the forewings. In the male genitalia, it differs from all close rela-



Figures 11–20. Adults. 11–16 Lygephila kazachkaratavika 11 neotype, ♂, Balamurum 12 ♀, Kazakhstan, Baigacum 13 ♂, Kazakhstan, Taldy-Kurgan reg. 14 ♀, Kazakstan, Baigacum 15 ♂, Kyrgyzstan, Issyk Kul 16 ♀, Kyrgyzstan, Issyk Kul 17, 18 L. lubrosa lubrosa 17 neotype, ♂, Kazakhstan, Ili river 18 ♀, Kazakhstan, Baigacum 19, 20 L. lubrosa orbonaria 19 neotype, ♂, Tajikistan, Pianj river 20 ♂, Tajikistan, Pianj river.



Figures 21–26. Clasping apparatus. Lygephila lubrica.



Figures 27–32. Clasping apparatus and genitalia slide. Lygephila lubrica.



Figures 33–38. Clasping apparatus. 33, 34 *Lygephila lubrosa lubrosa* 33 neotype 35, 36 *L. lubrosa orbonaria* 35 neotype 37, 38 *L. kazachkaratavika* 37 neotype.



Figures 39–41. Vesica structure of *Lygephila lubrica.* **39, 40** Mongolia, Hovd aimak, slide No. OP1957m **39** lateral view **40** lateral view opposite side **41** Russia, Altai, Kupchegen, slide No. OP1962m, lateral view.



Figures 42–45. Vesica structure. 42,43 *Lygephila kazachkaratavika*, neotype, Kazakhstan, Balamurum, slide No. OP2009m 42 lateral view 43 lateral view opposite side 44, 45 *L. lubrosa lubrosa*, neotype, Kazakhstan, Ili river, slide No. OP2082m 44 lateral view 45 lateral view opposite side.



Figures 46–63. Female genitalia. 46–57 Lygephila lubrica 58–61 L. lubrosa lubrosa 58 ventral view 59 dorsal view 60 lateral view 61 lateral view 62, 63 L. kazachkaratavika.

tives by its wider dilatation of the uncus, and the more acute apex of the valva (males); the longer antrum with a deep slit-like cleft on the posterior margin is diagnostic for females.

Description. Wingspan 41–44 mm. Head and body brownish grey; collar dark chocolate brown. Forewing contrastingly marked, variable in coloration from mottled light greyish brown to uniform dark brown; noctuid pattern well developed; subbasal line traceable; antemedial line arched, consisting of three elongated patches edged by light fascia basally; medial shade waved, bifurcated from below cell to anal margin, with two costal patches; reniform stigma somewhat triangular, black, sometimes with satellite streak-like spots on outer margin; orbicular stigma absent; postmedial line distinct; subterminal line with light fascia; terminal line a row of black dots. Hindwing ochreous; transverse line distinct; small discal spot present on underside; border between pale proximal part and dark outer third diffuse; fringes ochreous.

Male genitalia (Figs 37, 38, 42, 43). Uncus stem short, stout, distal part dilated, terminated in fine tip; anal tube membranous with oval hardening of tissue - scaphial crown on scaphium and with sclerotized plate on subscaphium; valva elongated, relatively wide with parallel margins in the middle and convergent basally and distally; clasper digitiform, undulate, placed subapically, not reaching costa. Aedeagus straight, long, tubular, with small sclerotized plate on ventral side of carina. Vesica globular, multidiverticulate, membranous; basal diverticulum small; medial diverticulum large, cupola shaped with two hemispherical chambers medially and basally; 1st terminal diverticulum large, more or less wedge shaped, with one part densely scobinated and membranous cauliflower-like, opposite part bearing numerous small pockets; 2nd terminal diverticulum tubular, elongated, scobinated on top; 3rd terminal diverticulum medium sized with lateral bulging; 4th terminal diverticulum large, conical, situated opposite to 3rd medial diverticulum; terminal tube membranous, as long as aedeagus, opening point of terminal tube located subbasally near carina.

Female genitalia (Figs 62, 63). Ovipositor relatively large, broad, papillae anales hairy with very long setae on apical edges. Apophyses anteriores long and thin, apophyses posteriores thin, somewhat longer than apophyses anteriores. Ostium broad, antrum tapering, funnel shaped, posterior margin deeply incised producing slit-like cleft; ductus bursae small; appendix bursae small; corpus bursae membranous, ovoid.

Distribution. Kazakhstan, Kyrgyzstan.

Lygephila lupina (Graeser, 1890), stat. n.

Figs 64, 65

Toxocampa lupina Graeser, 1890, Berliner Entomologische Zeitschrift, 35: 71–84. (TL: [Russia, Judish Autonomy, Radde] Raddefka) Synonymy: Lygephila mirabilis (Bryk, 1948), syn. n. Eccrita mirabilis Bryk, 1948 (TL: N Korea, Shuotsu)

Type material examined. ♀ Type, Amur Centr. (Radde), [18]87, ex. coll. Püngeler, slide No. OP1931f (coll. MNHU).

