RESEARCH ARTICLE



Description of a new species of Ectinorus (E. spiculatus) (Siphonaptera, Rhopalopsyllidae) from Argentina and a review of the subgenus Ichyonus Smit, 1987

Michael W. Hastriter^{1,†}, Richard D. Sage^{2,‡}

1 Monte L. Bean Life Science Museum, Brigham Young University, 290 MLBM, P.O. Box 20200, Provo, Utah 84602–20200, U.S.A. **2** Museum of Vertebrate Zoology, University of California, Berkeley, California 94720, U.S.A.

† urn:lsid:zoobank.org:author:8ED93E69-50DF-49B2-B990-93A1C7956A96‡ urn:lsid:zoobank.org:author:3BED27CD-FBB1-4FA0-9E7B-0DE416705F4E

Corresponding author: Michael W. Hastriter (michaelhastriter@comcast.net)

Guest	editor: Rober	rt E. Lewis	Received	10 June 2011		Accepted 21 Ju	ly 2011		Published	18 Aug	gust 2011
urn:lsid:zoobank.org:pub:627A1A7C-BF4A-45B4-B47C-F4371048F8ED											

Citation: Hastriter MW, Sage RD (2011) Description of a new species of *Ectinorus (E. spiculatus)* (Siphonaptera, Rhopalopsyllidae) from Argentina and a review of the subgenus *Ichyonus* Smit, 1987. ZooKeys 124: 1–18. doi: 10.3897/zooKeys.124.1688

Abstract

A new species, *Ectinorus spiculatus*, is described from *Phyllotis xanthopygus* (Waterhouse) and *Akodon iniscatus* Thomas from Neuquén Province, Argentina. Habitat characteristics are presented for the type locality. A change in status of four additional subspecies of the *Ectinorus* subgenus *Ichyonus* Smit is provided. *Ectinorus onychius onychius* Jordan & Rothschild, *E. onychius deplexus* Smit and *E. onychius angularis* Smit and Rosický were elevated to specific status. *Ectinorus (Ichyonus) onychius fueginus* was relegated as a junior synonym of the nominate species. *Phyllotis xanthopygus, Abrothrix olivaceus xanthorhinus, Loxodontomys micropus* Waterhouse, and *Euneomys chinchilloides* (Waterhouse) are new host records for *E. onychius*. A key to the three species of *Ichyonus* is included.

Keywords

Argentina, Flea, Ectinorus spiculatus, Ichyonus

Introduction

This study is a continuation of natural history studies of small mammals and their ectoparasites conducted in Neuquén and Río Negro Provinces, Argentina by the junior author (RDS). Among specimens collected during these studies, a new species of Ectinorus Jordan was discovered and is described herein. A synopsis of the genus Ectinorus is summarized in Hastriter and Sage (2009) and includes a comprehensive listing of known species and their distribution. Specimens of the subgenus Ichyonus Smit were also found in Neuquén Province which stimulated a further assessment of this enigmatic subgenus. Smit (1987) recognized four subspecies in the subgenus Ichyonus and his evaluation of these four taxa was based on relatively few specimens. The nominate subspecies comprised a good series of males and females, but only five specimens are representative of the other three subspecies. Of the latter three only males are known for two of them. With the availability of additional material, the status of taxa within this subgenus could be further evaluated. Systematic changes are addressed here. Including the new description that follows and a revision of available material representing Ichyonus, the genus is now comprised of 38 species [subgenera Ectinorus (34), Ichyonus (3) and Panallius Jordan (1)].

Materials and Methods

Techniques for trapping small mammals and processing them for ectoparasites are outlined in detail in Hastriter and Sage (2009). Fleas were mounted on glass microscope slides in accordance with Hastriter and Whiting (2003). Dissections of male genitalia follow the procedures of Hastriter (2004). Images were prepared using an Olympus BX61 Compound Microscope, Olympus CC12 digital camera accompanied with an Olympus Microsuite[™] B3SV program. Land marks used for flea measurements are described in Hastriter and Eckerlin (2003). Anatomical terms for flea anatomy are adapted from Rothschild and Traub (1971) and mammal nomenclature follows those of Wilson and Reeder (2005). Acronyms for repositories for "material examined" and type specimens follow:

BMNH	British Museum of Natural History, London
BYU	Monte L. Bean Life Science Museum, Brigham Young University, Provo,
	Utah
CMNH	Carnegie Museum of Natural History, Pittsburgh, Pennsylvania
JCB	Jean-Claude Beaucournu personal collection, Rennes, France
MACN	Museo de Ciencias Naturales "Bernardino Rivadavia" de la Ciudad de Bue-
	nos Aires, Republica Argentina

Results

Rhopalopsyllidae

Key to the Ectinorus subgenus Ichyonus Smit

1	Males
_	Females (<i>E. deplexus</i> unknown)
2	Four stout setae on dorso-posterior margin of telomere and without subtend-
	ing sinus; ventro-caudal hook on telomere extending upward less than 1/4 the
	length of the telomere (Fig. 3). Upper portion of acetabulum at about mid-
	point of anterior margin of telomere (Fig. 3). Lobe on caudal margin of distal
	arm of ninth sternum lacking or only slightly indicated. Crochet hyaline and
	rounded at apex (Fig. 6); (see exception in diagnosis)
_	Seven to eleven stout setae on dorso-posterior margin of telomere; with dis-
	tinct subtending sinus (Figs 1-2). Hook on telomere extending more than
	1/4 the length of the telomere. Upper margin of acetabulum either distinctly
	above or below middle of anterior margin of telomere. Lobe on caudal mar-
	gin of distal arm well developed
3	Mesal surface of hind femur with row of 11-12 setae. Crochet expanded,
	rounded at apex with wide sclerotization along ventral margin; ventro-caudal
	margin with series of convoluted folds (fold similar but less apparent in Chil-
	ean specimens). Apex of ventro-caudal hook of telomere extending upwards
	more than half the length of telomere; upper margin of acetabulum far below
	middle of anterior margin of telomere (Fig. 1) E. angularis
_	Mesal surface of hind femur with row of 17 setae. Crochet not expanded, but
	somewhat rectangular and truncate at apex; ventro-caudal angle with small
	lobe (Fig. 5). Apex of ventro-caudal hook of telomere not reaching half length
	of telomere; upper margin of acetabulum above middle of anterior margin of
,	telomere (Fig.2)
4	Anal stylet short, length twice width (2.1×) (Fig. 7) <i>E. angularis</i>
-	Anal stylet longer, length greater than three times width (range: $3.2-3.6\times$,
	average: 3.5×) (Fig.8) <i>E. onychius</i>

Ectinorus (Ichyonus) angularis Smit & Rosicky

http://species-id.net/wiki/Ectinorus_(Ichyonus)_angularis Figs 1, 4, and 7

Ectinorus onychius angularis Smit and Rosicky, 1972:366; Smit 1987:122; Beaucournu and Gallardo 1992:100; Alarcon 2000:13. sp. n.

Material Examined. Chile, [Magallanes Region]: Estancia Pudeto, W of Lago Sarmiento, [51°05'S, 73°00'W, ex *Akodon* sp.], 18 II 1969, M. Rozehnal (1 pair of paratypes) (BMNH).

Diagnosis. Male distinguished from other species of *Ichyonus* by large lobular crochet that has a heavily sclerotized ventral margin and convoluted folds on the ventrocaudal margin (Fig. 4). The ventro-caudal hook of the telomere is also much longer and robust, its apex hooking upward well beyond middle of telomere (Fig. 1). This extends less than half the length of the telomere in other species. The upper portion of the acetabulum far below middle of anterior margin of telomere (Fig. 1). The single known female specimen may be distinguished from females of *E. onychius* by a much shorter anal stylet (*cf* Figs 7 and 8).

Remarks. Known only from a single collection in the extreme southern limits of Chile; little can be said of its host preferences. The type locality of *E. angularis* occurs at the lowest elevation of the three species of *Ichyonus* at ~40m. The female is indistinguishable from females of *E. onychius* with the exception of the much shorter anal stylet. The anal stylet on both sides of the single specimen examined appears the same. Two additional females (paratypes) reportedly exist in the Czechoslovak Academy of Sciences, Prague, but they could not be obtained for examination. Smit and Rosicky (1972) examined all three of the known females at the time of their description. Although they did not state that the anal stylet was similarly short in all three specimens, Smit (1987:124, fig. 263) subsequently illustrated the anal stylet of the allotype. Additional collections will refute or substantiate the value of this character.

Ectinorus (I.) deplexus Smit

http://species-id.net/wiki/Ectinorus_(Ichyonus)_deplexus Figs 1, 5, and 9

Ectinorus onychius onychius Jameson and Fulk, 1977:402 (mis-identification) *Ectinorus onychius deplexus* Smit, 1987:120; Beaucournu and Gallardo 1992:100; Alarcon 2000:13. sp. n.

Material Examined. Chile, Santiago Province: La Parva, 3000m, ex *Eunomys noelli* (sic!) [*Euneomys noei*] = *Euneomys mordax* Thomas, Geo. Fulk (\mathcal{J} holotype and \mathcal{J} paratype) (BMNH).



Figures 1–8. I *Ectinorus angularis*, telomere, paratype **2** *Ectnorus deplexus*, telomere, paratype **3** *Ectinorus onychius*, telomere, holotype **4** *Ectinorus angularis*, crochet, paratype **5** *Ectinorus deplexus*, crochet, holotype **6** *Ectinorus onychius*, crochet, holotype **7** *Ectinorus angularis*, anal stylet, paratype **8** *Ectinorus onychius*, anal stylet, paratype. Scale = 100μ

Diagnosis. Female unknown. Setae on mesal surface of hind femur longer and more numerous (17) than other species (Fig. 9). Similar to *E. angularis* in the number of large setae on the caudal margin of the telomere, but distinguished by the much shorter length of the telomere from its apex to the upper margin of the acetabulum (Fig. 2).

Remarks. *Ectinorus deplexus* is known only from the type locality at an elevation that is more than three times that of the other two species of the subgenus *Ichyonus*. El-

evational limitations may prove to be a factor in the distribution of other *Ichyonus* species. The two males are also notably larger (2.8mm) than males of *E. onychius* (2.1mm; n=11) and *E. angularis* (2.4mm; n=2).

Ectinorus (I.) onychius (Jordan & Rothschild)

http://species-id.net/wiki/Ectinorus_(Ichyonus)_onychius Figs 3, 6, and 8

Parapsyllus onychius Jordan & Rothschild, 1923:352; Dalla Torre 1924:19; Del Ponte and Riesel 1939:545.

Ectinorus onychius (Jordan and Rothschild), Jordan 1942b:11.

- Ectinorus onychius onychius (Jordan and Rothschild), Jordan 1942a:434; Da Costa Lima and Hathaway 1946:149; Smit 1955:337 (Q description); Johnson 1957:142; Smit 1963:427; Giménez, Ciccarelli and de la Barrera 1964:129, 138, 139; Jameson and Fulk 1977:402 (misdetermination, originally referred to *E. onychius deplexus*); Smit 1987:116; Beaucournu and Gallardo 1988:100; Beaucournu and Alcover 1989:491; Beaucournu and Kelt 1990:648, 665; Beaucournu and Gallardo 1992:100; Alarcon 2000:13; Beaucournu and del Carmen Castro 2003:468. stat. n.
- *Ectinorus onychius fueginus* Jordan, 1942a:434; *Ectinorus onychius fueginus* Jordan, Da Costa Lima & Hathaway, 1946:149; Johnson, 1957:142; Smit 1987:121; Beaucournu and del Carmen Castro, 2003:468. syn. n.

Material Examined. Argentina, Chubut Province: Cholila, ex Ctenomys haigi Thomas, 23 I 1920, H.E. Box (d holotype); Cholila, ex Akodon iniscatus Thomas, 23 I 1920, H.E. Box (d paratype) (BMNH). Puerto Madryn, 6, 9 IV 1978, A. Kovacs (13, 19) (JCB). Mendoza Province: Puesto "Pugin", Algattolito (32°53'S, 67°18'W), 620m, ex "rats", D.F. Giménez (♀) (BMNH). Neuquén Province: Laguna Blanca National Park, Locality 40, 1.97 km W, 3.84 km N Mellizo Sud, (39°2'27.5"S, 70°19'24.5"W) inside clumps of "molle" (Schinus polygama) on sandy soil, 1290m, ex Abrothrix olivaceus xanthorhinus, 17 II 2006, R.D. Sage (3, RDS-17963/F-276); Laguna Blanca National Park, Locality 74, 0.04 km E, 3.22 km S Cerro de la Laguna, (39°03'5.03"S, 70°22'30.42"W), Nassauvia shrubland on dry slope above Laguna Blanca, south side of Península de la Laguna Blanca, 1285m, ex A. iniscatus (♂), 20 II 2007, R.D. Sage(♂, RDS-18370/F-282) (BYU); Laguna Blanca National Park, Locality 52, 0.27 km E, 3.93 km N Cerro de la Laguna, (38°59'9.84"S, 70°22'7.02"W), grass scrubland (Berberis sp. and Colletia sp.) on east facing slope of rimrock, 1340m, ex *Euneomys chinchilloides* (Waterhouse) (♂), 27 VII 2007, R.D. Sage (2 Q, RDS-18493) (MACN); Laguna Blanca National Park, Locality 76, 2.24 km W, 3.12 km S Cerro Mellizo Sud, (39°6'10.92"S, 70°19'33.42"W), lava outcrops with *Colliguaja* sp., 1320m, ex *Phyllotis xanthopygus* (Waterhouse) (\mathcal{J}), 13 III 2007, R.D. Sage. (2, RDS-18397) (BYU); Currhué Chico, ex Akodon oliva*ceus = Abrothrix olivaceus* Waterhouse, 22 IV 1988, J.C. Beaucournu (3); Lago Cur-

rhué, Akodon longipilis = Abrothrix longipilis Waterhouse, IV 1988, J.C. Beaucournu (\mathcal{E}); Lago Norquínco, ex *Ab. longipilis*, 1 V 1988, J.C. Beaucournu (2 \mathcal{Q}) (JCB). Río Negro Province: Trap line #6, Península Llao Llao, 0.4 km N Park Guard Station between Lagos Perito Moreno and Escondido, (41°02'54.7"S, 71°33'56.5"W) in green bamboo/coihue forest, 831m, ex *Loxodontomys micropus* Waterhouse (\mathcal{Q}), 6 V 2005, R.D. Sage (d, RDS-17339/F-278) (BYU); San Pedro, nr. Bariloche, ex "nest of *Rattus norvegicus*", 20 II 1954, J.M. de la Barrera (3∂, 1♀); San Pedro, nr. Bariloche, ex Hesperomys sp. [most likely a representative of either O. longicaudatus or A. longipilis], 27 II 1954, J.M. de la Barrera $(3^{\land}_{\circ}, 2^{\bigcirc}_{\circ})$; San Pedro, nr. Bariloche, ex *Euneomys dabbenei* = *E. chinchilloides*, 8 VII 1953, J.M. de la Barrera (\bigcirc paratype, "neallotype" on slide); Península of San Pedro, Bariloche, ex Akodon varius neocenus = Akodon neocenus Thomas [surely refers to A. iniscatus, since A. neocenus is only known to occur 150 km east of the Bariloche region, with A. iniscatus filling in the intervening area], 20 III 1960, J.M. de la Barrera (Q); Península of San Pedro, Bariloche, ex Oryzomys longicaudatus philippii = Oligoryzomys longicaudatus (Bennett), 20 III 1960, J.M. de la Barrera (\mathcal{Q}); Bariloche, Nahual Huapi, ex *Rattus* sp., J.M. de la Barrera (1 male); El Bolsón, ex "nest of rodent", G. Topali & Don J. Szabo (♂); San Carlos de Bariloche, ex Akodon olivaceus beatus = Ab. olivaceus, 14 I 1965, J.M. de la Barrera (\mathcal{Q}) (BMNH); San Pedro, nr Bariloche, ex *Oligoryzomys longicaudatus*, 26 II 1954, J.M. de la Barrera (♂); El Bolsón, 20 I 1961, Topal Gy. No. 58 (♀) (CMHN). Tierra del Fuego Province: Estancia Viamonte, ex Belonopterus chilensis = Vanellus chilensis Molina, 15 X 1931, P.W. Reynolds (Ectinorus onychius fueginus d holotype, new synonymy herein) (BMNH). Chile, Aisén Region: Chico Aerodromo, ex Akodon xanthorhinus = Ab. o. xanthorhinus, III 1987, J.C. Beaucournu (Kelt-3976, 3°_{\circ} , 1°_{\circ}); Puerto Ibanéz, El Salto, ex Akodon longipilis = Ab. longipilis, III 1987, J.C. Beaucournu (Kelt-3563, \mathcal{E} , \mathcal{Q}) (BYU).

