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Broadening the definition of the genus *Thalassaphorura* Bagnall, 1949 (Collembola, Onychiuridae) with a new aberrant species from China

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Abstract

A new species belonging to the tribe Thalassaphorurini, *Thalassaphorura problematica* **sp. n.**, is described from Northeast China. The new species is closest to the large genus *Thalassaphorura* by its simple vesicles in PAO and its furcal rudiment, but it does not fit the definition of the genus by the absence of chaeta d0 on head, the number of chaetae in the distal whorl of tibiotarsi and the labium type. We discuss the relative weakness of these last characters at generic level, which lead us to assign the new species to *Thalassaphorura* instead of erecting a new genus. The diagnosis of *Thalassaphorura* is broadened accordingly.

Keywords

Thalassaphorurini, head chaetotaxy, tibiotarsi, labium

Introduction

The tribe Thalassaphorurini was established by Pomorski (1998), characterized by a furcal rudiment in a form of a finely granulated area with four small chaetae in two rows posteriorly. During recent investigations on Collembola in Northeast China, we recorded seven species belonging to three genera of the tribe Thalassaphorurini: *Allonychiurus* (*A. songi* Sun & Wu, 2012), *Sensillonychiurus* (*S. changchunensis* Sun & Wu, 2012, *S. pseudoreducta* Sun & Wu, 2012, *S. reducta* Sun & Wu, 2012), and *Thalassaphorura* (*T. encarpata* (Denis, 1931), *T. lifouensis* (Thibaud & Weiner, 1997), *T. macrospinata* Sun & Wu, 2012). Meanwhile, we also found a new species of the tribe Thalassaphorurini, with a combination of morphological characters that did not fit any of the known genera of the tribe. In the present paper, we assign the new species to the genus *Thalassaphorura* according to its simple vesicles in PAO and its furcal rudiment, rather than erecting a new genus. We broaden accordingly the diagnosis of *Thalassaphorura* and discuss the inconsistent characters of the new species. An updated key to the genera of the tribe Thalassaphorurini is provided.

Material and methods

Specimens were collected by Berlese extraction of forest soil and humus, cleared in lactic acid and then mounted in Marc André II solution. They were studied using a Nikon Eclipse 80i microscope. The material is deposited in the Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun.

Labial types are named after Fjellberg (1999). Labium areas and chaetal nomenclature follow Massoud (1967) and D'Haese (2003). Chaetae on anal valves are named after Yoshii (1996). Chaetae on the furcal area are classified in accordance with Weiner (1996). Tibiotarsus chaetotaxy formula follows Deharveng (1983), and is expressed as: total number of chaetae (number of chaetae in row C, number of chaetae in row B, number of chaetae in row A+T), for example 14 (1, 7, 6).

Abbreviations used in descriptions

Ant.—antennal segments, PAO—postantennal organ, Th.—thoracic segments, Abd.—abdominal segments, ms—microsensillum, pso—pseudocellus, psx—parapseudocellus, Sp—posterior S-chaeta on Abd. V tergum, ^{m}—unpaired pseudopore or parapseudocellus.

The pseudocelli, parapseudocelli and pseudopores formula are the number of pseudocelli, parapseudocelli or pseudopores by half-tergum (dorsally) or half-sternum (ventrally) as follows: head anterior, head posterior/Th. I, Th. II, Th. III/Abd. I, Abd. II, Abd. III, Abd. IV, Abd. V (for instance: 32/133/33343).

Systematics

Family Onychiuridae Börner, 1913
Genus *Thalassaphorura* Bagnall, 1949

***Thalassaphorura problematica* sp. n.**
http://zoobank.org/17661043-729B-4277-827D-E4F25F48FBC9
http://species-id.net/wiki/Thalassaphorura_problematica

Type material. Holotype female; paratypes 9 females and 3 males on slides—China, Heilongjiang: Wulindong town, 46°33'N, 133°40'E, 16 Aug 2010, forest soil and humus, Wu Donghui, Liu Dong, Yuan Xiaoqiang and Yuan Yabin leg.