Additional material examined. 1 \bigcirc , [RFE], Ussuriysk dist., Kajmanovka, 20.VII. [19]82, leg. Ivanov, slide No. 0321Matov (coll. ZISP). 1 \bigcirc , [China], Tapaishan im Tsinling, Sued-Shensi, Ca. 1700 m, 14.7.1936, H. Höne, slide No. OP2402f, 1 \bigcirc , [China], Tapaishan im Tsinling, Sued-Shensi, Ca. 1700 m, 10.8.1936, H. Höne, slide No. OP2403f (coll. ZFMK).

Note. There is a lot of confusion between *L. mirabilis* and *L. vulcanea* (Butler, 1881) in the literature with regard to illustrations of the adults and genitalia. The taxonomic clarification of this problem will be given in a separate publication.

Taxonomy. The identity of *L. lupina* was unclear for a long time. *Lygephila lupina* was described, according to the original description, from Radde, central Amur [Khabarovsk region] (Graeser 1890) on the basis of a single male from the collection of Taylor Tancré, in comparison with *Lygephila maxima* (Bremer, 1861). The Püngeler collection, now in MNHU, contains a female specimen with a type label (Fig. 64). One can be convinced from the information given by the handwriting of Püngeler on the opposite side of the collecting label (made in May 1905) that this is a true type specimen from the Tancré collection and it is a female, not a male. Thus, Graeser was mistaken about the sex of the type specimen. The study of the genitalia of the type specimen reveals that *L. lupina* is conspecific with the species known as *L. mirabilis* (Bryk, 1948). *Lygephila mirabilis*, therefore, is a junior synonym of *L. lupina*, syn. n.

Diagnosis. The distinctive features of *L. lupina* and *L. vulcanea* (Fig. 66) are given in the works of Sviridov (1990) and Kononenko (1996) (under the names *L. vulcanea* and *L. mirabilis*). The main external differences between the two species are found in the colouration and shape of the forewing: *L. lupina* is broader winged and paler in colouration, usually ochreous brown to buff coloured, whereas *L. vulcanea* is darker, deep brown to claret brown, most often with a clearly visible violaceous shade and the forewing apex is somewhat more pointed. In the majority of the specimens the reniform stigma of *L. lupina* is stronger, sharper, and more distinctly marked against the paler background. The female genitalia differ from those of *L. vulcanea* (Fig. 69) by the shallower incision of the posterior margin of the antrum.

Description. Wingspan 44–49 mm. Head and body brownish grey; collar dark chocolate brown. Forewing brownish grey with sparse dark brown irroration; subbasal line indistinct; antemedial line arched with costal patch; reniform stigma large, dark brown, consists of 5 or 6 streak-like spots; orbicular stigma absent; postmedial and subterminal lines distinct; terminal line a row of black dots. Hindwing brownish; small discal spot present on underside; outer third dark brown; fringes as ground color.

Female genitalia (Figs 67, 68). Ovipositor long, papillae anales large, hairy with long setae on apical edges. Apophyses anteriores relatively short, apophyses posteriores thin, longer than apophyses anteriores. Antrum long, narrow anteriorly, dilated posteriorly, ostium broad, posterior margin with small U-shaped cleft. Corpus bursae membranous, ovoid.

Distribution. Russian Far East, China, Korea.



Figures 64–69. 64–66 Adults. 64, 65 *Lygephila lupina* (=*mirabilis*) **64** \bigcirc , Type, Russia, Raddefka **65** Russia, Kajmanovka **66** *L. vulcanea* \bigcirc , Japan **67–69** Female genitalia **67, 68** *Lygephila lupina* (=*mirabilis*) 67 Russia, Raddefka, slide No. OP1931f **68** Russia, Kajmanovka, slide No. 0321Matov **69** *L. vulcanea*, Japan, slide No. OP2442f.

Acknowledgements

I would like to express my profound gratitude to László Ronkay, Donald Lafontaine and Alberto Zilli for reading the manuscript and for their critical comments, to Gábor Ronkay (Budapest, Hungary), Péter Gyulai (Miskolc, Hungary) and Gottfried Behounek (Grafing bei München, Germany) for access to their extensive private collections. I'm grateful to Johann Stumpf (Lauda-Koenigshofen, Germany), Armin Becher (Freudenberg, Germany), Marek Dvořák (Smrčná, Czech Republic), Luboš Srnka (Lehota pod Vtáčnikom, Slovakia), Matjaž Černila (Kamnik, Slovenia), Aidas Saldaitis (Vilnius, Lithuania) and particularly Stanislav Korb (Nizhniy Novgorod, Russia) for providing material from their collections for the examination; to Keitaro Eda (Shizuoka, Japan) for granted Lygephila material from Japan; to Vlad Proklov (London, UK) for faunistics information of L. lubrica; to Vadim Zolotukhin (Uljanovsk, Russia) for his kind help in finding the material for study; to Evgenij Rutjan (Kiev, Ukraine) and Damir Sharafutdinov (Dushanbe, Tajikistan) for help in finding literature; to Anton Volynkin (Barnaul, Russia) for genitalia photos of L. lubrica from Altai; to Alexey Matov (ZISP, St. Petersburg, Russia) for adult and genitalia photos of *L. lubrica*, *L. lubrosa* and *L. mirabilis* and the great help in finding literature; to Martin Lödl and Sabine Gaal-Haszler (NHM, Vienna), Wolfram Mey (MNHU, Berlin), Axel Hausmann (ZSM, München) and especially to Dieter Stüning (ZFMK, Bonn) for access to the museum collections and for their help in finding literature.

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