Diagnosis. *Ectinorus onychius* males differ from either *E. angularis* and *E. deplexus* by the lack of a strong lobe on the ventro-caudal margin of the distal arm of the ninth sternum, by fewer strong setae on the caudal margin of the telomere with only 4 (sometimes 5) (Fig. 3), and the hyaline crochet is longer than wide and round on the apex (without sclerotized margins or special feature at ventro-caudal margin) (Fig. 6). Note: the crochet of *E. onychius* specimens from Aisén Region, Chile is more similar to the single exemplar of *E. angularis* in Magallanes Region, Chile than the more northern populations of *E. onychius* in Chubut, Neuquén, and Rio Negro Provinces, Argentina. Never-the-less, populations further north in Argentina and those in Aisén Province, Chile are clearly distinct from *E. angularis* by the greater superior position of the acetabulum on the telomere. Female distinguished from *E. angularis* by the much longer anal stylet (*cf.* Figs 7 and 8)

Remarks. There is insufficient morphological evidence to support the erection of *E. onychius fueginus* to full specific status, nor to recognize this single specimen as a subspecies. It is considered a junior synonym of *E. onychius* from which it is indistinguishable. Our taxonomic re-interpretation extends the geographic range of *E. onychius* from northeastern Mendoza Province to the extreme southern province



Figure 9. Ectinorus deplexus, paratype, hind femur. Scale = 100µ

of Tierra del Fuego, and from the Atlantic coast (Puerto Madryn) to steppe habitat at the Argentina/Chilean border. Additional collections in the southern parts of the Monte phytogeographic biomes of Mendoza/La Pampa/Río Negro Provinces and in the south-central parts of the Patagonian region are needed to establish the distribution and true range of this species. The finding of the record on a plover, [Belonopterus chilensis = Vanellus chilensis] (Tierra del Fuego Province) is certainly accidental and is the only known record of the subgenus found on an avian host. Representatives of Ichyonus appear on many small sigmodontid rodents and without specificity. There is only one report of this species on a non-sigmodontid rodent, i.e., on the histricomorphid genus Ctenomys. The nearly total absence of this flea in our extensive study of ectoparasites of species of Ctenomys would suggest this is an accidental association. In addition to hosts previously reported on E. onychius (in lit.), our findings on P. xanthopygus, Ab. olivaceus xanthorhinus, L. micropus, and E. chinchilloides are new host records. The reference to Ab. xanthorhinus as a subspecies of Ab. olivaceus is our attempt to identify the distinct race of this enigmatic sigmodontid complex. This flea is clearly more dependent on terraine, habitat, elevation, and microclimatic conditions than on host specificity.

A single specimen (RDS-17339) was collected from *L. micropus* in the region of the Valdivian, evergreen rainforest. The specific site is in a mature forest of *Nothofagus dombeyi* (Mirb.) ("coihue") with a dense understory of the bamboo *Chusquea culeou* Desvaux.("caña coihue") on a south-facing hillside (Fig. 24). There were many fallen and rotting trunks of the "coihue" on the ground, and a thick leaf litter comprised mostly of the bamboo leaves. The soil is dark in color and rich in humus. The environment is cool and moist. *L. micropus* was the third-most abundant of the six rodent species in this habitat, with *Ab. longipilis* being the most common species (40 percent of the total collected at this site) from which *E. onychius* was not collected. The second specimen (RDS-18370) was collected from *A. iniscatus* on the large peninsula that juts into Laguna Blanca in LBNP. The peninsula has been protected from livestock grazing for 15 years and has a comparatively very dense development of the Patagonian steppe vegetation. In particular the eastern (leeward) side of the peninsula is

densely covered with spiny shrub *Nassauvia glomerulosa* D. Don ("uña de gato") and bunch grasses. The soil consists of fine, windblown sand, with little organic matter. The Laguna Blanca area is cool, dry, and strong winds are frequent. On this protected peninsula, *A. iniscatus* is the most abundant species, followed by *Eligmodontia morgani* J.A. Allen. Two additional specimens (RDS-18493) were collected from, *Eu. chinchilloides* along the edge of a black basalt rimrock with a dense growth of the Patagonian steppe shrub *Colletia hystrix* (Clos.) ("espino negro") and large bunch grasses (Fig. 25). The ground consisted of blocks of the broken basaltic rock and wind-blown sand. *Phyllotis xanthopygus* was the more common of the five species of rodents trapped in this habitat.

Measurements of the anal stylet of eight specimens were conducted. The range of their length was $58-71\mu$ (average: 63μ) and width was $16-20\mu$ (average: 18μ) with an average ratio of $3.5\times$ (length:width). This ratio is substantially greater than that of the single female of *E. angularis* (2.1×). It is doubtful that the shorter anal stylet is an anomalous condition, since the stylet on both sides are similarly short.

Ectinorus spiculatus Hastriter & Sage, sp. n.

urn:lsid:zoobank.org:act:C739CAB5-FAA9-4D33-8266-D7C5827DCA9F http://species-id.net/wiki/Ectinorus_spiculatus Figs 10–23

Type Material. Argentina, Neuquén Province:, 1 km SSW from Route 40 on dirt road to Estancia Llamuco (38°44'1.2"S, 70°17'55.26"W), vegetation on sandy soil with basaltic rimrock, 1074m, ex *P. xanthopygus* (\mathcal{Q}), 14 IV 2008, R.D. Sage, Holotype \mathcal{J} (RDS-18861); Laguna Blanca National Park, Locality 76, 2.24 km W, 3.12 km S Cerro Mellizo Sud, (39°6' 10.92"S, 70°19'33.42"W), lava outcrops with *Colliguaja* sp., 1320m, ex *P. xanthopygus* (\mathcal{J}), 14 III 2007, R.D. Sage, allotype \mathcal{Q} (RDS-18407); same data as allotype except ex *A. iniscatus* (\mathcal{J}), 13 III 2007, paratype \mathcal{Q} (RDS-18403). Holotype and allotype are deposited in the Museo de Ciencias Naturales "Bernardino Rivadavia" de la Ciudad de Buenos Aires, Republica Argentina; paratype \mathcal{Q} deposited in the Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah, U.S.A.

Diagnosis. Males key to *Ectinorus hertigi* (Johnson) in Smit's (1987:78) key, while females key to *Ectinorus barrerai* Jordan. Morphologically the male is closely allied with *E. hertigi* but may be distinguished from it and all other species of the subgenus *Ectinorus* by the bilobed apex of the basimere and details of the aedeagus (Figs 13, 16). The presence of seven segments in the labial palpus of females (male with five) is the basis for its similarity with *E. barrerai*; however, their similarity is limited. If one continues onward in the key using five segments in the labial palpus (versus 6 to 8), females key out to *E. hertigi* also. Females share many similarities with *E. hertigi* (few with *E. barrerai*) for which they may be separated by an oblique flattened region of the spermatheca at the cribriform area and a very long bursa copulatrix that is reflected postad in a semi-circular arc (Fig. 23).



Figures 10–13. *Ectinorus spiculatus* sp. n., male, holotype **10** Overview **11** Head, pronotum and forecoxa **12** Enlargement of frontal tubercle (insert) **13** Terminal segments. Scale Fig. $10 = 500\mu$; Figs 11 and $13 = 200\mu$

Description. Chaetotaxy and structural references include only one side of specimen. Head (Figs 10–12). Frons evenly rounded; thickened throughout. Frontal tubercle quadrate; capsule heavily sclerotized but thin caudad. Two placoids between frontal tubercle and sclerotized antennal suture. Eye large, darkly pigmented, sinuate. Ocular setae four; laterals large, middle two much smaller. Tentorium clearly visible anterior to eye. Preantennal setae; one near oral angle, two (large and small) anterior to eye. Third



Figures 14–16. *Ectinorus spiculatus* sp. n. **14** Male, holotype, thorax and metepimeron **15** Female, allotype, spermatheca and bursa copulatrix **16** Male, holotype, aedeagus. Scale Fig. 14 = 200μ ; Figs 15–16 = 100μ

segment of maxillary palpus shorter than others; maxilla acutely sharp. Five segmented labial palpus extending to apex of coxa, apical two segments twice length of either second or third segments; apex blunt with array of fine setae. Antennal scape with apical row of six fine setae; pedicel with three minute dorsal setae; clavus extending onto prosternasome. Post-antennal area with four rows of setae (1, 1, 1, 6 plus intercalaries; female with only two minute setae anterior to main row). Two placoids; occipital groove moderately deep. Row of 18 setules along dorsal margin of antennal groove. Genal lobe bluntly rounded with three small apical setae; five larger marginal setae below eye. Thorax (Figs 10, 11, 14). Pro-, meso-, and metanota each with two rows setae. Eleven to 12 pseudosetae under mesonotal colar. Dorsal apex of metanotum curled downward. Cervical link-plate truncate at apex. Prosternasome grooved for retention of antennal apex; without setae. Mesepimeron with four setae and mesepisternum with two; mesosternum heavily sclerotized along ventral margin with incomplete suture between mesepisternum. Pleural rod bifurcate dorsally. Lateral metanotal area with two large, two small setae. Pleural arch and ridge well developed. Metepisternum and metasternum, fused into one; one large seta. Furca long and delicate. Metepimeron with two vertical rows of setae; anterior with two (dorsal minute), posterior of three (same arrangement in female). Legs (Fig. 10, 19). Fore coxa with 28 lateral setae; one long seta at posterior



Figurs 17–23. *Ectinorus spiculatus* sp. n., female, allotype **17** Overview **18** Dorsal and ventral anal segments **19** Hind tarsal segments **20** Head, pronotum, and forecoxa **21** Enlargement of frontal tubercle (insert) **22** Spiculated medial surface of hind tibia (insert) **23** *Ectinorus spiculatus* sp. n., female, paratype, seventh sternum, spermatheca, and bursa copulatrix. Scale Fig. 17 = 500μ; Figs 18–20 and 23 = 200μ

margin. Oblique break mid coxa indicated only at ventro-caudal margin. Two guard setae at femoral-tibial joints; lateral of two long equal on fore femur; shorter on mid and hind femora. Fore and mid femora with two lateral rows of setae; hind femur with single



Figure 24. Habitat from which *Ectinorus onychius* was collected from *Loxodontomys micropus*.

lateral row of 12 setae. Lateral sculpturing of hind femur very fine. Margin of fore, mid, and hind tibiae with 5, 6, and 6 dorsal notches, respectively. Number of setae in respective dorsal notches: fore tibia (beginning with proximal notch) (2, 2, 3, 2, 3), mid tibia (2, 2, 2, 3, 2, 3), hind tibia (2, 2, 2, 2, 2, 3). Lateral setae of each tibia, respectively (6, 6, 8). Inner (mesal) surface of hind tibia adorned with spicules. First hind tarsus with three long setae; two extending to and one extending beyond segment three. Second hind tarsus with two setae extending beyond distotarsomere. Distotarsomeres with four pair lateral plantar bristles; apical pair smallest. Pre-apical plantar bristles two; one small, one larger. Ungue symmetrical. Unmodified Abdominal Segments (Fig. 10). Dorsum of tergum I heavily sclerotized with distinct hump (absent in female); anterior lateral margin thick and sclerotized. Two rows setae. Terga II-III with two rows setae; terga IV-VII with single row. Ventral most setae of terga II-VII not extending below level of round spiracles. Single antesensilial bristles extending from pedestal beneath apical flange of tergum VII. Sternum II with lateral patch of 7-8 small setae. Sterna II-VII with single rows of setae (1, 2, 3, 3, 3, 3). Modified Abdominal Segments (Male) (Figs 10,13). Sensilium with 17 sensilial pits; surrounded by wide sclerotized area bearing single seta on caudal margin. Spiracle VIII vermiform, curved upward with three small setae dorsad. Tergum VIII large and highly specialized; lateral and apical surfaces with coarsely reticulated pattern. Tergum VIII envelops basal portion of basimere while curling under and behind apical portion of basimere and telomere to form an unusual conical sharp lobe. Caudal margin is adorned with eleven long setae; ventro-caudal margin with two long setae and smaller marginal setae cephalad. Sternum VIII with lateral row of eight long



Figure 25. Habitat from which Ectinorus onychius was collected from Euneomys chinchilloides.

setae; ventral apex with thick incrassation. Dorsad to incrassation extends a moderately sharp projection. Apex of basimere (tergum IX) with two asymmetrical lobes divided by a deep sinus. Dorsal lobe of basimere with numerous setae; ventral lobe with two stout setae. Robust processus basimeris ventralis present; group of stout setae at apex. Length of telomere more than five times width; bluntly rounded at apex, sides parallel. Numerous small setae line margin. Manubrium tapered, curving upward to acute point. Lateral portion of basimere with triangular, darkly sclerotized, caudally directed structure (Fig. 16). (A patch of fine setae are present on each side and appear to be present on a lobe ventrally located on the ventral margin of tergum VIII and may be associated with triangular sclerotization above. (Without dissection of genitalia, this anatomy could not be deciphered for certain). Distal arm of sternum IX long with parallel sides, expanding at tip; lateral setae present on upper third. Notable group of 9–10 long setae on caudally expanded lobe. Vestigial tendon of sternum IX affixed to apical sclerotization of sternum VIII. Aedeagus (Fig. 10, 13, 16). Similar to that of E. hertigi, but median dorsal lobe greatly reduced and lateral lobes expanded. Dorsal armature immense (seen behind basal portion of telomere sandwiched between conical lobe of tergum VIII, Fig. 13), ventral armature reduced. Sclerotized inner tube long, slightly curved ventrad; with annular ring at midpoint. Aedeagal apodeme bluntly rounded at apex; penis rods barely extend beyond apex of apodeme. Modified Abdominal Segments (Female) (Figs 15, 17, 18, 23). Seventh sternum with lateral row of five setae; caudal margin entire with



Figure 26. Habitat from which Ectinorus spiculatus holotype was collected from Phyllotis xanthopygus.

ventral margin incised, creating an indistinct rounded ventral lobe. Single antesensilial bristle arising from strong pedicel. Tergum VIII with group of eight setae above spiracle VIII. Spiracle VIII vermiform, slightly ballooned at base. Lateral row of six long setae on tergum VIII; marginal group of 20 plus setae at apical margin. Sternum VIII with apical rounded lobe, without setae. Sensilium with broad sclerotized ring; 16 sensilial pits. Anal stylet with apical long seta plus one seta longer than anal stylet. Length of anal stylet twice width. Hilla twice length of bulga; hilla approximate width of bulga. Bulga flattened on cribriform region; cribriform area not protruding into bulga. Bursa copulatrix extremely long; curved caudally in circular arc.

Length: Male holotype: 2185 μ ; female allotype: 2533 μ ; and female paratype: 2175 μ .

Etymology. The specific epithet *spiculatus* is derived from the characteristic presence of spicules on the mesal surface of the hind tibia.

Remarks. The single male and two females were all collected from different host specimens. The authors feel confident that both sexes belong to the same taxon for the following reasons: 1) Both male and female have spicules on the mesal surface of the hind tibiae, 2) both sexes have very similar head chaetotaxy and shape of the genal lobe, 3) the second tarsal segment possesses three long setae, two of which extend beyond segment four, 4) a pair was collected at the same locality (Laguna Blanca National Park) and the other female within close proximity, within 35 km, 5) the male at one locality and female from the other were from the same host species (*P. xanthopy-gus*), and 6) terraine, habitat, and elevations for both localities were nearly the same.

The holotype (RDS-18861) was collected from *P. xanthopygus* along the edge of a rimrock of a dark-red basaltic flow from a nearby, unnamed cinder cone (Fig. 26). Deep drifts of unconsolidated, wind-blown sand, filled the fissures in this broken-rock habitat. A dense growth of *Colliguaja integerrima* Gillies & Hook ("coliguay") and bunch grasses were the dominant plants. The area was cold, dry, and at times very windy. Only *P. xanthopygus* and an undescribed species of *Ctenomys* ("tuco-tuco") were trapped at the type locality. It should be noted that *Ectinorus lareschaei* Hastriter and Sage, 2009 was also collected from the same host specimen as this holotype. Paratypes RDS-18403 and RDS-18407, were collected from *A. iniscatus* and *P. xanthopygus*, respectively, in Laguna Blanca National Park at the southern edge of the lava flow forming the cinder cone volcano, Cerro Mellizo Sud. Deep sandy soil fills in the small fissures in the lava flow and there is a sparse growth of the Patagonian steppe vegetation, mostly bunch grasses and smaller shrubs such as *C. integerrima* and *N. glomerulosa*. *P. xanthopygus* was the more common of the five small mammals trapped here, including the mouse opossum *Thylamys pallidior* (Thomas).

Acknowledgments

We express our appreciation to Claudio Chehébar, Gustavo Iglesias, and Eduardo Ramilo of the Delegación Regional Patagonia of the Argentina National Park Service, for approval of projects APN No. 538 and 696. Much of the collection was completed in the Laguna Blanca National Park. For the assistance and friendship of the Park staff members Fernando Zanona, Federico Bracamonte, and Carlos Quilaqueo, we are indebted. Additionlly, we thank the staff of the Subsecretario de Medio Ambiente, Municipalidad de S. C. de Bariloche and the Dirección de Fauna Silvestre, Provincia de Río Negro for permits to make collections in Río Negro Province. Specimens were exported to the senior author by permit of the Dirección de Fauna Silvestre, Buenos Aires, Argentina and we express our appreciation to Dr. Alejandro Tablado, Curador Colección Nacional de Invertebrados, Museo Argentino de Ciencias Naturales, Argentina and Cecilia Li Puma of Dirección de Fauna Silvestre for assisting with the administrative details necessary for exportation of scientific material. We are grateful to Professor Jean-Claude Beaucournu, Rennes, France, Theresa Howard (British Museum of Natural History, London) and John E. Rawlins (Carnegie Museum of Natural History, Pittsburgh, PA) for loan of specimens. We continue to be indebted to Michael F. Whiting and staff of the Monte L. Bean Life Science Museum for provision of work space, supplies, and equipment without which these studies would not be possible.

References

Alarcón ME (2000) Estado actual del conocimiento de los siphonapteros presents en Chile (Insecta: Siphonaptera) Gayana 64(1): 1–17.