Diagnosis. Pso formula as 32/133/33343 dorsally, 11/000/00010 ventrally; psx formula as 0/000/112001+1^{m} ventrally; Ant. III sensory organ with two granulated clubs (inner one bigger than outer); labium with 5 proximal chaetae; labial type AB; tibiotarsi of legs I–III with 14 (1, 7, 6) chaetae each; male ventral organ present on ventral tube as modified distal chaetae; anal spines 1.1–1.2 times as long as inner edge of hind unguis.

Description. Body white in alcohol. Size 1000-1300 μm in females, 800–1100 μm in male; holotype: 1050 μm. Body subcylindrical, body sides parallel.

Pseudocellar formula: 32/133/33343 dorsally, 11/000/00010 ventrally, subcoxa 1 of legs I-III with 2, 2 and 2 pso respectively (Fig. 1A, B). Parapseudocellar formula: 0/000/112001+1^{m} (each of anal valve with one psx) ventrally, absent dorsally (Figs 1A, B, 2G). Pseudopore formula: 0/011/11110 dorsally, 00/111/0001^{m}0 ventrally (Fig. 1A, B).

Head. Antennae short and distinctly segmented, as long as head. Length ratio of Ant. I: II: III: IV as about 1: 1.5: 1.5: 1.5. Subapical organite of Ant.IV with globular apex; basolateral ms at about 1/3 length from base, above the second proximal row of chaetae (Fig. 1F). Ant. III sensory organ composed of 5 papillae, 5 guard chaetae, 2 sensory rods and 2 granulated clubs, the inner bigger than the outer, and a lateral ms (Figs 1D, F). Ant. II with 13 chaetae. Ant. I with 8 chaetae. Antennal base well marked. PAO composed of 20–24 simple vesicles (Fig. 1C). Dorsal cephalic chaeta d0 absent (Figs 1A, 2A). 3+3 p-chaetae present between two inner posterior pso, p1 anterior to others. Mandible with strong molar plate and 4 apical teeth. Maxilla bearing 3 teeth and 6 lamellae. Maxillary palp simple with 1 basal chaeta and 2 sublobal hairs. Labral formula 4/1,4,2;. Labium with 5 proximal, 4 basomedian (E, F, G, f) and 5 basolateral (b, c, d, e, e') chaetae (Fig. 2B); labial type AB, papillae A–E respectively with 1, 4, 0, 3 and 2 guard chaetae (Fig. 1E). Head ventrally with 4+4 postlabial chaetae along ventral groove (Fig. 2B).

Body chaetotaxy. S-chaetae subcylindrical, apically rounded, 11/011/222121 dorsally, 11/000/000110 ventrally (Figs 1A, B); subcoxae 2 of legs I, II and III with 0, 0, 1 S-chaeta respectively. Tiny and blunt ms, present on Th. II–III. Ordinary chaetae differentiated into meso- and macrochaetae, ratio Sp: m1: p1 on Abd. V tergum = 1: 2–2.3: 0.8. Th. I tergum with 7–8+7–8 dorsal chaetae. Th. II–III terga with 4+4 chae-