- Beaucournu JC, Alcover JA (1989) Puces récoltées dans la province de Neuquén (Argentina); description de 4 nouveaux taxa (Insecta, Siphonaptera). Anales de Parasitologie Humaine et Comparee. 64(6): 489–505.
- Beaucournu JC, del Carmen-Castro D (2003) Contribution à un inventaire des puces d'Argentine (Insecta Siphonaptera). Beitrage zur Entomologie 53(2): 449–479.
- Beaucournu JC, Gallardo MN (1988) Puces nouvelles d'Argentine (Insecta, Siphonaptera). Revue Suisse de Zoologie 95(1): 99–112.
- Beaucournu JC, Gallardo MH (1992) Catalogue provisoire des puces du Chile (Insecta; Siphonaptera). Bulletin de la Société Française de Parasitologie 10(1):93–129.
- Beaucournu JC, Kelt DA (1990) Contribution a la faune du Chile: puces nouvelles ou peu connues de la partie sud (Insecta, Siphonaptera). Revue Suisse de Zoologie 97(3): 647–668.
- Costa Lima A da, Hathaway CR (1946) Pulgas: Bibliografia, catálogo e hospedadores. Monografias do Instituto Oswaldo Cruz, No. 4: 1–522.
- Dalla Torre CG (1924) Aphaniptera. Sonderabdruck aus den Berichten des naturwissenschafilich-medizinischen Vereines in Innsbruck 39:1–29.
- Del Ponte E, Reisel MA (1939) Notas sobre "Siphonaptera" argentines. II. Primera lista de species. Physis, Revista de la Sociedad Argentina de Ciencias Naturales, Buenos Aires 17: 543–551.
- Hastriter MW, Eckerlin RP (2003) *Jellisonia painteri* (Siphonaptera: Ceratophyllidae), a new species of flea from Guatemala. Annals the of Carnegie Museum 72: 215–224.
- Hastriter MW, Whiting MF (2003) Siphonaptera (Fleas), In: Resh VH, Carde R (Eds), Encyclopedia of Insects, Academic Press, 1040–1044.
- Hastriter MW (2004) Revision of the flea genus *Jellisonia* Traub, 1944 (Siphonaptera: Ceratophyllidae). Annals of the Carnegie Museum 73(4): 233–257.
- Hastriter MW, Sage RD (2009) A description of two new species of *Ectinorus* (Siphonaptera: Rhopalopsyllidae) from Laguna Blanca National Park, Neuquén Province, Argentina. Proceedings of the Entomological Society of Washington 11(3): 581–597. doi: 10.4289/0013-8797-111.3.581
- Jordan K (1942a) On Siphonaptera collected by Dr. J.M. de la Barrera in the province of Mendoza during 1939. Revista del Instituto Bacteriologico "Dr. Carlos G. Malbran" 10(4): 401–460.
- Jordan K (1942b) On *Parapsyllus* and some closely related genera of Siphonaptera. EOS, Revista Española de Entomologia 18: 7–29.
- Jordan K, Rothschild NC (1923) On the genera *Rhopalopsyllus* and *Parapsyllus*. Ectoparasites 1: 320–370.
- Rothschild M, Traub R (1971) A revised glossary of terms used in the taxonomy and morphology of fleas (reprinted from G.H.E. Hopkins and M. Rothschild, An Illustrated Catalogue of the Rothschild Collection of Fleas (Siphonaptera) in the British Museum (Natural History). Vol. 5, 8–85.
- Smit FGAM (1987) An illustrated catalogue of the Rothschild collection of fleas (Siphonaptera in the British Museum (Natural History), Vol. VII, Malacopsylloidea (Malacopsyllidae and Rhopalopsyllidae), Oxford University Press, British Museum (Natural History), Oxford and London, 5 plates, 380 pp.

- Smit FGAM, Rosicky B (1972) Some Siphonaptera from Chile. Folia Parasitologica (Praha) 19: 365–368.
- Wilson DE, Reeder DM (2005) Mammal species of the world. A taxonomic and geographic reference. 3rd Ed., The Johns Hopkins University Press, Baltimore.

RESEARCH ARTICLE



Tungurahualini, a new tribe of Neotropical leafhoppers, with notes on the subfamily Mileewinae (Hemiptera, Cicadellidae)

C. H. Dietrich

Illinois Natural History Survey, University of Illinois, 1816 S. Oak St., Champaign, IL 61820, USA

urn:lsid:zoobank.org:author:82FCB86C-54B4-456A-AE5E-D7847D271CB9

Corresponding author: C. H. Dietrich (dietrich@inhs.uiuc.edu)

Academic	editor: <i>Mick</i>	Webb	Received	17 May 2011		Accepted 25 July 2011	Publishee	d 18 August 2011
		urn:lsid	:zoobank.orş	g:pub:80965FF0	5-61	BF3-4C64-958B-DE17FDE8	9604	

Citation: Dietrich CH (2011) Tungurahualini, a new tribe of Neotropical leafhoppers, with notes on the subfamily Mileewinae (Hemiptera, Cicadellidae). ZooKeys 124: 19–39. doi: 10.3897/zookeys.124.1561

Abstract

A new cicadellid tribe, Tungurahualini, is recognized to include *Tungurahuala* Kramer, and a related new genus, *Ilyapa* gen. n., based on six new species. The tribe is included in subfamily Mileewinae, the concept of which is further expanded to include tribes Makilingiini Baker, and Tinteromini Godoy and Webb, taxa previously treated as separate subfamilies. Keys to tribes of Mileewinae (sensu lato) and genera of Tungurahualini are provided. A new species of *Tungurahuala*, *T. acuminata* sp. n., is also described and keys to species of *Tungurahuala* and *Ilyapa* are provided. The new tribe is presently recorded only from cloud forests in the northern Andes Mountains of South America.

Keywords

Homoptera, Auchenorrhyncha, morphology, identification, distribution

Introduction

The leafhopper subfamily Mileewinae comprises small to medium-sized, slender, usually darkly pigmented species that inhabit wet tropical forests worldwide. Most species appear to inhabit montane cloud forests where they occur on herbaceous vegetation in the understory. The group was established by Evans (1947) as a tribe of Cicadellinae (as "Tettigellinae") based

Copyright C. H. Dietrich. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

on the dorsal ocelli, narrow gena, and strongly convex frontoclypeus. Evans distinguished the tribe from other Cicadellinae based on the forewing "with a reduced clavus, a wide appendix and lacking vein M1+2 [= r-m1]." Young (1965) transferred the tribe from Cicadellinae to Typhlocybinae because he considered Mileewini to have "much more in common" with the latter subfamily, but did not mention particular characters that supported this new placement. In his subsequent comprehensive revision of world Cicadellinae, Young (1968) initially continued to treat Mileewini as a tribe of Typhlocybinae but later (Young 1986) followed Linnavuori and DeLong (1977) who elevated Mileewinae to status as a separate subfamily. This latter status was adopted by Dietrich (2005) in a key to cicadellid family-group taxa. Two other taxa most recently treated as separate subfamilies (Young 1986, Dietrich 2005), Makilingiinae Baker and Tinterominae Godoy & Webb, inhabit similar wet tropical montane forest habitats and resemble Mileewinae in having dorsal ocelli, reduced forewing venation, and also the hind wing submarginal vein very close to the wing margin distally (Dietrich 2005). Both are currently known only based on their type genera: Makilingia Baker, previously known only from the Philippines but recently recorded from Thailand (Dietrich, unpublished); and *Tinteromus* Godoy & Webb, previously known only from Costa Rica, but recently recorded from Colombia and Peru (D. M. Takiya, unpublished).

Phylogenetic analyses of both morphological and molecular data (Dietrich 1999, 2004, Dietrich et al. 2001, 2005, 2010) have consistently placed Mileewinae, Makilingiinae, and Tinterominae in a well-supported clade also comprising subfamilies Evacanthinae (sensu Dietrich 2004), Cicadellinae (sensu Young 1968), Signoretiinae (sensu Dietrich 2005), and Typhlocybinae but have not adequately resolved the relationships among major lineages within this clade. However, because the first three mentioned taxa share a unique combination of morphological traits (see below), they may represent a monophyletic group. Thus, they are here treated as tribes of a single subfamily, Mileewinae (sensu lato).

In a recent taxonomic review and morphology-based phylogenetic analysis of Evacanthinae sensu lato (including tribes Balbillini, Evacanthini, Nirvanini, and Pagaroniini), Dietrich (2004) excluded the enigmatic Andean genus *Tungurahuala* Kramer from that subfamily, placing it in Cicadellinae based on the structure of the ovipositor. Further morphological study of *Tungurahuala* and several South American species representing a new genus (described below), suggests that these two genera are most closely related to Mileewinae. The unique features uniting these two genera and their lack of apparent close relationship to other known leafhoppers support their placement in a new tribe, described below.

Methods

Morphological terminology follows Oman (1949) and Kramer (1950) except that groups of macrosetae on the legs are named using the system of Rakitov (1998) and terminology for nymphs follows Dmitriev (2002). Setal rows on the femur and tibia are referred to according to their position, assuming that the leg is fully extended perpendic-

ular to the sagittal plane of the body, as follows: AD=anterodorsal; AM=anteromedial; AV=anteroventral, PD=posterodorsal; and PV=posteroventral. Individual setae are numbered sequentially, beginning with the most distal. Numerical formulae are used to indicate the numbers of setae on the pro- and mesothoracic tibiae and the metathoracic femur. For tibiae the formula given is AD+PD; for the femur, the formula follows standard practice in which, for example, 2+2+1 means that a distal pair, a penultimate pair, and a single antepenultimate seta are present. Measurements are given in millimeters for males and females, respectively; body length is measured from the apex of the head to the apex of the forewing in repose. Digital photographs were taken using a Q-Imaging Micropublisher digital camera mounted on a stereo or compound microscope.

Specimens examined are deposited in the following institutions: Humboldt Institute, Villa Leyva, Colombia (HIC); Illinois Natural History Survey (INHS); North Carolina State University, Raleigh (NCSU); and Universidad de San Marcos, Lima, Peru (USML).

Results

Subfamily Mileewinae

htp://species-id.net/wiki/Mileewinae

Mileewinae Evans, 1947 Makilingiinae Evans, 1947, syn. n. Tinterominae Godoy & Webb, 1994, syn. n.

Redescription. Head with lorum extended little or no farther dorsad than clypeal suture; gena partially or entirely concealing triangular proepisternum; anteclypeus strongly convex, tapered distally; frontoclypeus without median longitudinal carina; ocelli on crown anteromesad of eyes, well separated from anterior margin; crown glabrous or punctate, without oblique lateral submarginal carinae; antennal base near anterodorsal corner of eye. Forewing (Figs 19, 21, 23, 25, 27, 29) with crossvein s (= "r" of Evans 1947) absent (outer anteapical cell absent); inner apical cell elongate, more or less parallel-sided, extended to apical margin. Hind wing (Figs 20, 22, 24, 26, 28, 30) with submarginal vein very close to or coincident with edge of wing along apical and costal margins; R2+3 complete. Front femur (Figs 33, 34) row AV without enlarged basal setae; hind femoral setal formula 2+1+1; hind tarsomere I pecten with 4 or fewer setae, including 0-2 platellae. Male subgenital plates (Figs 37-45) usually elongate, depressed basally, expanded medially or distally in lateral view (triangular in Makilingiini), macrosetae when present well separated from lateral margin and scattered or uniseriate; style apex (Fig. 47) usually cheliform, apophysis usually with preapical tooth; connective Y- or T-shaped, usually with median anterior lobe. Female second valvulae (Figs 71, 75) with distal paired blades comprising 50% or more of entire length of ovipositor.

Notes. Mileewinae, as here redefined, are most readily distinguished from other Cicadellidae by the following combination of features: head with ocelli on crown distant from eyes and margin, frontoclypeus without median longitudinal carina; forewing with only two anteapical cells; hind wing submarginal vein very close to margin at wing apex; female second valvulae with paired distal blades occupying 50% or more of their length. They key to couplet 46 in Dietrich's (2005) key to tribes of Cicadellidae, which comprises the three previously recognized taxa included here as tribes. Comparative study of morphological characters indicates that *Tungurahuala*, previously placed in Nirvaninae (Kramer 1965), and a related new genus, *Ilyapa*, are not closely related to other nirvanines and are more closely allied to Mileewinae (Dietrich 2004). Mileewinae previously included only the nominotypical tribe but is here redefined to include tribes Tinteromini and Makilingiini (both previously treated as separate subfamilies), as well as a new tribe, Tungurahualini, described below.

The hind wing venation is very similar among the tribes here included in Mileewinae, although the extant Old World genera currently included in Mileewini (Mileewa Distant, Ujna Distant, and Processina Yang, Deitz & Li) share a unique pattern in which vein R2+3 is complete but does not extend to the wing apex (Fig. 22); thus in these three genera there appear to be only three closed apical cells reaching the wing apex instead of the usual four (Fig. 26). Interestingly, this pattern does not occur in the New World mileewine genus Amahuaka Melichar, or in the two genera of Mileewini described from Eocene Baltic amber (Gebicki and Szwedo 2001), all of which have vein R2+3 extended to the wing apex (Fig. 26). Gebicki and Szwedo (2001) suggested that, based on the wing venation, Orsalebra robusta Young belongs in Mileewinae; however, species of Orsalebra have the ocelli on the anterior margin of the crown and are similar to Alebrini (Typhlocybinae) in other respects, so Young's (1952) original placement in Alebrini appears to be correct. As noted by Takiya (2007), Vidanoana Young, a genus endemic to Chile and currently placed in Cicadellini has hind wing venation similar to that of Mileewinae (Young 1977). The placement of this genus needs to be re-evaluated through further comparative study beyond the scope of the present paper.

Key to tribes of Mileewinae

Tungurahualini, trib. n.

3

urn:lsid:zoobank.org:act:E466750A-38A1-4798-A4E4-4E169D37F6E8 htp://species-id.net/wiki/Tungurahualini

Type genus. *Tungurahuala* Kramer

Description. Medium-sized leafhoppers (~6-8 mm), body depressed, head produced, face horizontal in profile, antenna shorter than width of head, frontoclypeus with transverse carina forming distinct shelf in lateral view (Figs 11-12, 31-32); anteclypeus extended to or slightly beyond lower margin of gena; lorum with lateral margin not extended to lateral margin of gena; rostrum short, not surpassing front trochanters. Front femur (Figs 33-34) with AM1 and AV1 enlarged, row AV with 0-1 preapical setae; intercalary row with 12 or more slender setae; hind tarsomere I without platellae, pecten with four tapered macrosetae. Forewing (Figs 27, 29) vein R with three branches; two r-m crossveins present; appendix absent or very narrow; hind wing vein R2+3 complete, extended to wing apex. Male pygofer (Fig. 37) with well developed ventral appendage, dorsal appendage absent, several macrosetae present distally; anal tube usually with paired ventrolateral processes; valve (Fig. 38) short, transverse, broadly fused to pygofer; subgenital plate broadest near base in ventral view, expanded medially in lateral view, with numerous scattered stout submarginal setae; connective stem as long as or longer than arms; style (Figs 47-51) cheliform with preapical lobe greatly enlarged and preapical tooth distinct. Female first valvulae with dorsal sculpturing strigate (Fig. 70) or concatenate (Fig. 73); second valvulae (Figs 71, 74) with small serrations between larger teeth.

Notes. Tungurahualini resemble other Mileewinae in having the ocelli on the crown distant from the margin, the frontoclypeus and clypellus strongly convex, the forewing with the inner apical cell elongate and parallel-sided, the hind femur with macrosetal formula 2+1+1, and the second valvulae with the toothed distal blades longer than the



Figures 1–18. Mileewinae, scale bars = 1 mm 1–10 Tungurahualini, dorsal habitus 1 *Tungurahuala* basilisca, male from Colombia 2 *T. acuminata*, male 3 same, female 4 *Ilyapa bifida*, male 5 *I. loca*, male 6 *I. longispina*, male 7 *I. ochrescens*, male 8 *I. recurvata*, male 9 *I. viridis*, male 10 same, 5th instar nymph 11–12 Tungurahualini, lateral habitus 11 *T. acuminata*, male 12 *I. viridis*, male 13–18 Other Mileewinae 13 *Makilingia* sp., male from Thailand, dorsal habitus 14 same, *Mileewa margheritae*, male 15 same, *Tinteromus* sp., male from Colombia (full length of antenna not shown) 16 *Makilingia* sp., lateral habitus 17 same, *Mileewa margheritae* 18 same, *Tinteromus* sp.



Figures 19–30. Mileewinae, wings 19–20 *Makilingia* sp. (Thailand), fore- and hind wing 21–22 same, *Mileewa margheritae* 23–24 same, *Tinteromus* sp. (Colombia) 25–26 same, *Amahuaka* sp. (Mexico) 27–28 *Tungurahuala acuminata* 29–30 same, *Ilyapa viridis*.

basal fused section. They differ from other Mileewinae in having the head depressed with the face horizontal, the frontoclypeus with a transverse carina (Figs 31–32) forming a distinct shelf in profile, the first hind tarsomere pecten with spiniform setae (platel-lae absent), and the subgenital plate with numerous scattered macrosetae (see also Key).

A previous cladistic analysis (Dietrich 2004) consistently grouped the two genera included in Tungurahualini together in a clade also comprising the single included repre-



Figures 31–45. Tungurahualini 31–32 head, anteroventral view 31 *Tungurahuala acuminata* 32 *Ilyapa viridis* 33–34 prothoracic femur, anterior view 33 *T. acuminata* 34 *I. viridis* 35–36 hind tarsomere I, ventral view 35 *T. acuminata* 36 *I. viridis* 37 *T. acuminata*, genital capsule, lateral view 38 same, valve and subgenital plates, ventral view 39 *Ilyapa bifida*, genital capsule, lateral view 40 same, *I. loca* 41 same, *I. longispina* 42 same, *I. ochrescens* 43 *I. ochrescens*, left subgenital plate, ventral view 44 *I. recurvata*, genital capsule, lateral view 45 same, *I. viridis*.

sentative of Makilingiini. A more recent morphology-based analysis of the entire family Cicadellidae (Dietrich et al. 2010) placed Tungurahualini within a paraphyletic assemblage, comprising the other tribes here included in Mileewinae, that gave rise to Cicadellinae and Typhlocybinae. More detailed analysis of this entire lineage will be needed to further elucidate the phylogenetic status of Mileewinae and its included tribes. Tungurahualini resemble Nirvanini (Evacanthinae, sensu Dietrich 2004) in having the body depressed with the head strongly produced and the face horizontal. The male genitalia also resemble those of Nirvanini, particularly the structure of the style (apex foot-shaped) and aedeagus (base with paired dorsal processes). However, unlike Nirvanini (and other Evacanthinae), the crown of the new tribe lacks a distinct marginal carina, the ocelli are distant from the margin, the face lacks a median longitudinal carina, crossvein $r-m_1$ is present in the forewing, and the front femur lacks enlarged basal setae in row AV. The presence of spiniform setae rather than platellae at the apex of the first hind tarsomere is an unusual trait shared with some Oriental Evacanthini, but evacanthines differ in having the crown distinctly elevated mesad of the eyes, with marginal and submarginal carinae, and ocelli near the crown margin.

Species of Tungurahualini are presently known only from cloud forests in the northern and central Andean regions of the New World tropics.

Key to genera of Tungurahualini

1	Head and pronotum uniformly black dorsally; crown margin pentagonal in
	dorsal view Tungurahuala Krame
_	Head and pronotum pale orange, white or green with distinct reddish orang
	markings; crown margin parabolic in dorsal view

Tungurahuala Kramer

htp://species-id.net/wiki/Tungurahuala

Tungurahuala Kramer 1965: 68. Type species *T. basiliscus* Kramer by original designation. New placement.

Redescription. Elongate, strongly depressed, leafhoppers (Figs 1–3, 11). Coloration dark brown to black; face with dull yellow band extended from lora across base of clypellus and apex of clypeus. Crown unevenly convex, coarsely granulose and densely clothed with minute setae, pentagonal in dorsal view; marginal carina present apically, becoming obsolete posterolaterally; median longitudinal carina weakly delimited; ocelli on crown anterad of eyes, slightly closer to lateral margin than to midline; antennal ledge broad, depressed, coincident with lateral margin of crown; flagellum slightly shorter than crown width; mesal margin of eye entire; lateral frontal suture absent dorsad of antennal ledge; frontoclypeus (Fig. 31) rugulose medially with well developed muscle scars laterally, oblique anteroventral section separated from nearly horizontal posteroventral section by transverse ridge; transclypeal suture indistinct; anteclypeus tapered, weakly convex, apex wider than lorum; lorum well separated from ventral genal margin; gena angulately produced, largely concealing proepisternum. Pronotum depressed, rugulose and minutely setose, narrower than head, lateral margins strongly



Figures 46–52. *Tungurahuala*, male genitalia **46** *T. basilisca* (specimen from PNN Chingaza Alto de la Bandera, Cundinamarca, Colombia), genitalia, lateral view **47** same, ventral view (only right style shown) **48** *T. basilisca* (specimen from PNN Chingaza Churrascales, Cundinamarca, Colombia), genitalia, lateral view **49** same, ventral view **50** *T. acuminata*, genitalia, lateral view **51** same, ventral view **52** same, aedeagus, posteroventral view.

carinate, carina even with eye, margins subparallel in dorsal view; exposed part of mesonotum and scutellum together wider than long. Forewing (Fig. 27) opaque basally, gradually becoming translucent distally; veins raised and well delimited, with marginal setae; costal flange well developed basally; R three-branched (rarely with 1–2 supranumerary branches), branches not reflexed; crossvein s absent (outer anteapical cell open distally); two r-m and three m-cu crossveins present; inner apical cell narrow; appendix absent. Hind wing (Fig. 28) with cell distad of r-m crossvein broadened distally. Prothoracic femur (Fig. 33) stout, AM1 and AV1 well developed, intercalary row with ~13 close-set preapical setae; tibia short, weakly expanded distally, dorsal rows with few indistinct, widely spaced setae, AV well developed, PV with few scattered setae. Mesothoracic femur longer and wider than prothoracic femur, compressed, AV and PV each with several irregular setae, tibial rows with numerous poorly differentiated setae. Metathoracic tibia row AV with setae evenly spaced from base to apex; tarsomere I with several scattered plantar setae. Male sternum III apodemes well developed; pygofer (Fig. 37) short with scattered macrosetae dorsolaterally, with long, slender process densely clothed with minute spicules arising from ventrolateral margin and extending mesad into genital capsule, then dorsad; anal tube well sclerotized, broader than long in dorsal view, venter flat; valve (Fig. 38) short, straplike, narrowly fused to pygofer; plates triangular, depressed, extended well beyond posterior margin of pygofer, with lateral band and irregular submedial row of macrosetae, dorsolateral margin weakly sinuate in lateral view, base weakly constricted in ventral view; aedeagus (Figs 46–52) in lateral view with shaft split into dorsal gonopore-bearing section and tapered ventral process; connective (Figs 47–51) trilobed basally; style with large preapical lobe and attenuated, hooked apex. Female sternite VII (Fig. 65) subtruncate, concealing base of ovipositor; first valvulae (Fig. 69) slender, with dorsal and ventral preapical sculpturing irregularly strigate; second valvulae (Fig. 71) with basal fused area short, distal blades large, dorsal margin ascending in straight line, then gradually descending toward apex, declivous portion with ~7 widely spaced conical teeth and intervening serrations, apical fourth serrate, without teeth; third valvulae without macrosetae. Nymph unknown.

Notes. Kramer (1965) described *Tungurahuala* based on a single male specimen of *T. basiliscus* Kramer from Baños, Tungurahua, Ecuador, placing it in Nirvaninae but noting that it is "vastly different from any previously described" genus. Recent Malaise trap sampling in Colombia has yielded additional specimens of the type species and a new, closely related species, including the first known female specimen of the genus. The genus may be distinguished from its only known relative, *Ilyapa*, by the characters noted in the key.

Key to species of *Tungurahuala* (males)

1	Anal tube without distinct apical ventrolateral spines; aedeagal shaft (Figs 46, 48) broad in lateral view, ventral process with dorsolateral lobes
	<i>basilisca</i> Kramer
_	Anal tube with pair of distinct apical ventrolateral spines (Fig. 37); aedeagal shaft (Fig. 50) narrow in lateral view, ventral process without dorsolateral
	lobesacuminata, sp. n.

Tungurahuala basilisca Kramer

htp://species-id.net/wiki/Tungurahuala_basilisca Figs 1, 46–49

Tungurahuala basiliscus Kramer 1965: 68 [incorrect original spelling]

Redescription. Length male 7.6–7.9. Head (male) approximately ¼ length of forewing. Forewing dark brown to black. Male anal tube with apical ventrolateral spines very weak-ly developed or absent. Aedeagus with gonopore-bearing shaft in lateral view broad, apex obliquely truncate, posteroapical margin concave; ventral process with pair of micro-

trichiate dorsolateral lobes, in ventral view abruptly expanded preapically, apex broadly bilobed, in lateral view with apex convergent toward shaft and with dorsal margin entire.

Material examined. Holotype male: ECUADOR: Mt. Tungurahua, Baños, 2500m, August 20, 1937 (W. Clarke-Macintyre) [USNM]. Other material: 1 male, COLOMBIA, Cundinamarca, PNN Chingaza Charrascales, 4°31'N, 73°45'W, 2990m, Malaise, 4–18 October 2001 (L. Cifuentes), M.2551 [HIC]; 1 male, Cundinamarca, PNN Chingaza Alto de la Bandera, 4°31'N, 73°45'W, 3660m, Malaise, 30 March -12 April 2001 (L. Cifuentes), M.1585 [HIC]; 1 male, same data except 27 December 2001–11 January 2002 (E. Raigoso), M.3023 [INHS].

Notes. The specimens examined from Colombia are here considered conspecific with the holotype from Ecuador, although there is slight variation among specimens in size, coloration, and the shape of the aedeagus. Given the small number of specimens available, it seems prudent to consider these minor variations to be intra-specific, despite the considerable geographic disjunction among the known populations.

Although Kramer (1965) did not explicitly indicate the gender of the name *Tungu-rahuala*, it is here interpreted as feminine due to its ending (ICZN Art. 30.2.4). Thus, Kramer's original spelling of the species name, which has a masculine ending, is incorrect and the spelling here emended to agree in gender with the genus name.

Tungurahuala acuminata sp. n.

urn:lsid:zoobank.org:act:FD180D56-E0FC-4CCC-9C28-4F4C8FB090FE htp://species-id.net/wiki/Tungurahuala_acuminata Figs 2, 3, 11, 27, 28, 31, 33, 35, 37, 38, 50–52, 65, 69–71

Description. Length male 7.5–7.6 mm, female 7.6 mm. Head (male) approximately 1/3 as long as forewing (female with head proportionately longer compared to forewing). Forewing dark brown with tan pigment along costal area, mostly tan in female. Male anal tube with apical ventrolateral spine well developed. Aedeagus with gonopore-bearing shaft slender, apex rounded; ventral process acuminate, without dorsolateral lobes, apex in ventral view narrower than shaft.

Material examined. Holotype male: COLOMBIA, Boyacá, SFF Iguaque Lagunillas, 5° 25'N, 73° 27'W, 3380m, Malaise 2–18 May 2001 (P. Reina), M.1756. Paratypes: 1 male, same data except 28 June–19 July 2001, M.1966 [HIC]; 1 male, Boyacá, SFF Iguaque Qda. Carrizal, 5° 25'N, 73° 27'W, 3350m, Malaise 13 – 30 July 2000 (P. Reina), M.379; 1 male, same data except 21 January–9 February 2001; 1 male and 1 female, Boyacá, SFF Iguaque Cabaña Mamarramos, 5° 25'N, 73° 27'W, 2855m, Malaise 7–21 January 2001 (P. Reina), M.1252; 1 male, same data except 21 December 2000–7 January 2001, M.1072 [HIC, INHS].

Etymology. The name refers to the acuminate ventral process of the aedeagus.

Notes. This species resembles *T. basilisca* in overall structure, but the head is proportionately longer and the coloration of the forewing is lighter overall, although variable among specimens examined.

Ilyapa gen. n.

urn:lsid:zoobank.org:act:7A6B2217-9F1E-4D41-B680-BFD78F3FE553 htp://species-id.net/wiki/Ilyapa

Type species. I. longispina, sp. n.

Description. Medium sized, depressed leafhoppers (Figs 4–10, 12). Coloration pale orange or green with red/orange markings dorsally; crown with pair of oblique red maculae mesad of ocelli, pronotum with semicircular orange macula anteriorly, face and thoracic sternites black, legs dark basally and pale distally, abdomen heavily marked with dark brown dorsally and with varying amounts of dark brown pigmentation ventrally. Crown depressed, finely granulose, without setae, weakly pentagonal in dorsal view, marginal and medial carinae absent; ocelli on crown anterad of eyes slightly closer to lateral margin than to midline; antennal ledge broadly depressed, coincident with crown margin; flagellum slightly shorter than crown width; mesal margin of eye emarginate; frontoclypeus granulose with oblique anterodorsal section separated from nearly horizontal posteroventral section by distinct transverse ridge; muscle scars distinct laterally; clypeal suture obsolete medially; anteclypeus convex, tapered, apex narrower than lorum; lorum well separated from ventral genal margin; gena weakly produced laterally, partially concealing proepisternum. Pronotum weakly convex with irregular transverse striations, lateral margins divergent posterad, slightly wider than head, strongly carinate, carina even with eye. Exposed part of mesonotum and scutellum wider than long. Forewing (Fig. 29) opaquely sclerotized except apical cells, veins distinct but without marginal setae; R three branched, crossvein s absent; two r-m and 3-4 m-cu crossveins present; apical cells 2 and 3 very short; inner apical cell relatively broad; appendix very narrow. Hind wing (Fig. 30) with cell distad of r-m crossvein parallel-sided or narrowed distally. Prothoracic femur (Fig. 34) slender, AM1 large, located on ventral margin, AV1 well developed, row AV without preapical setae; intercalary row with ~17 slender, close-set setae; tibia 1+1, PV absent. Mesothoracic femur equal in length but wider than prothoracic femur; AV and PV with few widely separated setae, tibial row PD with apical seta, other rows with few irregularly spaced setae. Metathoracic femur macrosetal formula 2+1+1, rarely 2+1+1+1, tibia and tarsus as in *Tungurahuala*. Male with apodemes of sternite III well developed; pygofer (Figs 39-45) short with scattered macrosetae dorsolaterally, with ventral process glabrous, slender, arising posteroventrally and curved posterodorsad; anal tube in dorsal view as long as broad, flat ventrally, with or without pair of ventrolateral processes distally; valve short, rectangular, narrowly fused to pygofer; plates depressed basally, expanded and slightly compressed distally with band of macrosetae extended from lateral margin of base posteriorly across middle of apex; aedeagal shaft (Figs 53–62) arcuate posteriorly, often asymmetrical, gonopore apical; connective trilobed anteriorly, stem broad and depressed; style sinuate with large, sparsely setose preapical lobe, apophysis acuminate with ventral preapical tooth. Female seventh sternite (Figs 65-68) longer than sixth and concealing basal half of ovipositor in repose, posterior margin produced; first valvulae (Fig. 72-73) slender, dorsal sculpturing concatenate, second valvulae (Fig. 74) similar to those of *Tungurahuala* but with dorsal teeth more numerous, prominent and closely spaced, and dorsum at base of blade evenly rounded rather than angulate.

Fifth instar nymph (Fig. 10) with overall form and chaetotaxy similar to that of adult except coloration pale greenish yellow with median dorsal longitudinal white stripe; crown with acrometope well delimited, longer than wide; metope well delimited; ocellar precursors well delimited and positioned as in adult on coryphe anteromesad of eyes, well separated from margin; face with distinct transverse shelf corresponding to epistomal suture (as in adult), cibarial muscle scars distinct; dorsum glabrous with scattered sparse, minute setae; enlarged setae absent; hind tarsomere I with three apical platellae.

Etymology. The name *Ilyapa* is based on that of the Inca god of thunder, lightning, and rain, but the gender is here considered feminine due to its ending.

Notes. This genus is closely related to *Tungurahuala*, as indicated by the similarities in cephalic structure (ocelli distant from margin, frontoclypeus with transverse carina), forewing venation (crossvein s lacking), leg chaetotaxy (hind tarsomere I pecten with tapered macrosetae), and male genitalia (pygofer with recurved posteroventral process, style with strong preapical lobe). It differs from *Tungurahuala* in the characters noted in the key.

The genus is described based on six species from the Andean region of South America. The species inhabit cloud forests and have been collected by sweeping grasses and other herbaceous vegetation in the understory. They are readily distinguished by differences in coloration, head proportions, and the structure of the male genitalia.

Key to species of Ilyapa (males)

1	Aedeagal shaft (Figs 59-60) bilaterally symmetrical, compressed and broad in
	lateral viewochrescens, sp. n.
_	Aedeagal shaft strongly asymmetrical, elongate, slender and tubular
2	Aedeagus (Figs 55–56) with depressed, apically truncate ventral process aris-
	ing near base; gonopore-bearing shaft elongate, spinelike, evenly tapered dis-
	tally, without processloca, sp. n.
_	Aedeagus without basal process, shaft with one or more distal processes 3
3	Aedeagus (Figs 58, 64) with distal process extended laterad at approximately
	right angle in ventral view
_	Aedeagus with distal process not extended laterad at right angle, either
	strongly recurved ventrad or more or less continuing in line with shaft5
4	Distal process of aedeagus bifid (Fig. 64)viridis, sp. n.
_	Distal process of aedeagus unbranched (Fig. 58) longispina, sp. n.
5	Aedeagus (Fig. 61) with single distal process strongly recurved ventrad, apex
	branched; anal tube with several small, irregular ventrolateral teeth (Fig. 44)
_	Aedeagus (Figs 53-54) with two distal processes more or less aligned with
	shaft, asymmetrically curved, one process with angulate projection near base;
	anal tube with pair of short triangular projections near base (Fig. 39)
	bifida, sp. n.

Ilyapa bifida sp. n. urn:lsid:zoobank.org:act:26BD4DCA-8225-43E5-AD09-1D484CC59C40 htp://species-id.net/wiki/Ilyapa_bifida Figs 4, 12, 39, 53, 54

Description. Length male 6.6–6.9 mm, female 7.0 mm. Crown pale orange-yellow, orange-red maculae broad, overlapping ocelli, anterior margin forming acute angle; pronotum and opaque areas of forewing bright green (mottled with yellow in specimens removed from ethanol), pronotum with semicircular macula distinct. Male pygofer processes extended mesad and curved dorsad but not or only slightly crossing midline; anal tube process short, triangular. Aedeagus asymmetrical, shaft tubular, in lateral view V-shaped basally, arched and sinuate distally, terminating in two slender, bladelike processes continuing in line with shaft but asymmetrically curved, one process with angulate projection near base. Female seventh sternite with posterior margin slightly produced, rounded medially. Fifth instar nymph pale olive green dorsally with broad white median longitidinal stripe extended entire length of body; venter white.

Material examined. Holotype male: PERU, Pasco, Yanachaga-Chemillén N.P., Refugio El Cedro, 2420 m, 10°33'07"S 75°21'27"W, 10 October 2002 (D. M. Takiya) PE07 [USML]. Paratypes: 1 male, same data except 12 October 2002 (R. Rakitov) [INHS].

Etymology. The species name refers to the bifid apex of the aedeagus.

Notes. This species may be distinguished by its relatively long crown and the bifid apex of the aedeagus.

Ilyapa loca sp. n.

urn:lsid:zoobank.org:act:8E950B3B-7F16-47E6-96F5-C5729EA156C0 htp://species-id.net/wiki/Ilyapa_loca Figs 5, 40, 55–56

Description. Length male 6.1 mm. Coloration as described for *I. bifida* except crown margin white, orange-red maculae broader, and pronotum almost entirely orange; apical margin of crown forming obtuse angle. Male pygofer processes short, broad, and digitiform; anal tube without processes. Aedeagus highly asymmetrical; gonopore-bearing shaft acuminate, extended to left posterodorsad and gradually curved mesad, apex without process; ventral process depressed, apex expanded, truncate, and even with style apices. Female unknown.