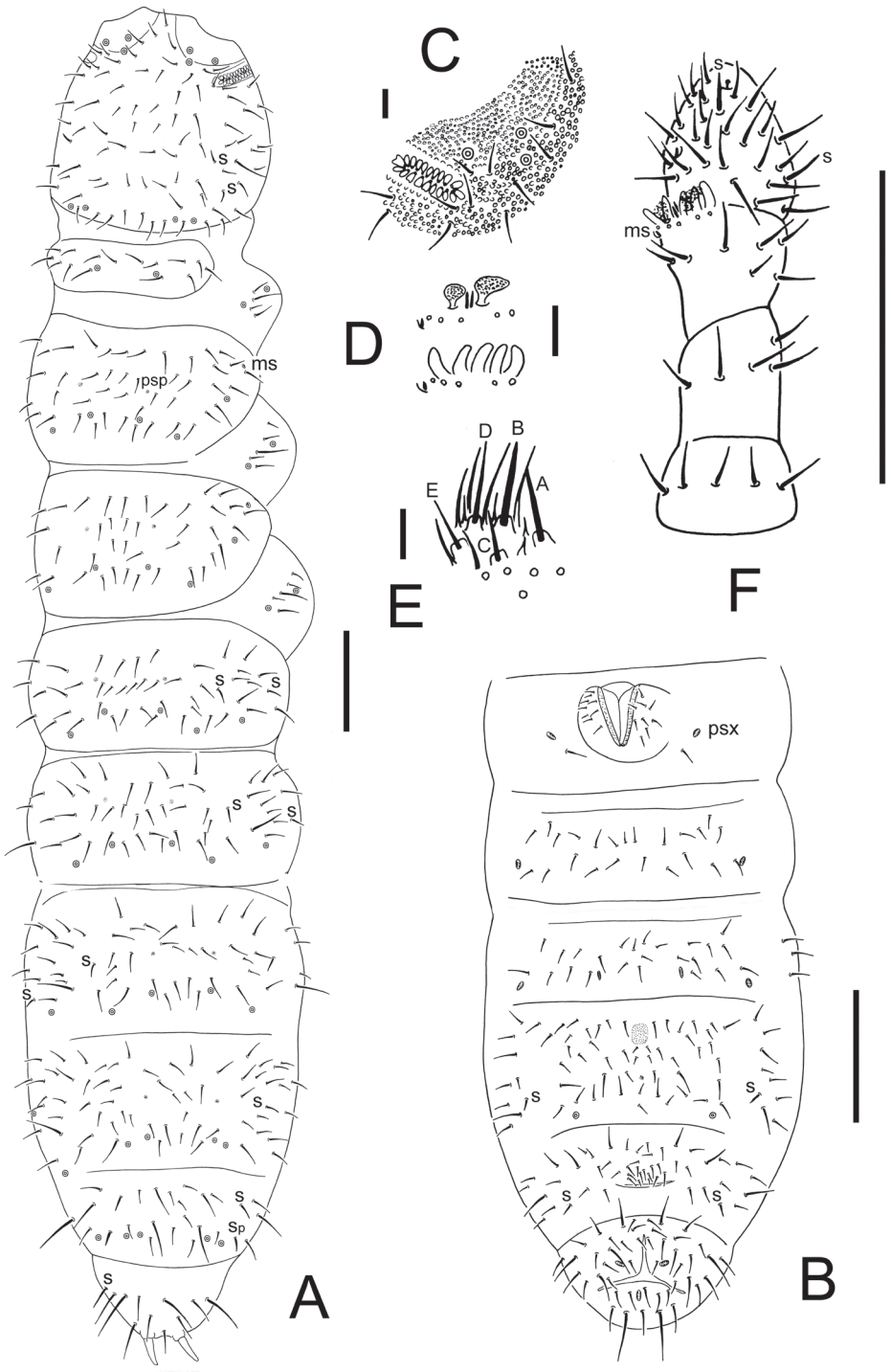

Figure 1. *Thalassaphorura problematica* sp. n. **A** dorsal side of body **B** ventral side of Abd. I–VI **C** PAO **D** clubs and papillae of AIIIO **E** Labium **F** Antenna. Scales: 0.1 mm (**A–B, F**), 0.01 mm (**C–E**).

tae and Abd. I–III terga with 3+3 chaetae along axis respectively (Fig. 1A). Abd. IV–V terga with one axial chaeta (p0) each, sometimes with asymmetric chaetae along axis. Abd. VI tergum with two axial chaetae (a0 and p0) (Figs 1A, 2C). Sterna of Th. I, II, and III with 0+0, 1+1, 1+1 chaetae respectively.

Appendages. Subcoxa 1 of legs I–III with 4, 5 and 5 chaetae, subcoxa 2 with 0, 4 and 4 chaetae respectively. Tibiotarsi of legs I, II and III with 14 (1, 7, 6) chaetae each (Fig. 2E). Unguis without teeth. Unguiculus short, about 0.3 times as long as inner edge of unguis, with inner basal lamella (Fig. 2E). Ventral tube with 1+1 basal and 8–11+8–11 distal chaetae (8–10+8–10 in female, 11+11 of which 9+9 modified in males) (Fig. 2D). Furca reduced to a field of fine granulation with 4 small dental chaetae arranged in 2 rows posteriorly; only one manubrial row of chaetae present posteriorly to dental chaetae (Fig. 2F).

Genital plate with 14–15 chaetae in females, 33–36 chaetae in male. Anal valves with numerous acuminate chaetae; each lateral valve with a0 and 2a1; upper valves with chaetae a0, 2b1, 2b2, c0, 2c1, 2c2 (Fig. 2G). Anal spines set on distinct papillae, 1.1–1.2 times as long as inner edge of hind unguis.

Derivatio nominis. Named for its unusual characters among *Thalassaphorura*.

Discussion. The new species is closest to the genus *Thalassaphorura* by its simple vesicles in PAO and the furcal rudiment. However, it does not match the definition of this genus proposed by Sun et al. (2010), nor those given previously by Weiner (1996), Fjellberg (1999) or Pomorski (1998) for three characters: absence of chaeta d0 on head, 6 chaetae in the distal whorl of tibiotarsi of all legs, and labium type AB. In order not to erect a new genus in a tribe in need of revision (Sun et al. 2011) and for a species otherwise very similar to existing *Thalassaphorura*, we placed our new species in the genus *Thalassaphorura* and broadened its diagnosis.

The new species belongs to the species-group of *Thalassaphorura* which has modified ventral chaetae in the adult male ("male ventral organ"), including the species *T. petiti* Sun & Wu, 2013, *T. bisetosa* Sun & Wu, 2013, *T. qinlingensis* Sun & Wu, 2013, *T. macrospinata* Sun & Wu, 2012 and *T. qixiaensis* Yan, Shi & Chen, 2006, all described from China. These species can be distinguished easily by the position or the number of modified chaetae of the male ventral organ, dorsal and ventral pso formula, and ventral psx formula.

Assigning the new species to this genus led us to re-examine three important taxonomic characters that separate the new species from most other *Thalassaphorura*.

The distal tibiotarsal chaetae have been recently checked in the genera *Allonychiurus*, *Onychiurus* and *Thalassaphorura* (Sun et al. 2010; Sun et al. 2011; Sun and Zhang 2012), showing that this character has a limited taxonomical value to discriminate these genera. In addition, paratypes of *T. petaloides* (Rusek, 1981) from Iraq and specimens of the same species from southern China were found to actually have 15 (1, 7, 7), 14 (1, 7, 6) and 14 (1, 7, 6)) chaetae on tibiotarsi I, II and III. Together with reduced tibiotarsal chaetotaxy of the new species described here, this leads us to extend the diagnosis of *Thalassaphorura* to species with 6, 7 or 9 chaetae in the distal row of tibiotarsus.