Material examined. Holotype male, PERU, Chanchamayo, 25 July 1960 (Young and Ramirez) [NCSU].

Etymology. The species name means "crazy" and refers to the bizarre, asymmetrical aedeagus.

Notes. This species may be distinguished by its relatively short crown and by the presence of a long, unpaired ventral process arising from the base of the aedeagal shaft.

Ilyapa longispina sp. n.

urn:lsid:zoobank.org:act:00015CA5-8E24-4357-A657-7B8D3B5F6638 htp://species-id.net/wiki/Ilyapa_longispina Figs 6, 41, 57–58, 67, 72–74

Description. Length male 5.9–6.2 mm; female 7.1–7.5 mm. Coloration as described for *I. bifida* except crown margin mostly white; apical margin of crown forming approximately right angle. Male pygofer processes slender, crossing posteromedially; anal tube with pair of retrorse posterolateral spines; plate apex angulate mesally. Anal tube processes long with apices curved ventrad; pygofer processes shorter, not meeting medially; apical process of aedeagus more elongate, with minute preapical spine posteriorly. Female seventh sternite with posterior margin trilobed with median lobe acute and larger than lateral lobes.

Material examined. Holotype male: PERU, Chanchamayo, 25 July 1960 (Salazar and Ramirez) [NCSU]. Paratypes: 1 male, same locality, 22 July 1960 (C. Ramirez) [INHS]; 3 females, same locality except 21 and 25 July 1960 [NCSU, INHS]. Other material: 3 males, PERU, Ucayali, 8 km E Abra La Divisoria, 1250 m, 9°9'57"S 75°48'11"W, 25 October 2002 (R. A. Rakitov) sweeping, 02-39-2; 1 male, 1 female, PERU, Pasco, Yanachaga-Chemillén N.P., Puesto de Control Huampal, 1050 m, 10°1'09"S 75°34'27"W, 8 October 2002 (R. A. Rakitov) on grass; 1 male, same data except Refugio El Cedro, 2420 m, 10°33'07"S 75°21'27"W, 10 October 2002, D.M. Takiya, PE07; 1 female PERU, Junin, 1 km S Minapichita, 2100 m 11°6'1"S 75°25'30"W, 19 October 2002 (C. H. Dietrich) sweeping, 02-20-1 [INHS].

Etymology. The species name refers to the long distal spine of the aedeagus.

Notes. This species may be distinguished by its moderately long crown and by the elongate, laterally directed distal spine of the aedeagus.

One specimen from Yanachaga-Chemillén National Park, Peru, has the distal spine of the aedeagus extended to the right, mirroring the condition found in other examined specimens of this species. Specimens examined from Yanachaga-Chemillén National Park have the anal tube processes considerably shorter than those in the type series from Chanchamayo, but such variation is here considered to be intraspecific. Based on the material available for study, this is the most widespread and common species of the genus.

Ilyapa ochrescens sp. n.

urn:lsid:zoobank.org:act:1D932E94-61AA-400E-B8D3-40F45CEF0D3D htp://species-id.net/wiki/Ilyapa_ochrescens Figs 7, 42, 59–60

Description. Length male 6.5–6.8 mm. Nearly uniform pale orange dorsally, orangered maculae slender; apical margin of crown forming acute angle, pronotal macula indistinct. Male pygofer processes robust, not crossing posteromedially; anal tube without ventrolateral processes; aedeagal shaft symmetrical, pillarlike, compressed, in lateral view extended dorsad and bent posterad at right angle, with pair of acute anterodorsal processes extended anterad, posterodorsal extension with ventral margin irregular, apex expanded with ventral spine. Female unknown.

Material examined. Holotype male: COLOMBIA, Cundinamarca, PNN Chingaza Valle Del Fraylejon, 4°31'N, 73°45'W, 3170m, Malaise 31 August–13 September 2000 (A. Pérez), M.732. Paratypes: 1 male, same coordinates, Chingaza Bosque Palacio, 2930m, Malaise 8–22 December 2000 (A. Cifuentes), M.1027; 1 male, same data except 3–16 March 2001(C. Vinchira and A. Cifuentes), M.1492 [HIC].

Etymology. The species name refers to the mostly orange coloration of the dorsum.

Notes. This species may be distinguished by its relatively long crown, predominantly orange coloration, and broad, strongly compressed aedeagal shaft.

Ilyapa recurvata sp. n.

urn:lsid:zoobank.org:act:987F8A00-CC54-494D-AE56-7FDAD4F92AF3 htp://species-id.net/wiki/Ilyapa_recurvata Figs 8, 44, 61–62

Description. Length male 5.9 mm. External morphology and male terminalia similar to those of *I. longispina*, except as follows: anal tube without pair of ventrolateral processes but with several irregular teeth; aedeagus with distal process strongly recurved ventrad and anterad, branched near midlength with one branch about half length of other. Female unknown.

Material examined. Holotype male: PERU, Huánuco, Carpish Pass, 2600 m, 9°43'3"S 76°5'38"W, 27 October 2002 (C. H. Dietrich) sweeping, 02-45-1 [USML].

Etymology. The species name refers to the strongly recurved apex of the aedeagus.

Notes. This species may be distinguished by its relatively short crown and strongly recurved aedeagal apex.

Ilyapa viridis sp. n.

urn:lsid:zoobank.org:act:D530E660-0B0C-4159-9EDF-4FD394B048C4 htp://species-id.net/wiki/Ilyapa_viridis Figs 9, 29–30, 32, 34, 45, 63–64, 68

Description. Length male 5.8–6.0, female 7.0 mm. Crown pale yellow medially, white laterally, orange/red maculae broad, overlapping ocelli; anterior margin forming approximately right angle; pronotum and opaque areas of forewing dark green (mottled with yellow in specimens removed from ethanol), pronotum with semicircular macula distinct. Male pygofer processes slender, crossing posteromedially; anal tube with pair of retrorse posterolateral spines. Aedeagus asymmetrical, shaft narrow, tubular and gradually tapered distally, in lateral view narrowly U-shaped with distal part



Figures 53–64. Ilyapa, male genitalia, lateral and ventral views 53–54 I. bifida 55–56 I. loca 57–58 I. longispina 59–60 I. ochrescens 61–62 I. recurvata 63–64 I. viridis.



Figures 65–74. Tungurahualini, female terminalia 65–68 sternite VII 65 *Tungurahuala acuminata* 66 *Ilyapa bifida* 67 *I. longispina* 68 *I. viridis* 69–71 *T. acuminata* 69 first valvula 70 same, detail of dorsal sculptured area 71 second valvulae 72–74 *I. longispina* 72 first valvula 73 same, detail of dorsal sculptured area 74 second valvulae

attenuated and arcuate; apex with slender bifurcate process extended to left at right angle to shaft. Female seventh sternite posterior margin with small acute median tooth.

Material examined. Holotype male: PERU, Pasco, Yanachaga-Chemillén N.P., 10°32'39.7"S, 75°22'00.1"W, 2300m, 10–13 October 2002 (D. Takiya, C. Peña, R. Rakitov) Malaise trap across Rio San Alberto [USML]. Paratypes: 1 male and 1 female,

same data; 2 males, Yanachaga-Chemillén N.P., 10°32'S, 75°21'W, S. Alberto Valley ca. Refugio El Cedro, 2270–2420m, 12 October 2002 (R. Rakitov); 2 males, 1 female, same data except 10 October 2002 (D. M. Takiya); 8 females, same data except 12 October 2002 (D. M. Takiya) [INHS].

Etymology. The species name refers to the mostly pale green coloration of the dorsum.

Notes. This species closely resembles *I. longispina* in external morphology and in the male genitalia, but may be distinguished by the shorter, distinctly branched distal aedea-gal process and by the distinctly smaller median lobe of female abdominal sternite VII.

In one examined male specimen, the configuration of the aedeagus is the mirror image of that of the other specimens.

Acknowledgments

I am indebted to Paul Freytag and Michael Sharkey (University of Kentucky) for access to their Malaise trap samples from Colombia, to Robert Blinn (NCSU) for the loan of additional specimens, to Carlos Peña and Pedro Lozada for help with field work in Peru, to Daniela Takiya and Dmitry Dmitriev for helpful discussions, and to Mick Webb and Chandra Viraktamath for constructive criticism of the manuscript. This work was supported in part by grants from the U.S. National Science Foundation.

References

- Dietrich CH (1999) The role of grasslands in the diversification of leafhoppers (Homoptera: Cicadellidae): a phylogenetic perspective. In: Warwick C (Ed) Proceedings of the Fifteenth North American Prairie Conference. Natural Areas Association, Bend, Oregon, 44–48.
- Dietrich CH (2004) Phylogeny of the leafhopper subfamily Evacanthinae with a review of Neotropical species and notes on related groups (Hemiptera: Membracoidea: Cicadellidae). Systematic Entomology 29: 455–487. doi:10.1111/j.0307-6970.2004.00250.x
- Dietrich CH (2005) Keys to families of Cicadomorpha and subfamilies and tribes of Cicadellidae (Hemiptera: Auchenorrhyncha). Florida Entomologist 88: 502–517. doi:10.1653/0015-4040(2005)88[502:KTTFOC]2.0.CO;2
- Dietrich CH, Rakitov RA, Holmes JL, Black IV WC (2001) Phylogeny of the major lineages of Membracoidea (Insecta: Hemiptera: Cicadomorpha) based on 28S rDNA sequences. Molecular Phylogenetics and Evolution 18: 293–305. doi:10.1006/mpev.2000.0873
- Dietrich CH, Dmitriev DA, Rakitov RA, Takiya DM, Zahniser JN (2005) Phylogeny of Cicadellidae (Cicadomorpha: Membracoidea) based on combined morphological and 28S rDNA sequence data. In: Purcell A (Ed) Abstracts of Talks and Posters: 12th International Auchenorrhyncha Congress, Berkeley, CA, 7–12 August, 2005, S13–14.
- Dietrich CH, Dmitriev DA, Rakitov RA, Takiya DM, Webb MD, Zahniser JN (2010) Phylogeny of Cicadellidae (Hemiptera: Cicadomorpha: Membracoidea) based on morphological

characters. In: Soulier-Perkins A, Ouvrard D, Bourgoin T (Eds) 13th International Auchenorrhyncha Congress Abstracts, Museum National d'Histoire Naturelle, Paris, 48–49.

- Dmitriev DA (2002) General morphology of leafhopper nymphs of the subfamily Deltocephalinae (Hemiptera: Cicadellidae). Acta Entomologica Slovenica 10: 65–82.
- Evans JW (1947) A natural classification of leaf-hoppers (Jassoidea, Homoptera) Part 3. Jassidae. Transactions of the Royal Entomological Society of London 98: 105–271. doi:10.1111/j.1365-2311.1947.tb01054.x
- Gebicki C, Szwedo J (2001) The first record of fossil Mileewinae from Eocene Baltic amber (Hemiptera: Membracoidea: Cicadellidae). Annales Zoologici 511: 417–422.
- Godoy C, Webb MD (1994) Recognition of a new subfamily of Cicadellidae from Costa Rica based on a phenetic analysis with similar taxa (Hemiptera Homoptera Auchenorrhyncha). Tropical Zoology 7: 131–144.
- Kramer JP (1965) Studies of Neotropical leafhoppers. I. (Homoptera: Cicadellidae). Proceedings of the Entomological Society of Washington 67: 65–74.
- Kramer S (1950) The morphology and phylogeny of Auchenorrhynchous Homoptera (Insecta). Illinois Biological Monographs 20: 1–111.
- Linnavuori R, DeLong DM (1977) Studies of the Neotropical Mileewaninae (Homoptera: Cicadellidae). Journal of the Kansas Entomological Society 50: 410–421.
- Oman PW (1949) The Nearctic leafhoppers–a generic classification and check list. Memoirs of the Entomological Society of Washington 3: 1–253.
- Oman PW, Knight WJ, Nielson MW (1990) Leafhoppers (Cicadellidae): a Bibliography, Generic Check-list, and Index to the World Literature 1956–1985. CAB International Institute of Entomology, Wallingford, 368 pp.
- Rakitov RA (1998) On differentiation of cicadellid leg chaetotaxy (Homoptera: Auchenorrhyncha: Membracoidea). Russian Entomological Journal 6: 7–27.
- Takiya DM (2007) Systematic studies on the leafhopper subfamily Cicadellinae (Hemiptera: Cicadellidae). PhD thesis, University of Illinois at Urbana-Champaign, Urbana, Illinois.
- Young DA (1965) Western Hemisphere Mileewanini (Homoptera, Cicadellidae). Zoologische Beiträge 11: 369–380.
- Young DA (1968) Taxonomic study of the Cicadellinae (Homoptera: Cicadellidae). Part 1. Proconiini. Bulletin of the United States National Museum 261: 1–287.
- Young DA (1977) Taxonomic study of the Cicadellinae (Homoptera: Cicadellidae). Part 2. New World Cicadellini and the genus *Cicadella*. North Carolina Agricultural Experiment Station Technical Bulletin 239: 1–1135.
- Young DA (1986) Taxonomic study of the Cicadellinae (Homoptera: Cicadellidae), Part 3. Old World Cicadellini. North Carolina Agricultural Research Service Technical Bulletin 281: 1–639.

RESEARCH ARTICLE



Notes on the genus Apteroloma of China with description of a new species (Coleoptera, Agyrtidae)

Liang Tang^{1,†}, Li-Zhen Li^{1,‡}, Jan Růžička^{2,§}

I Department of Biology, Shanghai Normal University, 100 Guilin Road, 1st Educational Building 323 Room, Shanghai, 200234 P. R. China 2 Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences in Prague, Kamýcká 129, CZ-165 21 Praha 6, Czech Republic

turn:lsid:zoobank.org:author:F45FE527-E59A-4702-A87E-B45BC33ED4C7
turn:lsid:zoobank.org:author:BBACC7AE-9B70-4536-ABBE-54183D2ABD45
urn:lsid:zoobank.org:author:EC96049B-28B7-4242-B0F7-DBFFE32A2450

Corresponding author: Liang Tang (monkey_zzz1980@163.com)

Academic editor: Jan Klimaszewski	Received 3 July 2011	Accepted 1 August 2011	Published 18 August 2011
-----------------------------------	----------------------	------------------------	--------------------------

urn:lsid:zoobank.org:pub:123EBD18-7505-4781-B3B5-AD9F4A192BF8

Citation: Tang L, Li L-Z, Růžička J (2011) Notes on the genus *Apteroloma* of China with description of a new species (Coleoptera, Agyrtidae). ZooKeys 124: 41–49. doi: 10.3897/zookeys.124.1774

Abstract

A new species, *Apteroloma zhejiangense* **sp. n.**, is described from Zhejiang Province, China. The habitus and sexual characters of the new species are illustrated. *Apteroloma jinfo* Růžička, is reported for the first time from China: Hubei and Hunan Provinces, and *A. potanini* (Semenov, 1893) from Ningxia and Henan Provinces. Presence of *A. kozlovi* Semenov-Tian-Shanskij & Znojko in Semenov-Tian-Shanskij, 1932 in South Korea is confirmed based on re-examined material.

Keywords

Agyrtidae, Apteroloma, China, Coleoptera, new species, Palaearctic region, taxonomy

Introduction

Apteroloma Hatch, 1927 is a large genus of Agyrtidae with 29 species in the world and 8 species in China. The Chinese species of the genus were recently revised by Růžička et al. (2004) and Růžička and Pütz (2009).

Copyright Liang Tang et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

In this paper, a new species from Zhejiang Province is described which considerably expands the distribution of the genus to East China. New province records of several *Apteroloma* species from China are provided and *A. kozlovi* from South Korea is confirmed based on re-examined material.

Material and methods

For examination of genitalia, the last two male abdominal segments and last female abdominal segment were detached from the body after softening in hot water. The aedeagus together with other dissected pieces were mounted in Euparal (Chroma Geselschaft Schmidt, Koengen, Germany) on plastic slides. Photos of sexual characters were taken with Canon G7 attached to Olympus SZX 16 stereomicroscope; habitus photos were taken with a Canon macro photo lens MP-E 65mm attached to Canon EOS40D camera.

The measurements are abbreviated as follows:

- **EL** length of elytra (measured from caudal tip of scutellum to elytral apex)
- **EW** combined maximum width of elytra
- **PBW** pronotal basal width
- PML pronotal medial length
- PMW pronotal maximum width
- **TBL** total body length.

The specimens treated in this study are deposited in the following public and private collections:

- JRUC Jan Růžička collection, Praha, Czech Republic
- MPEC Michel Perreau collection, Paris, France
- NMPC National Museum, Praha, Czech Republic (Jiří Hájek)
- NSMT National Science Museum, Tokyo (Shûhei Nomura)
- SHNU Department of Biology, Shanghai Normal University, P. R. China (Liang Tang)

Taxonomy

Apteroloma zhejiangense Tang, Li & Růžička, sp. n.

urn:lsid:zoobank.org:act:115949B4-715B-4D54-8634-A515CED52E1D http://species-id.net/wiki/Apteroloma_zhejiangense Figs 1, 2, 5–8

Type locality. China, Zhejiang Province, Anji County, Longwangshan N. R. [ca. 30°22'N, 119°30'E].