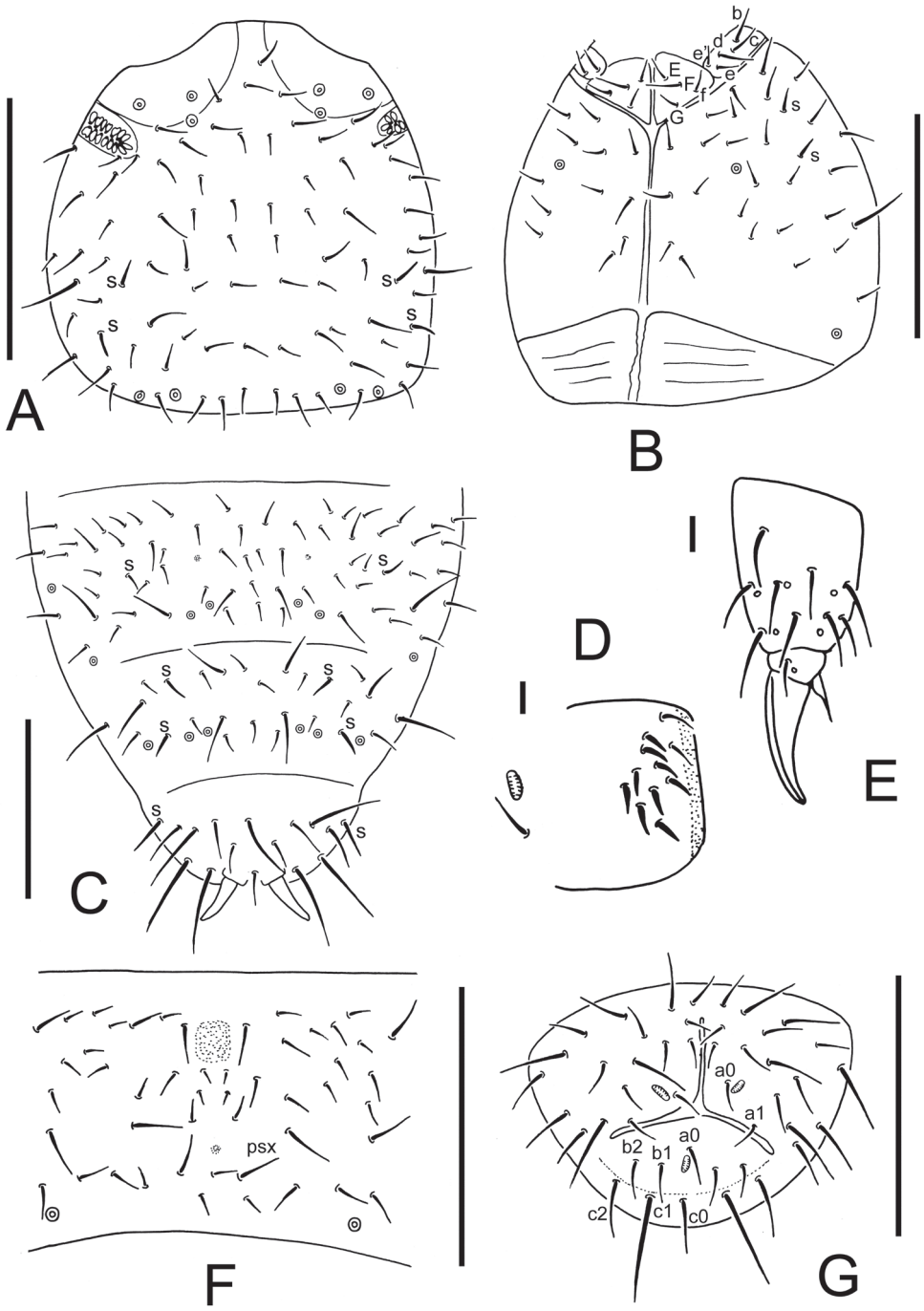

Figure 2. *Thalassaphorura problematica* sp. n. **A** dorsal side of head **B** ventral side of head **C** Abd. IV–VI terga **D** ventral tube (showing male ventral organ) **E** distal part of leg III **F** furca **G** anal valves. Scales: 0.1 mm (**A–C** and **F–G**), 0.01 mm (**D–E**)

Chaeta d0 on head is considered as a stable character at the generic level. It is present in all species of *Thalassaphorura* (Sun et al. 2011) except *T. jailolonis* (Yoshii & Suhardjono, 1992) from Malukku (Indonesia) and the new species *T. problematica* sp. n. The species *jailolonis* was described in *Jailolaphorura* Yoshii & Suhardjono, 1992 (a subgenus of *Onychiurus* , upgraded to genus level by Weiner in 1996), but was subsequently transferred to *Thalassaphorura* by Bellinger et al. (1996–2013) according to a personal communication of Pomorski in 2002. This assignation is however uncertain because the chaetotaxy of the furcal rest is unknown in *T. jailolonis*. At this point, we consider that the diagnosis of the genus *Thalassaphorura* should provisionally state that d0 is present or absent on head, waiting for a re-examination of *T. jailolonis* on fresh material.

The third character, labium type, is not stable in several genera of Thalassaphorurini, being AC or ABC in *Allonychiurus* and *Sensillonychiurus* (Babenko et al. 2011), and even A, AC or ABC in *Thalassaphorura* (Sun et al. 2010). In our new species, labium is still of another type – AB. Moreover, labial type is undescribed in many species. This high intra-generic variability implies that this character should not be considered diagnostic at a generic level among Thalassaphorurini.

An amended diagnosis of the genus *Thalassaphorura* and an updated key of the genera of Thalassaphorurini integrating these changes are given below.

***Thalassaphorura* Bagnall, 1949**
http://species-id.net/wiki/Thalassaphorura

Type species: *Onychiurus thalassophilus* Bagnall, 1937

Diagnosis. Postantennal organ oval, with numerous simple vesicles perpendicular to the long axis; antennal basis more or less indicated; clubs of AIIIO smooth, ribbed or granulated; Ant. IV with S-chaetae differentiated or not, ms close to the second row of chaetae, and no bulb on Ant. IV; labral chaetae formula 4/1,4,2; no multiplication of dorsal pseudocelli, 3 (rarely 4 or 2) pseudocelli in the antenno-basal group, 3–4 (rarely 2 or 5) pseudocelli per half-tergum on Abd. IV, 3 (rarely 4 or 2) pseudocelli per half-tergum on Abd. V (1–3 in a postero-internal group, one in a postero-lateral group); chaeta d0 on head present, rarely absent; Th. I usually with pseudocelli; Abd. VI with one or two axial chaetae (a0 or m0, or both); anal spines present or absent; distal whorl of tibiotarsal chaetae as 6, 7 or 9, no clavate tenent hairs; furcal rudiment as a finely granulated area with 4 small dental chaetae in two rows posteriorly, one manubrial row of chaetae present posteriorly to dental chaetae.

Key to genera of the tribe Thalassaphorurini

1 Postantennal organ with simple vesicles ..... ***Thalassaphorura***
– Postantennal organ with compound vesicles **2**

2(1) Chaeta d0 on head present.....**3**
– Chaeta d0 on head absent.....**5**
3(2) Multiplication and unusual position of anterior pso on head and on Abd. IV–V .....***Micronychiurus*** **Bagnall, 1949**
– Low number of dorsal pso in usual position.....4
4(3) Anal spines absent.....***Agraphorura*** **Pomorski, 1998**
– Anal spines present .....***Allonychiurus*** **Yoshii, 1995**
5(2) Distal whorl of tibiotarsi with 11 chaetae.....6
– Distal whorl of tibiotarsi with 7 or 9 chaetae .....
.....***Sensillonychiurus*** **Pomorski & Sveenkova, 2006**
6(5) Abd. V–VI terga fused, Abd. III sternum not divided in two sub-sterna.....
..... ***Detriturus*** **Pomorski, 1998**
– Abd. V–VI terga not fused, Abd. III sternum divided in two sub-sterna.....
..... ***Spinonychiurus*** **Weiner, 1996**

Notes. The genus *Thibaudichiurus* Weiner, 1996 was synonymized with the genus *Allonychiurus* by Babenko et al. (2011), but is still assigned to the tribe Thalassaphorurini by Bellinger et al. (1996–2013). Here we prefer to accept the synonym. The genus *Dungeraphorura*, of uncertain tribal position, has closer relation to the genera of the tribe Thalassaphorurini by three key characters, simple vesicles in postantennal organ, presence of d0 chaeta on head and 9 distal chaetae on tibiotarsi (Gulgenova and Potapov 2012). In the present key, we don't include this genus because of its furcal rudiment, reduced to a cuticular pocket while it is reduced to a finely granulated area in the tribe.

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Lumicella, a new genus of the tribe Empoascini (Hemiptera, Cicadellidae, Typhlocybinae) from China

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§ *http://zoobank.org/8A2F8AB5-D18F-43F8-B33A-DDE57F2B305B*
| *http://zoobank.org/72814FDB-6F9B-4A56-B2F4-2DB95D631784*

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Abstract

Lumicella rotundata **gen. et sp. n.** is described based on specimens from Fujian Province, China. Habitus photos and illustrations of male genitalia of this new species are provided. Differences between the new genus and closely related genera are discussed.