Type material. China: Zhejiang Province: Holotype. Male, glued on a card, with genitalia mounted on a plastic slide in Euparal, with labels as follows: "Mt. Longwang-



Figures 1–4. Adult of *Apteroloma* in dorsal and ventral view. **1**, **2** *A. zhejiangense* sp. n. (paratype female) **3**, **4** *A. jinfo* (male from Hubei). Scales = 1 mm.

shan N. R. / 950–1200m / Zhejiang Prov. / 25-IV-2004 / Liang Tang leg." "Holotype / *Apteroloma zhejiangense* / Tang, Li & Růžička det. 2011" [red handwritten label] (SHNU) and "Paratype / *Apteroloma zhejiangense* / Tang, Li & Růžička det. 2011" [yellow printed label] for the rest of the type series. **Paratypes.** 1 male and 1 female, Longwanshan, 3.X.2003, Hu & Tang leg. (SHNU); 1 male, Longwanshan, 950–1200m,



Figures 5–12. Sexual structures of *Apteroloma*. 5–8 *A. zhejiangense* sp. n. 9–12 *A. jinfo* (Hubei); 5, 9 aedeagus laterally 6, 10 aedeagus ventrally 7, 11 apex of aedeagus ventrally 8, 12 female ventrite VIII ventrally. Scales = 0.5 mm.

25.IV.2004, Liang Tang leg. (SHNU); 1 male, Longwanshan, 300–500m, 26.IV.2004, Li & Hu leg. (SHNU); 4 males and 1 female, Longwangshan, 950–1200m, 25.IV.2004, Liang Tang, Jia-Yao Hu & Li-Long Zhu leg. (SHNU); 6 males and 7 females, Long-

wangshan N. R., alt. 950–1200m, 25.IV.2006, Liang Tang, Rui-Fen Lin, Xin Yuan, Jin-Wen Li & Shan-Jia Shen leg. (1 pair in JRUC, rest in SHNU).

Description. Measurements of the male holotype: TBL=7.3 mm, PMW/ PML=1.49, PMW/PBW 1.19, EL/EW 1.33, EW/PMW 1.53.

Body large, 6.7–7.7 mm in length; dorsum in mature specimens dark brown; antennae, mouthparts, lateral portion of pronotum and legs uniformly ferruginous; dorsal surface shiny, with fine transverse microsculpture (pronotum discally with nearly isodiametric microsculpture); pronotum and elytra with scattered short erect setation; each mandible with two large acute teeth on inner edge before apex.

Pronotum widest in middle; anterior margin weakly emarginate; lateral margins distinctly bordered; weakly explanate; sides flat, only moderately raised and weakly sinuate posteriorly (Fig. 1); base wide, without impressions. Disc with scattered fine punctures, lateral and posterior areas heavily, densely punctured.

Elytra broadly oval. Each elytron with nine regular striae, third stria with ca. 49– 59 medium-sized punctures; lateral margin smooth, without serration; epipleural keel narrow. Elytral epipleura with strong and dense punctures (Fig. 2). Metathoracic wings fully developed.

Male. Aedeagus evenly rounded with elongate, straight apex in lateral view (Fig. 5); sides before apex broadened, with blunt tip in dorsal view (Figs 6, 7).

Female. Ventrite VIII regularly rounded posteriorly, spiculum ventrale short, narrow and truncate anteriorly (Fig. 8). Ovipositor with transverse valvifer without setae; triangular, heavily sclerotized coxite bearing numerous setae; and stylus modified into strongly curved, apically glabrous scrapers.

Diagnosis. The new *Apteroloma* species, is most similar to *A. jinfo*, both species share the following characters in combination: body large (6.7–7.7 mm in length), dorsum dark brown with uniformly ferruginous appendages (Figs 1, 3), pronotum with wide base (Figs 1, 3), aedeagus with elongate apex in lateral view (Figs 5, 9) and sides before apex only slightly broadened in dorsal view (Figs 7, 11), female ventrite VIII with narrow spiculum ventrale (Figs 8, 12).

The recently published key of Chinese *Apteroloma* (Růžička and Pütz 2009) should be modified at the couplet #5 as follows to accommodate the new species:

5	Large species (body length 6.7–7.7 mm)54
_	Small species (body length 5.4–6.8 mm)
5a	Pronotum with sparse punctation discally, sides distinctly sinuate posteriorly
	(Fig. 3). Elytra with fine punctation (third stria with 62-67 punctures, Fig
	3). Elytral epipleura with fine punctures (Fig. 4). Aedeagus with narrow, elon
	gate tip (Fig. 11). Ventrite VIII with spiculum ventrale elongate (Fig. 12)
	Central China (Chongqing, Hubei, Hunan) (Fig. 13)
	A. jinfo Růžička, Schneider & Háva, 2004
_	Pronotum with dense punctation discally, sides weakly sinuate posteriorly
	(Fig. 1). Elytra with strong punctation (third stria with 49–59 punctures, Fig
	1). Elytral epipleura with strong punctures (Fig. 2). Aedeagus with subapi

cally broadly rounded, blunt tip (Fig. 7). Ventrite VIII with spiculum ventrale short (Fig. 8). Eastern China (Zhejiang) (Fig. 13)..... *A. zhejiangense* sp. n.

Etymology. Adjective; named after Zhejiang Province, where the new species was discovered.

Distribution. China (Zhejiang) (Fig. 13). So far known only from the type locality. **Bionomics.** All specimens of the new species were collected by sifting leaf litters in broad-leaved forest, at altitudes 300–1200 m.

Apteroloma jinfo Růžička, Schneider & Háva, 2004

http://species-id.net/wiki/Apteroloma_jinfo Figs 3, 4, 9–12

Apteroloma jinfo Růžička et al. 2004: 116.

Material examined. China: Chongqing municipality: data of type series as in Růžička et al. (2004: 116); **Hubei Province:** 8 males and 8 females, Wufeng County, Houhe N. R. [ca. 30°33'N, 108°55'E], 30.IV.2004, Li-Zhen Li leg. (1 pair in JRUC,



Figure 13. Distribution of *Apteroloma zhejiangense* sp. n. (empty circle) and *A. jinfo* (filled circles) in China.

rest in SHNU); **Hunan Province:** 3 males, Longshan Xian, Huoyan Xiang, Huoyan, entrance to Feihu Dong cave [ca. 29°12'N, 109°18'E], 20.IX.1997, Y. Nishikawa leg. (1 male JRUC, 2 males NSMT).

Variation. Aedeagus has distinctly triangular, sub-sinuate apex in dorsal view in specimens from the type locality (Růžička et al. 2004: 117, Fig. 3), and straight apex with narrower tip in dorsal view in males from Hubei and Hunan Provinces (Fig. 11). Posterior margin of female ventrite VIII in specimens of Hubei Province is regularly rounded (Fig. 12), not distinctly truncate as observed in specimens from the type locality (Růžička et al. 2004: 117, Fig. 10). However, we consider variability of both characters to fall within variability range of the same species.

Distribution. China (Chongqing, Hubei, Hunan) (Fig. 13). The type locality (Jinfo Shan) was erroneously located in Sichuan Province by Růžička et al. (2004) and Růžička & Pütz (2009). First records from Hubei and Hunan Provinces.

Apteroloma potanini (Semenov, 1893)

http://species-id.net/wiki/Apteroloma_potanini

Pteroloma potanini Semenov 1893: 338.

Apteroloma potanini: Semenov-Tian-Shanskij 1932: 338; Růžička et al. 2004: 122; Růžička & Pütz 2009: 642.

Material examined. China: Ningxia Province: 1 male, Longde County, Qingliang, 35°36'19"N, 106°11'02"E, alt. 2400m, 26.VI.2008, Zi-Wei Yin leg. (SHNU); 1 male, Jingyuan County, Dongshanpo Forestry, Haizigou, 35°37'13"N, 106°15'42"E, alt. 2300m, 24. VI. 2008, Zi-Wei Yin leg. (SHNU); Henan Province: 2 specimens: Quanbaoshan, Xiong'er Shan, 34°07'N, 111°25'E, alt. 1700m, 23.–24.V.2010, broadleaved forest, beside forest path, first specimen in fly and second one at a big stone covered by moss, M. Perreau det. (MPEC); 1 specimen: same data, but alt. 1850m, pitfall trap (baited with beer and antifreeze) (MPEC).

Distribution. China (Sichuan, Shaanxi, Gansu, Ningxia, Hubei, Hebei, Henan). First records from Ningxia and Henan Provinces.

Apteroloma kozlovi Semenov-Tian-Shanskij & Znojko, 1932

http://species-id.net/wiki/Apteroloma_kozlovi

Apteroloma kozlovi Semenov-Tian-Shanskij & Znojko in Semenov-Tian-Shanskij 1932: 341.

Garytes coreanus Mroczkowski 1966: 434 (synonymy by Růžička et al. (2004)).

Material examined. China: Shaanxi Province: 1 male, 110 km NNE Xian, Huayin vill., Hua Shan Mt., 34°29.5'N, 110°05.1'E, alt. 1275m, 8.–9.V.2011, granite cliff,

valley of small brook, gravel stones close to water, M. Balke & J. Hájek leg. (NMPC); **South Korea:** 1 female, GanWeon-do, ChungChong [= Gangwon-do province, Chuncheon, ca. 37°52'N, 127°44'E], 17.V.1984, K. Morimoto leg. (NSMT); 2 males, KangWeon-do, HongCheon-gun, Pupan-myon [= Gangwon-do province, Hoengseong-gun county (centroid ca. 37°29'N, 127°59'E), Pupan myeon township (not located)], 20.V.1992, S. Nomura leg. (NSMT); 4 males, 2 females, Gyeongsamnam-do, Macheong-meon, Samjeong-li [= Gyeongsangnam-do province, Macheong-myeon township, Samjeong-ri village, ca. 35°21.5'N, 128°56.7'E], 9.V.1991, S. Nomura leg. (NSMT).

Note. The examined material from South Korea (reported as *A. potanini* by Nomura and Lee 1993) is verified here to belong to *A. kozlovi*. *Apteroloma kozlovi* was considered as junior subjective synonym of *A. potanini* by Schawaller (1991), and resurrected as a valid species only by Růžička et al. (2004).

Distribution. China (Qinghai, Shaanxi, Shanxi, Hebei, Beijing); North Korea; South Korea.

Acknowledgements

We would like to express our sincere gratitude to Dr. A. F. Newton (Chicago, USA) for improving the manuscript and sending us papers, to Dr. W. Schawaller (Stuttgart, Germany) for kindly sending us papers, to all Chinese collectors mentioned in this paper. Michel Perreau (Paris, France) is thanked to share unpublished data on *Apteroloma potanini* and Jiří Hájek (NMPC) and Shûhei Nomura (NSMT) for loan of material of *Apteroloma* under their care. The research was supported by National Natural Science Foundation of China (No. 30870323), National Natural Science Foundation of Shanghai (No. 10ZR1421600) to L. Tang and L.-Z. Li, and by grant of the Grant Agency of the Czech Republic (No. 206/07/1053) to J. Růžička.

References

- Hatch MH (1927) Studies on the carrion beetles of Minnesota, including new species. Technical Bulletin, University of Minnesota Agricultural Experiment Station 48: 3–19.
- Mroczkowski M (1966) Contribution to the knowledge of Silphidae and Dermestidae of Korea (Coleoptera). Annales Zoologici, Warszawa, 23: 433-443.
- Nomura S, Lee C E (1993) A Record of the Family Agyrtidae (Coleoptera) from South Korea. Esakia 33: 49–50.
- Růžička J, Pütz A (2009) New species and new records of Agyrtidae (Coleoptera) from China, India, Myanmar, Thailand and Vietnam. Acta Entomologica Musei Nationalis Pragae 49: 631–650.
- Růžička J, Schneider J, Háva J (2004) A revision of *Apteroloma* (Coleoptera: Agyrtidae) from China. Acta Societatis Zoologicae Bohemicae 68: 113–126.

- Schawaller W (1991) Agyrtidae (Coleoptera) aus dem Himalaya und den angrenzenden Gebieten. Stuttgarter Beiträge zur Naturkunde, Serie A (Biologie) 468: 1–22.
- Semenov A (1893) Revisio specierum ad Silphidarum genera Pteroloma Gyllh. et Lyrosoma Mannh. spectantium. Trudy Russkago Entomologicheskago Obshschestva 27: 335–346.
- Semenov-Thian-Shanskij A (1932) Le caractère zoogéographique du groupe des Pterolomini (Coleoptera, Silphidae) dans le faune paléarctique. Doklady Akademii Nauk SSSR 1932: 338–341.

RESEARCH ARTICLE



Myrmica elmesi (Hymenoptera, Formicidae) a new species from Himalaya

Himender Bharti[†], Yash Paul Sharma[‡]

Department of Zoology, Punjabi University Patiala, India 147002

turn:lsid:zoobank.org:author:5CFEBC9B-3CA9-4459-83A6-6D7B61B984B7 turn:lsid:zoobank.org:author:9C75D8FC-E870-4628-A8D9-861E3385D2A7

Corresponding author: Himender Bharti (himenderbharti@gmail.com, himender_bharti@yahoo.com)

Academic editor: Donat Agosti | Received 20 May 2011 | Accepted 1 August 2011 | Published 17 August 2011 urn:lsid:zoobank.org:pub:8916F83C-26C3-49D6-8B3F-9CF54678D6D4

Citation: Bharti H, Sharma YP (2011) Myrmica elmesi (Hymenoptera, Formicidae) a new species from Himalaya. ZooKeys 124: 51–58. doi: 10.3897/zookeys.124.1586

Abstract

Myrmica elmesi **sp. n.** is described from Himalaya. This species belongs to the *pachei* group of *Myrmica* species and is distinct from the species described in this group hitherto, which is represented by 14 species including three from Indian Himalaya. *Myrmica elmesi* is the fourth species of the diverse *pachei* group found in Himalaya; it was collected from the transitional zone and is described with notes on its ecology, this gains significance in the sense that ecology of most of the old world *Myrmica* is either unknown or poorly known.

Keywords

Ants, taxonomy, Myrmica elmesi, new species, pachei group, ecology, Himalaya

Introduction

Genus *Myrmica* in the old world is represented by 142 valid species. These species are well distributed in Palearctic zone and South-east Asian tropical and subtropical regions. The central Asian mountains which comprise Hindu Kush, Karakorum, South-western slope of Himalaya (Afghanistan, Pakistan, India, Nepal and Bhutan) comprise 33 species representing 7 species groups. 31 species (94%) are endemic to this region. The diversity of this region is quite interesting because the species of this

region show a plesiomorphic state of features (Radchenko and Elmes 2010). However, unfortunately the diversity from Indian Himalaya is very poorly represented in this census. There are reasons galore for this, but the most significant being lack of material collected from this region. The diversification of the major ant lineages, as elucidated by Moreau et al. (2006), occurred from the beginning of the early Paleocene to the late Cretaceous, 60 to 100 million years ago in the age of angiosperms. Interestingly, the same time span coincides with the geological history of Himalaya; the initial mountain building processes were underway about 70 million years ago when the North moving Indo-Australian plate collided with the Eurasian plate, followed by a second phase of mountain development about 65 million years ago. Upliftment of Himalaya as an isolation barrier has led to lot of endemism, thus many species groups which remain concentrated here, diversified in this region only (Bharti 2008a, b, 2011; Bharti and Sharma 2011; Radchenko and Elmes 2001, 2010). In last decade or so, the author and his team have started exploring Himalayan fauna and as per expectations the region has quite a number of undescribed/unnoticed species (reasonably unique too) which can contribute a lot to understanding the Myrmica of old world and would provide an insight into many unresolved questions.

Although the species groups in Myrmica as created by Radchenko and Elmes (Radchenko and Elmes 2001, 2010) are arbitrary divisions they seem to be correct as verified by molecular studies (Jansen et al. 2009, 2010) and appear to be monophyletic. Myrmica elmesi sp. n. which is described here, belongs to the pachei group, which is characterized by an alitrunk dorsum atleast partly with transverse rugosity; scape gradually though distinctly curved at base, not angled, with no trace of lobe or carina; anterior clypeal margin rounded or slightly prominent with no medial notch; petiole with a relatively short peduncle (Radchenko and Elmes 2010). Earlier this group was considered rare and unusual however Radchenko and Elmes (2008, 2009) while examining *Myrmica* fauna of China have found that it is more diverse than previously expected. Now it is represented by 14 species which are found in Himalaya, southwestern and southern China. It is supposed that like the *rugosa* group, the *pachei* group is derived from a *ritae* like ancestor that adapted to somewhat cooler temperate, open conditions that are found at slightly lower altitudes on more northern mountains in the Oriental region, where local isolation has led to a variety of local forms (Radchenko and Elmes 2010). Myrmica elmesi sp. n. is quite different from previously described species of *pachei* group; it was collected from the transitional zone of Himalaya and is described with notes on its ecology. This gains significance in the sense that ecology of most of the old world Myrmica is either unknown or poorly known and furthermore this is the fourth species of the diverse *pachei* group found in Himalaya.

Materials and methods

The specimens were collected through winkler's extractor and were preserved in 70% alcohol. Then the mounted material was analyzed on Nikon SMZ-1500 stereo zoom

microscope. For digital images, MP evolution digital camera was used on same microscope with Auto-Montage (Syncroscopy, Division of Synoptics, Ltd) software. Later, images were cleaned as per requirement with Adobe Photoshop CS.

We used measurements and indices proposed by Radchenko and Elmes (1998, 2010):

- **HL** length of head in dorsal view, measured in a straight line from the anterior point of median clypeal margin to mid-point of the occipital margin.
- HW maximum width of the head in dorsal view behind the eyes.
- **FW** minimum width of the frons between the frontal carinae.
- FLW maximum width between the external/outer borders of the frontal lobes.
- **SL** maximum straight-line length of antennal scape in profile.
- **AL** diagonal length of the alitrunk seen in profile, from the neck shield to the posterior margin of propodeal lobes (workers) and from the anterio-dorsal point of alitrunk to posterior margin of propodeal lobes (queens).
- **AH** Height of alitrunk, measured from upper level of mesonotum perpendiculary to the level of lower margin of mesopleuron (queens and males).
- **PL** maximum length of petiole from above, in dorsal view, measured from the posterolateral margin of petiole to the articulation with propodeum, the petiole should be positioned so that measured points lay on the same plane.
- **PPL** maximum length of post-petiole in dorsal view between its visible anterior and posterior margins.
- **PW** maximum width of petiole in dorsal view.
- **PPW** maximum width of postpetiole from above/in its dorsal view.
- **PH** maximum height of petiole in profile, measured from the uppermost point of petiolar node perpendicularly to the imaginary line between anteroventral(just behind the subpetiolar process) and posteroventral points of petiole.
- **PPH** maximum height of postpetiole in profile from the uppermost to the lowermost point, measured perpendicularly to the tergo-sternal suture.
- **ESL** maximum length of propodeal spine in profile, measured along the spine from its tip to the deepest point of the propodeal constriction at the base of the spine.
- ESD distance between tips of spines from above/in dorsal view.
- **PNW** maximum width of pronotum from above/in dorsal view.