Keywords

Homoptera, Auchenorrhyncha, leafhoppers, taxonomy, distribution

Introduction

The fauna of Empoascini in China is very rich and diverse, this is associated with China's high biodiversity. To date, 31 genera of this tribe have been described in Chinese fauna (Matsumura 1931; Dworakowska 1971, 1973, 1982, 1993, 1995; Zhang 1990; Qin 2003; Zhang and Qin 2004, 2005; Qin and Zhang 2003, 2008; Qin et al. 2010, 2011a, 2011b, 2013); Qin and Zhang (2008) provided a key to the genera

of the tribe from China. However, our knowledge of the Chinese fauna of this tribe is still incomplete with many genera and species remaining to be described. In this paper, a new genus and species is described based on our recent examination of unidentified materials collected from southern China, as well as habitus photos and drawings of male genitalia of the new species.

Material and methods

The specimens examined in this study are deposited in the Entomological Museum, Northwest A&F University, Yangling, Shaanxi, China (NWAFU). The entire male abdomen of the examined specimens were removed and cleared in 10% NaOH and drawn from preparations preserved in glycerin. External morphology was observed under an Olympus SZX-10 microscope. Photographs of the specimens were made using a Nikon SMZ 1500 microscope with a Retiga 2000R camera (CCD). Images were produced using the software Auto-Montage Pro. The male genitalia were drawn using a Olympus PM-10AD, and wings were drawn with a Leica MZ-12.5 microscope. All the pictures were edited and enhanced using Adobe Photoshop CS7.0 (Adobe Systems). The body measurements are from apex of the vertex to the tip of the forewing.

Morphological terminology predominantly follows Zhang (1990) except for the nomenclature of the wing and setae on the subgenital plate, where we follow Dworakowska (1993) and Southern (1982) respectively.

Taxonomy

Lumicella **Lu & Qin, gen. n.**
http://zoobank.org/1CF76EB3-99CB-441A-B1F4-10178954ED2A
http://species-id.net/wiki/Lumicella

Type species. *Lumicella rotundata* Lu & Qin, sp. n., here designated.

Description. Body small. Head with eyes broader than maximum width of pronotum (Figs 1, 3). Vertex short, rounded anteriorly (Figs 1, 3), profile of transition to face rounded (Fig. 2), coronal suture long (Figs 1, 3). Face narrow and slightly convex in profile, lateral frontal suture present (Figs 2, 4). Forewing narrow, rounded apically, apical cells occupying less than one-third total length, all apical cell with separate bases, 2nd apical cell with margins subparallel but slightly broadened at apex, c and r cells nearly equal in width, narrower than m and cua cells; veins RP, MP' arise from r cell and MP"+CuA' from m cell (Fig. 9). Hindwing with bifurcation point of CuA basad of coalescence of CuA with MP" (Fig. 10).

Male basal abdominal sternal apodemes developed, apically rounded and parallel sided (Fig. 8). Male pygofer elongate, strongly narrowing caudad, terminally with rigid microsetae on each side of lobe, ventral appendage present (Figs 5, 6, 11–13), dorsal

bridge short, less sclerotized in middle dorsocaudad (Fig. 6). Subgenital plate much exceeding pygofer side, A-group setae distinct, C-group setae arranged in a single row and reaching apex of plate (Figs 5, 11, 18, 19). Paramere slim, apophysis bearing prominent dentifer and a few slender setae (Figs 5, 7, 11, 19, 20). Connective lamellate (Fig. 17). Aedeagus without dorsal apodeme, preatrium well developed, shaft tubular and curved twice, gonopore apical on ventral side (Figs 15, 16). Anal tube process curved and narrowed terminally (Figs 5, 7, 11, 14).

Etymology. The generic name is an arbitrary combination of letters, and is regarded as feminine.