Indices used

Cephalic	CI = HL/HW
Frontal	FI = FW/HW
Frontal lobe	FLI = FLW/FW
Scape (1)	SI1 = SL/HL
Scape (2)	SI2 = SL/HW
Petiole (1)	PI 1 = PL/PH
Petiole (2)	PI2 = PL/HW
Petiole (3)	PI3 = PW/HW

Post-petiole (1)	PPI1 = PPL/PPH
Post-petiole (2)	PPI2 = PPH/PPW
Post-petiole (3)	PPI3 = PPW/PW
Post-petiole (4)	PPI4 = PPW/HW
Spine length	ESLI = ESL/HW
Spine width	ESDI = ESD/ESL

Results

Myrmica elmesi sp. n.

urn:lsid:zoobank.org:act:F5D15679-304A-475B-A2F8-5F60FF0B4022 http://species-id.net/wiki/Myrmica_elmesi Figs 1–3, Table 1

Type locality. India, Jammu and Kashmir, Machedi, 32.72364°N, 75.669464°E, 2000 meters above mean sea level. Collected 3rd August, 2008 by Yash Paul Sharma.

Holotype. Worker from type locality, triangle mounted. Deposited in Department of Zoology (Dr Himender Bharti's Collections- DST-YEG-562), Punjabi University Patiala, India.

Paratypes. 1 worker (with same data as of holotype) and 10 workers from India, Jammu and Kashmir, Sarthal, 32.812947°N, 75.762503°E, 2200 metres above mean sea level, all paratype triangle mounted. Collected 15th June, 2009 by Yash Paul Sharma. Deposited in Department of Zoology (Dr. Himender Bharti's Collections-DST-YEG-292, 293, 294, 296, 297, 298, 299, 300, 344, 345, 561) Punjabi University Patiala, India. One of the paratypes will be deposited at Natural History Museum, London.

Description (worker).Worker measurements: FLW 0.40, FW 0.39, HL 1.10, HW 0.88, SL 0.94, PL 0.51, PPL 0.41, PW 0.24, PPW 0.34, PH 0.30, PPH 0.34, AL 1.57, TL 5.26.

Head distinctly longer than broad, with parallel sides and straight occipital margin. Mandibles with 9 teeth (apical and preapical ones are the largest), masticatory margin black, whole mandible finely longitudinally costulate, rugulose and punctated. Clypeus convex, longitudinally rugulose, anterior clypeal margin prominent and rounded medially, spaces between rugae minutely punctated but appear shiny. Frontal triangle somewhat deep, smooth and shiny. Frontal carinae short, partially cover the condylar bulb, almost straight, curving outwards to merge with rugae that surround antennal sockets. Antennae 12 segmented, funicular segments densely punctated, but two basal segments finely punctated; scape slender, narrow, weakly curved at base, without any trace of angle or carina, running towards apex just extending beyond upper margin of head; antennae with oblique short semi-erect hairs, with pubescence developed only on 3 apical segments, antennal club 3 segmented. Eyes large, situated slightly below the midlength of head. Head dorsum longitudi-





Figures 1–3. I Head of *Myrmica elmesi* sp. n., dorsal view **2** Body of *Myrmica elmesi* sp. n., lateral view **3** Body of *Myrmica elmesi* sp. n., dorsal view.

		TT 1	Workers (12)									
		type	Mean±SD	Min	Max			Holo- type	Mean±SD	Min	Max	
	HL	1.10	1.09±0.07	0.89	1.17]	CI	1.25	1.24±0.08	1.07	1.30	
surements	HW	0.88	0.87±0.04	0.81	0.97		FI	0.44	0.44 ± 0.01	0.41	0.45	
	SL	0.94	0.91±0.04	0.85	1.00		FLI	1.03	1.05±0.01	1.03	1.08	
	PL	0.51	0.49±0.03	0.42	0.52		PI1	1.70	1.64±0.08	1.50	1.78	
	PH	0.30	0.30±0.01	0.28	0.32		PI2	0.58	0.56±0.03	0.52	0.60	
	PW	0.24	0.24±0.01	0.23	0.27	lice	PI3	0.27	0.28±0.01	0.26	0.30	
	PPL	0.41	0.40 ± 0.02	0.36	0.43	Ind	PPI1	1.21	1.14±0.05	1.06	1.21	
Me	PPH	0.34	0.35±0.02	0.33	0.39		PPI2	0.97	0.96±0.02	0.92	1.00	
	PPW	0.35	0.37±0.02	0.35	0.40		PPI3	1.46	1.53±0.07	1.44	1.63	
	FLW	0.40	0.40 ± 0.02	0.36	0.43		PPI4	0.40	0.42 ± 0.01	0.40	0.44	
	FW	0.39	0.38±0.02	0.35	0.40		SI1	0.85	0.84±0.04	0.80	0.96	
	ESL	0.24	0.22±0.02	0.19	0.26		SI2	1.07	1.03 ± 0.04	0.93	1.09	
	AL	1.57	1.51±0.07	1.33	1.57		ESLI	0.27	0.26±0.03	0.20	0.31	
	ESD	0.40	0.37±0.03	0.33	0.41		ESDI	1.67	1.67 ± 0.20	1.27	1.95	
	PNW	0.65	0.64±0.02	0.60	0.67							

Table 1. The mean, standard deviation, minimum and maximum values (in mm) of the measurements and indices made on samples of species (workers). The measurement codes are as indicated in the text and the numbers of individuals measured are given in parenthesis.

nally rugose, 11 rugae between frontal carinae at the level of eyes, occiput with reticulate sculpture, opaque; anterior clypeal margin fringed with setae; head, clypeus and mandibles with long hairs.

Promesonotal dorsum feebly convex, forming regular arch, not saddle shaped, promesonotal suture indistinct. Promesonotum transversally sinuously rugose in dorsal view, pronotum transversally striate laterally; metanotal groove broad, deep and longitudinally striate. Propodeal dorsum longitudinally striated, declivity smooth and highly polished, mesonotum and propodeum longitudinally striate laterally, propodeal lobes rounded apically; propodeal spines long, sharp, projected upward and divergent. Tibiae of hind and middle legs with well-developed pectinate spur.Petiole with short anterior peduncle, petiolar dorsum rounded; whole petiole punctated and finely reticulated, appears dull. Postpetiole slightly longer than broad, finely punctated and longitudinally striated. In profile, petiole high and narrow with a short tooth like subpetiolar process. Alitrunk, petiole and postpetiole with long hairs, except for propodeum.

Gaster smooth, highly polished and shiny, with long, erect hairs.

Etymology. The species is dedicated to Dr Graham Elmes for his contribution to the investigation of genus *Myrmica*.

Distribution and habitat. North-west Himalaya (India, Jammu and Kashmir).

Ecology. Species has been collected from leaf litter in both the habitats. The collection site at Machedi has a patchy *Cedrus* forest along with agricultural land surrounding the site; moreover the area has lot of anthropogenic activities with a dry type

of environment (mean temperature during collection period 32°C, relative humidity 36.62%, annual rain fall 970mm and thickness of leaf litter 2.1cm). The collection site at Sarthal has dense *Cedrus* forest with abundant leaf litter, no agricultural land, it remains snow clad from November to beginning of March and has very limited anthropogenic activities with only nomads visiting the area (mean temperature during collection period 22°C, relative humidity 66.38%, annual rain fall 1476mm and thickness of leaf litter 3.9cm) with comparatively wet environment.

This zone where the species is distributed is a transitional zone between sub temperate and temperate Himalaya and geographically it penetrates in to the Palearctic zone (whose boundary in Southern Asia is largely altitudinal, where an altitude of 2000–2500 meters above mean sea level forms the boundary between Palearctic and Indo-Malayan ecozones). Besides, Himalayan ecology is temperature-dependent. The snow line occurs at an average of 6000 meters above mean sea level and the timber line at an average of 3000 meters (the highest altitude at which the forest ends). With this sort of environment, the micro-climate plays an important role for ants like *Myrmica* which prefer to live under stones or in rare cases in leaf litter, as the soil temperature is comparatively higher to ambient temperature in these habitats (Bharti 2008b).

Conservation status. Yet to be ascertained, but population level could be low as collected from leaf litter only.

Discussion. Myrmica elmesi sp. n. (Figs 1–3) which belongs to pachei group, and is significantly different from all other described species of this group due to its transversally striated promesonotum (seen in dorsal view), longitudinally striated propodeal dorsum and PI1 1.64. In all the known species of *pache*i group hitherto, either the pronotal and propodeal dorsum are transversally striated, or the whole alitrunk dorsum has transverse rugae, or the mesonotal and propodeal dorsum has coarse/fine transverse rugosity. A PI1> 1.5 has been recorded in only one other species of the *pachei* group (i.e. PI1=1.68 for Myrmica weii Radchenko & Zhou, 2008 in Radchenko et al. 2008). Myrmica elmesi can be placed in to the recent key provided by Radchenko and Elmes (2010, page 736) in the following way 1-2-3 and at couplet 3;

3	Promesonotal dorsum with transverse rugae, propodeal dorsum with fine
	longitudinal striations
_	Either whole alitrunk dorsum with transverse rugae or pronotal and propo-
	deal dorsum with transverse rugae4
4	Pronotal and propodeal dorsum with transverse rugae, other part of alitrunk
	dorsum with longitudinal rugosity and reticulation
	varisculpta Radchenko & Rigato, 2009 in Radchenko and Elmes 2009
_	Whole alitrunk dorsum with transverse rugae

(Then couplet 5 onwards *Myrmica pachei* Forel, 1906, *Myrmica inezae* Forel, 1902 and *Myrmica villosa* Radchenko & Elmes, 1999 can be keyed out as given in the above mentioned key of Radchenko and Elmes 2010).

Acknowledgements

Sincere thanks to Professor Alex Radchenko for his comments and discussion on Himalayan *Myrmica*. Financial assistance rendered by Department of Science and Technology (grant no. SR/SO/AS-65/2007), Ministry of Science and Technology, Government of India, New Delhi for this work is gratefully acknowledged.

References

- Bharti H (2008a) Ants and Speciation Patterns. ANeT News letter, Kagoshima University, Japan, 10: 6–9.
- Bharti H (2008b) Altitudinal Diversity of Ants in Himalayan Regions (Hymenoptera: Formicidae). Sociobiology 52(2): 305–322.
- Bharti H (2011) List of Indian Ants (Hymenoptera: Formicidae). Halteres 3: 79-87.
- Bharti H, Sharma YP (2011) *Myrmica radchenkoi* a new species of ant (Hymenoptera: Formicidae) from Indian Himalaya. Sociobiology 58(2): 1–8.
- Jansen G, Savolainen R, Vepsalainen K (2009) DNA barcoding as a heuristic tool for classifying undescribed Nearctic *Myrmica* ants (Hymenoptera: Formicidae). Zoologica Scripta 38: 527–536. doi: 10.1111/j.1463-6409.2009.00386.x
- Jansen G, Savolainen R, Vepsalainen K (2010) Phylogeny, divergence-time estimation, biogeography and social parasite-host relationships of the Holarctic ant genus *Myrmica* (Hymenoptera: Formicidae). Molecular Phylogenetics and Evolution 56: 294–304. doi: 10.1016/j.ympev.2010.01.029
- Moreau CS, Bell CD, Vila R, Archibald B, Pierce NE (2006) Phylogeny of the ants: Diversification in the age of Angiosperms. Science 312: 101–103. doi: 10.1126/science.1124891
- Radchenko AG, Elmes GW (1998) Taxonomic revision of the *ritae* species-group of the genus Myrmica (Hymenoptera: Formicidae). Vestnik Zoologii 32(4): 3–27.
- Radchenko AG, Elmes GW (2001) A taxonomic revision of the ant genus *Myrmica* Latreille (Hymenoptera: Formicidae) from Himalaya. Entomologica Basiliensia 23: 237–276.
- Radchenko AG, Elmes GW (2003) A taxonomic revision of the socially parasitic *Myrmica* ants (Hymenoptera: Formicidae) of the Palearctic region. Annales Zoologici 53(2): 217–243.
- Radchenko AG, Elmes GW (2009) Taxonomic revision of the *pachei* species group of the genus *Myrmica* Latreille (Hymenoptera: Formicidae) Annales Zoologici 59(1): 67–92. doi: 10.3161/000345409X432592
- Radchenko AG, Elmes GW (2010) *Myrmica* ants (Hymenoptera: Formicidae) of the Old World. Natura Optima dux Foundation, Warsaw, Poland, 789pp.
- Radchenko A, Zhou S, Elmes GW, Rigato F (2008) Seven new *Myrmica* species (Hymenoptera: Formicidae) from China. Annales Zoologici 58(4): 767–784. doi: 10.3161/000345408X396701

RESEARCH ARTICLE



DNA barcoding and morphology reveal two common species in one: *Pimpla molesta* stat. rev. separated from *P. croceipes* (Hymenoptera, Ichneumonidae)

Anu Veijalainen¹, Gavin R. Broad², Niklas Wahlberg¹, John T. Longino³, Ilari E. Sääksjärvi¹

Lepartment of Biology, FI-20014 University of Turku, Turku, Finland 2 Natural History Museum, Cromwell Road, London SW7 5BD, UK 3 Lab 1, Evergreen State College, Olympia, Washington, 98505, USA

Corresponding author: Anu Veijalainen (anu.veijalainen@utu.fi)

Academic editor: Norman Johnson | Received 6 July 2011 | Accepted 26 July 2011 | Published 17 August 2011

Citation: Veijalainen A, Broad GR, Wahlberg N, Longino JT, Sääksjärvi IE (2011) DNA barcoding and morphology reveal two common species in one: *Pimpla molesta* stat. rev. separated from *P. croceipes* (Hymenoptera, Ichneumonidae). ZooKeys 124: 59–70. doi: 10.3897/zookeys.124.1780

Abstract

Correct species identification is the basis of ecological studies. Nevertheless, morphological examination alone may not be enough to tell species apart. Here, our integrated molecular and morphological studies demonstrate that the relatively widespread and common neotropical parasitoid wasp *Pimpla croceipes* Cresson, 1874 (Hymenoptera: Ichneumonidae: Pimplinae) actually consists of two distinct species. The name *Pimpla molesta* (Smith, 1879) stat. rev. is available for the second species. The two species were identified by DNA barcoding and minor differences in morphology and colouration. Our results support the previous notions that DNA barcoding can complement morphological identification and aid the discovery of cryptic species complexes.

Keywords

cryptic species, integrative taxonomy, Neotropics, parasitoid wasp, Pimplinae

Introduction

In the midst of global biodiversity loss and substantial taxonomic shortcomings, improved identification methods for hyperdiverse and poorly known invertebrate groups are good news. Integrating molecular methods with morphological species identifica-

Copyright Anu Veijalainen et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

tion can accelerate biodiversity inventories and facilitate spotting cryptic species (i.e. two or more distinct species classified as one due to morphological similarity; e.g. Smith et al. 2009). Since ecological research ultimately depends on the work of taxonomists, consistent species definitions are important for attaining reliable scientific results. In this paper, we use DNA barcoding to reveal a cryptic complex masked within a common and widespread neotropical parasitoid wasp species.

The parasitoid wasp family Ichneumonidae (Hymenoptera) may well be the largest animal family on earth, but it is considered taxonomically challenging and poorly known (Gauld 2000). The vast majority of ichneumonids are parasitoids; they lay their eggs in or on other arthropods and the developing parasitoid eventually kills the host. The tropical ichneumonid fauna is generally undersampled, although Ian Gauld's tremendous work on the Costa Rican ichneumonids yielded many practical keys for identifying Central American species (Gauld 1991, 1997, 2000, Gauld et al. 1998, 2002ab). Most neotropical ichneumonids are either rare or rarely collected, as even extensive sampling produces only a few specimens of most species (Sääksjärvi et al. 2004). However, as in any fauna, there are still some commonly encountered neotropical ichneumonids, such as the characteristic pimpline *Pimpla croceipes* Cresson, 1874 (Fig. 1), whose distribution has been reported to extend from Mexico through Central America to Colombia, Ecuador and Venezuela (Yu et al. 2005). With its wide distribution, it is not surprising that there has been a junior synonym of P. croceipes recognised, namely Pimpla molesta Smith, 1879 (Fig. 2), synonymized with P. croceipes by Cameron (1886) (see Gauld 1991).

When working through neotropical ichneumonid samples, we encountered a number of specimens that we identified as *P. croceipes* (hereafter *P. croceipes* sensu lato) according to the keys in Gauld (1991) and Gauld et al. (1998), but which showed some variation in colouration and sculpture. This variation could not be readily compartmentalised as distinct species but this led us to hypothesize that the specimens represented a complex of species morphologically close to one another. As integrating DNA barcoding with other species identification methods has proven an efficient method for separating cryptic ichneumonoid species complexes (Janzen et al. 2009, Smith et al. 2009), we tested our hypothesis by comparing the results of DNA barcoding and careful morphological examination.

Methods

The data consisted of specimens collected by the LLAMA project in Guatemala and Honduras (see below), currently on loan to the Zoological Museum, University of Turku (ZMUT) and later to be deposited in the collaborative institutions of the LLAMA project, and the collections of the Natural History Museum, London, UK (BMNH). The LLAMA specimens were studied using both molecular and morphological species identification methods, the BMNH specimens focusing exclusively on morphology. The images were taken in ZMUT using an Olympus SZX16 stereomicroscope attached



Figure 1. Pimpla croceipes female, lateral view.

to an Olympus E520 digital camera. The layer photos were combined using the programmes Deep Focus 3.1, Quick PHOTO CAMERA 2.3 and Combine ZP.

LLAMA

In the LLAMA samples, there were 97 specimens of *P. croceipes* s.l. The specimens were collected by Malaise traps as a part of the Leaf Litter Arthropods of Mesoamerica project (LLAMA; http://llama.evergreen.edu) led by JTL in Guatemala and Honduras from May to June 2009 and 2010, respectively (see online Supplementary material for geospatial information and sampling periods). The LLAMA project applied similar Malaise sampling effort at 17 study sites, ranging from 50–2400 m asl. Four or five Malaise traps were set for four days at each site. *Pimpla croceipes* s.l. specimens were collected at nine of the 17 sites (Suchitepequez: 4 km S volcano Atitlán, Sacatepequez: 5 km SE Antigua, Baja Vera Paz: Biotopo El Quetzal, Zacapa: 2 km SE La Unión, Ocotepeque: 13 km E Nueva Ocotepeque, Cortés: Parque Nacional Cusuco, Comayagua: 10 km E Comayagua, Olancho: Parque Nacional La Muralla, and Olancho: 9–11 km N Catacamas; Figs 3, 5). All nine sites were at mid to high elevation, 1200–2335



Figure 2. Pimpla molesta female, lateral view.

m asl. Habitats were all mature wet forest, typically diverse mesophyll cloud forest, but some sites with variable densities of pine, oak, and *Liquidambar*. Traps were generally located on forest edges or in small clearings. Sampling took place during the transition from dry season to wet season.

DNA barcoding

We extracted the DNA of all the LLAMA specimens of *P. croceipes* s.l. using the DNeasy[®] Blood & Tissue Kit (QIAGEN) and following the standard bench protocol for animal tissue in DNeasy Blood & Tissue Handbook 07/2006 (the samples were also incubated at 70° C for 10 minutes after adding the Buffer AL and vortexing). Next, we amplified the approximately 650-base fragment of the 5' end of the mitochondrial gene cytochrome *c* oxidase I (*cox1*, COI) well known as the barcode region for animals. Each PCR was done in a 20 μ l volume consisting of 1 μ l of DNA extract and 19 μ l of master mix (12.5 μ l dH₂O, 2.0 μ l 10× PCR Gold Buffer, 2.0 μ l MgCl₂ solution, 1.0 μ l primer LCO, 1.0 μ l primer HCO, 0.4 μ l dNTP, 0.1 μ l Ampli Taq



Figure 3. The LLAMA study sites where specimens of either *P. croceipes*, *P. molesta*, or both, were collected I Suchitepequez 2 Sacatepequez 3 Baja Vera Paz 4 Zacapa 5 Ocotepeque 6 Cortés 7 Comayagua 8 Olancho: "La Muralla" 9 Olancho: "Catacamas". See text for more specific descriptions of site locations.

Gold). The PCRs were run for 40 cycles with an annealing temperature of 50°C. The succesful PCRs were cleaned and sequenced by Macrogen (South Korea), after which we edited and aligned the sequences and constructed the neighbour-joining tree based on genetic distance calculated with the K2P model using MEGA v4 (Kumar et al. 2004). The LLAMA specimens for which we were not able to extract DNA or receive readable sequences were still included in the morphological studies. Sequences have been deposited in GenBank under accession numbers JN387917–JN387993. Specimens of two species of *Pimpla* from GenBank (*Pimpla aequalis* Provancher AF146681 and *Pimpla* sp. FN662469) were used as outgroups for the neighbour-joining analyses. Support was assessed using bootstrapping with 1000 pseudoreplicates.

BMNH collection

In the Natural History Museum, there were 237 Costa Rican specimens, 24 Mexican specimens, 2 from Panama and 1 from Venezuela, plus the holotype of *P. molesta*, from Costa Rica (Supplementary material). These were sorted into *P. croceipes* and *P. molesta* based on their morphological characters (Table 1).

Sex	Character	P. croceipes (Fig. 1)	P. molesta (Fig. 2)
F	Subalar prominence	Yellow/white	Black
F	Fore coxa	Yellow	Black or black and yellow
F	2nd tergite	More closely punctate (Fig. 6A)	More sparsely punctate (Fig. 6B)
М	2nd tergite	Transverse groove at or behind posterior 0.65 (Fig. 6C), curved; tergite usually more closely punctate	Transverse groove at posterior 0.55 (Fig. 6D), almost straight; tergite more sparsely punctate

Table 1. A comparison of the diagnostic morphological characters of *P. croceipes* and *P. molesta*.

Results

We obtained COI sequences for 77 specimens and the DNA barcoding results clearly group the specimens into two distinct species (Fig. 4). The two species differed from each other by 9.9% (K2P distance), and had virtually no intraspecific variation (0–0.5% variation). The molecular results, enabling us to preliminarily divide the successfully barcoded LLAMA specimens into two groups, greatly facilitated the morphological identification process, and we also found interspecific differences in the specimens' colouration and morphology (Table 1). Careful morphological examination and comparison with type material revealed that the two species each have available names: *P. croceipes* and *P. molesta* (stat. rev.). The ecological information that was available (e.g. altitude, habitat) could not be used to entirely predict the identity of the specimens as the distribution areas of the two species overlap in the total LLAMA dataset and both species could occur in the same traps (Supplementary material). This is in accordance with Gauld et al. (1998) who noted that many common Central American species of *Pimpla*, namely *P. croceipes* s.l., *P. croceiventris* (Cresson) and *P. sedula* Cameron, occur sympatrically.

We examined the holotype female of *Pimpla molesta* in BMNH and images of the lectotype female of *P. croceipes* (deposited in Philadelphia Academy of Sciences). Fortuitously, these two types correspond to our two morphotypes, designated on the basis of the molecular separation. We were therefore able to assign all of our specimens of *P. croceipes* s.l. to either *P. croceipes* or *P. molesta*. The species can be identified according to the diagnosis below and separated with the character differences summarized in Table 1 and illustrated in Figures 1, 2 and 6.

Males are relatively straightforward to separate on the basis of the sculpture on the second metasomal tergite, although colour pattern appears to offer no differences. Females are more difficult to separate but do show small differences in colour and metasomal sculpture. Table 1 should serve to separate almost all individuals. Both key easily to *Pimpla croceipes* using Gauld's (1991) and Gauld et al.'s (1998) keys to Costa Rican species and Gauld et al.'s (2002b) key to El Salvadorean species. Large females of *P. molesta* (fore wing length ~12mm) may have a partly yellow/white subalar prominence and only a small amount of black on the fore coxa. However, the subalar prominence is not entirely yellow or white which, coupled with the difference in sculpture of the second tergite, should allow separation of all individuals. Conversely, some small females



Figure 4. Neighbour-joining tree of the successfully DNA barcoded LLAMA specimens (species; sex; DNA voucher code; location). Numbers above the branches are bootstrap proportions. The two species *Pimpla croceipes* and *P. molesta* (stat. rev.) are clearly separated into two well-supported clusters. The results were further supported by morphological examination.



Figure 5. A frequency histogram of elevations of Malaise samples, for **I**) all LLAMA samples, **2**) those with *P. croceipes*, and **3**) those with *P. molesta*.

of *P. croceipes* (fore wing length ~8.5mm) have the fore coxa extensively dark marked and the subalar prominence a rather dull cream, but the subalar prominence is nevertheless entirely pale-marked and the sculpture differences still stand. Interestingly, the two *Pimpla* species seem to share a mimicry pattern with a *Lissonota* species (Ichneumonidae: Banchinae), represented by two males collected by Malaise trap in another sample at the Honduran site Comayagua and illustrated in Figure 7 (LLAMA sample code Ma-C-04-1-02). This is in accordance with the notion of Gauld et al. (2002a) that many of the Costa Rican montane *Lissonota* are involved in mimicry complexes characterized by black colouration with lower body parts being bright yellow.

In total, we studied 361 specimens (excluding the holotypes) and finally assigned them into 175 individuals of *P. croceipes* and 186 of *P. molesta* (Supplementary material). The two species were equally abundant in the LLAMA samples (*P. croceipes*: 48, *P. molesta*: 49 individuals). In BMNH, there are *P. croceipes* specimens from Costa Rica and Venezuela (Las Mercedes) and *P. molesta* specimens from Costa Rica, Mexico (several sites in Guerrero State) and Panama (Chiriquí).

Discussion

We confirm with morphological and molecular evidence and occurrence of sympatry that *P. croceipes* and *P. molesta* are two separate species. Gauld (1991) observed similar morphological variation as that reported here but, in the absence of molecular data, he concluded that these differences represented intraspecific variation among *P. croceipes* rather than in-



Figure 6 (A–D). Metasoma. 2nd tergite sculpture of *P. croceipes* female **A**, *P. molesta* female **B**, *P. croceipes* male **C**, and *P. molesta* male **D**.

terspecific variation between two species. He wrote (referring to *P. molesta* stat. rev., based on specimens in BMNH): "Variation: Small individuals tend to have the metasomal tergites very sparsely and weakly punctate; often such specimens lack black marks on the tegula and subalar prominence and a few individuals even have the scutellum black. Specimens from the wettest sites also often lack the yellow mark on the subalar prominence."

When cryptic species are discovered, linking previously compiled species data to the correct "new" species may be difficult (Schlick-Steiner et al. 2007). Gauld (1991) reports that in Costa Rica, the species (*P. croceipes* s.l.) is very common in humid areas at 800–1600 m asl where it comprises 7–35% of pimpline individuals in Malaise trap samples. According to our observations, both species are very common in Central American cloud forests: in the Guatemalan and Honduran LLAMA Malaise trap samples studied here, *P. croceipes* accounts for 22.2% and *P. molesta* for 22.7% of all the pimplines collected (50–2400 m asl). When the trap sites are plotted along an altitudinal gradient, there is a degree of altitudinal separation between the two species, with *P. croceipes* ranging from 1210 to 2010 m and *P. molesta* at somewhat higher altitudes, from 1480 to 2335 m (Fig. 5, Supplementary material). Costa Rican specimens in BMNH show a fairly clear altitudinal separation, with no specimens of *P. croceipes* having been collected above 1300 m, whilst 60% of the *P. molesta* specimens were collected above 1300 m (Supplementary material). All of the Mexican specimens in BMNH were



Figure 7. *Lissonota* sp. male (Ichneumonidae: Banchinae) collected at Comayagua (Honduras) showing a similar mimicry pattern to *P. croceipes* and *P. molesta*.

collected at relatively high altitude (1800–2900 m) and all proved to be *P. molesta*. The species' distribution ranges overlap as they were collected in the same LLAMA samples, yet the species were at their most abundant at different LLAMA sites; 19 out of 48 specimens (40%) of *P. croceipes* were collected at Zacapa, and 23 of 49 specimens (47%) of *P. molesta* at Sacatepequez. Both of the species were found in cloud forests at midelevations (approx. 1500–1750 m asl) (Fig. 5, Supplementary material). The biology of the species is not known, and we are not aware of any rearing records for them either. In fact, there are only very few host records for tropical American species of *Pimpla*, which may be due to their biology (see Díaz 2000, Gauld et al. 2002b). In other parts of the world, *Pimpla* species are known to be idiobiont endoparasitoids of lepidopteran pupae in concealed locations (e.g. in soil, leaf litter or leaf rolls).

We identify at least three sources of error that should be kept in mind while interpreting the presence of the two *Pimpla* species in specific study localities. First, the

69

sampling efficiency of Malaise traps may be influenced by the precise positioning of the trap in the sampling locality. Second, many species of ichneumonids are often rare in samples. For this reason, their presence or absence in a Malaise trap sample may be largely a coincidence. Third, pimplines are large parasitoids which are normally strong fliers. Thus, a Malaise trap may sample individuals that are just passing a forest patch instead of actually being resident there.

We have shown that the *P. croceipes* s.l., previously thought to be one species, is in fact two morphologically very similar but molecularly clearly different species which are both common and co-occur in Central America. As with other studies on neotropical parasitoids (Smith et al. 2007, 2008, Janzen et al. 2009), we found DNA barcoding to complement morphological identification and to aid the discovery of cryptic species complexes. Integrative taxonomy studies continually find cryptic species both from the temperate and tropical regions (Bickford et al. 2006). Whether cryptic parasitoid species in the tropics are prevalent enough to significantly raise the estimations of the total number of parasitoid species remains to be seen.

Acknowledgements

Project LLAMA was supported by National Science Foundation grant DEB-0640015. The Finnish Society of Forest Science, Jenny and Antti Wihuri Foundation, Smithsonian Institution Graduate Student Fellowship, and SYNTHESYS provided personal funding and travel grants to Anu Veijalainen. The lab work expenses were covered by The Academy of Finland grant 129811 to Niklas Wahlberg, and Kone Foundation grant (Biodiversity and multiple trophic interactions) to Ilari E. Sääksjärvi. Matthew Buffington (USDA) and Michael Sharkey (University of Kentucky) arranged the distribution of specimens, and the assistance from the TEGLab staff (University of Turku) was of great help. Thank you to Jason Weintraub (Academy of Natural Sciences, Philadelphia) for sending images of the lectotype of *Pimpla croceipes*. Norman Johnson, Jose Fernandez-Triana and one anonymous referee gave valuable comments on the manuscript.

References

- Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2006) Cryptic species as a window on diversity and conservation. TRENDS in Ecology and Evolution 22: 148–155. doi: 10.1016/j.tree.2006.11.004
- Díaz FA (2000) The Venezuelan species of *Pimpla* (Hymenoptera: Ichneumonidae). Journal of Hymenopteran Research 9: 246–253.
- Gauld I (1991) The Ichneumonidae of Costa Rica 1. Memoirs of the American Entomological Institute 47: 1–589.
- Gauld I (1997) The Ichneumonidae of Costa Rica 2. Memoirs of the American Entomological Institute 57: 1–485.

- Gauld ID (2000) The Ichneumonidae of Costa Rica 3. Memoirs of the American Entomological Institute 63: 1–453.
- Gauld ID, Ugalde Gómez JA, Hanson P (1998) Guía de los Pimplinae de Costa Rica (Hymenoptera: Ichneumonidae). Revista de Biologia Tropical 46: 1–189.
- Gauld I, Godoy C, Sithole R, Ugalde Gómez J (2002a) The Ichneumonidae of Costa Rica 4. Memoirs of the American Entomological Institute 66: 1–768.
- Gauld ID, Menjívar R, González MO, Monro A (2002b) Guía para la Identificación de los Pimplinae de cafetales bajo sombra de El Salvador (Hymenoptera: Ichneumonidae). The Natural History Museum, London, 76 pp.
- Janzen DH, Hallwachs W, Blandin P, Burns JM, Cadiou JM, Chacon I, Dapkey T, Deans AR, Epstein ME, Espinoza B, Franclemont JG, Haber WA, Hajibabaei M, Hall JPW, Hebert PDN, Gauld ID, Harvey DJ, Hausmann A, Kitching IJ, Lafontaine D, Landry JF, Lemaire C, Miller JY, Miller JS, Miller L, Miller SE, Montero J, Munroe E, Green SR, Ratnasingham S, Rawlins JE, Robbins RK, Rodriguez JJ, Rougerie R, Sharkey MJ, Smith MA, Solis MA, Sullivan JB, Thiaucourt P, Wahl DB, Weller SJ, Whitfield JB, Willmott KR, Wood DM, Woodley NE, Wilson JJ (2009) Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. Molecular Ecology Resources 9 (Suppl. 1): 1–26. doi: 10.1111/j.1755–0998.2009.02628.x
- Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics 5: 150–163. http:// www.kumarlab.net/pdf_new/KumarTamura03.pdf
- Sääksjärvi IE, Haataja S, Neuvonen S, Gauld ID, Jussila R, Salo J, Marmol Burgos A (2004) High local species richness of parasitic wasps (Hymenoptera: Ichneumonidae; Pimplinae and Rhyssinae) from the lowland rainforests of Peruvian Amazonia. Ecological Entomology 29: 735–743. http://www.sci.utu.fi/projects/amazon/The_team/Publications/individual_publications/Saaksjarvi_et_al_2004_EcolEnt.pdf
- Schlick-Steiner BC, Seifert B, Stauffer C, Christian E, Crozier RH, Steiner FM (2007) Without morphology, cryptic species stay in taxonomic crypsis following discovery. Trends in Ecology and Evolution 22: 391–392. doi: 10.1016/j.tree.2007.05.004
- Smith MA, Wood DM, Janzen DH, Hallwachs W, Hebert PDN (2007) DNA barcodes affirm that 16 species of apparently generalist tropicl parasitoid flies (Diptera, Tachinidae) are not all generalists. Proceedings of the National Academy of Sciences 104: 4967–4972. doi: 10.1073/pnas.0700050104
- Smith MA, Rodriguez JJ, Whitfield JB, Deans AR, Janzen DH, Hallwachs W, Hebert PDN (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. Proceedings of the National Academy of Sciences 105: 12359–12364. doi: 10.1073/pnas.0805319105
- Smith MA, Fernandez-Triana J, Roughley R, Hebert PDN (2009) DNA barcode accumulation curves for understudied taxa and areas. Molecular Ecology Resources 9: 208–216. doi: 10.1111/j.1755–0998.2009.02646.x
- Yu DS, van Achterberg K, Horstmann K (2005) World Ichneumonoidea 2004. Taxonomy, biology, morphology and distribution. Taxapad CD, Vancouver, Canada.