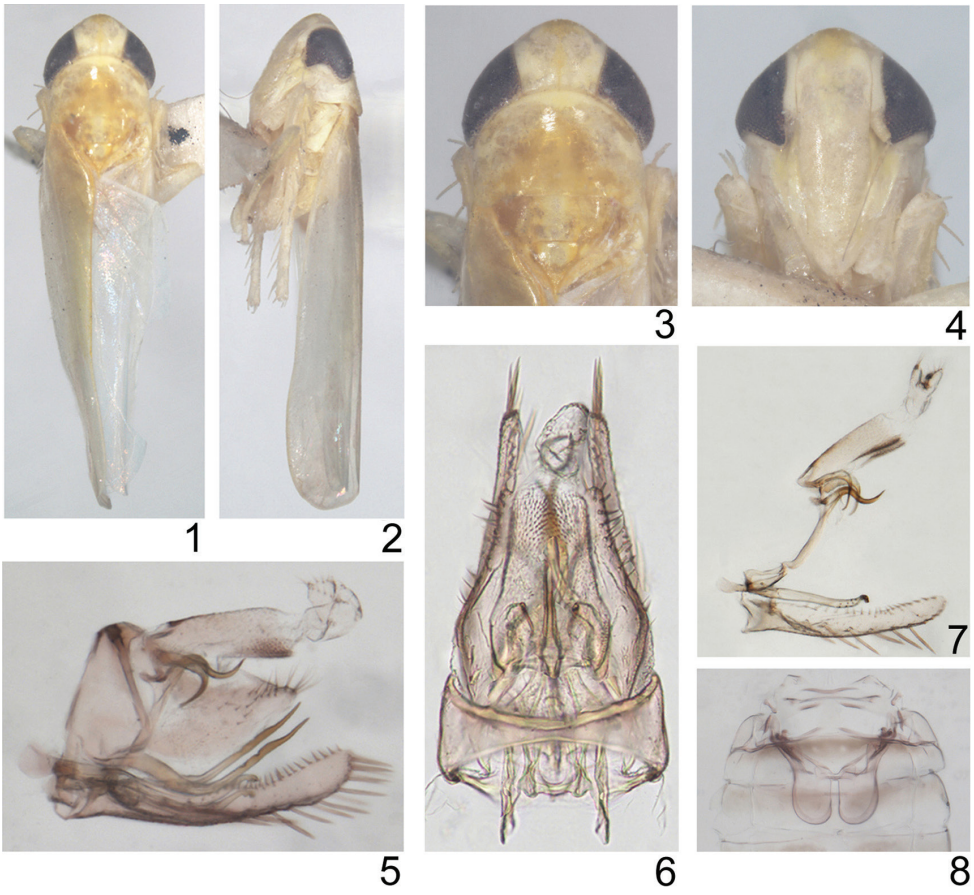
Discussion. In *Alebroides* Matsumura group, the new genus is similar to *Ghauriana* Thapa, *Membranacea* Qin & Zhang, *Dattasca* Dworakowska, *Luvila* Dworakowska, *Szara* Dworakowska, *Szuletaia* Dworakowska, *Luodianasca* Qin & Zhang, *Nikkotettix* Matsumura and *Znana* Dworakowska in having veins RP, MP' of forewing arise from r cell and MP"+CuA' from m cell, all apical cells in fore wing having separate bases (in *Nikkotettix* and *Znana,* 3rd apical cell stalked or sessile) and CuA in the hindwing branched apically. However, this new genus differs from *Membranacea*, *Luodianasca*, *Luvila* and *Szara* in the presence of the ventral pygofer appendage (ventral pygofer appendage absent in these four genera), from *Dattasca* and *Szuletaia* in having bifurcation point of CuA basad of coalescence of CuA with MP" (apicad of coalescence of CuA with MP" in *Dattasca* and *Szuletaia*), from *Znana* in having coronal suture not reaching apex of vertex (surpassing apex of vertex and reaching the level of ocelli on face in *Znana*); from *Ghauriana* in the subgenital plate having A-group setae (A-group setae undifferentiated in *Ghauriana*), from *Nikkotettix* in the absence of ventral process at the base of aedeagal shaft (with ventral process at the base of aedeagal shaft in *Nikkotettix*). The new genus also differs from *Membranacea* in the presence of anal tube appendage (anal tube appendage absent in *Membranacea*) and from *Luvila* in having the C-group setae of subgenital plate arranged in a single row subbasally (C-group setae arranged in two rows subbasally in *Luvila*).

Distribution. China (Fujian).

***Lumicella rotundata* Lu & Qin, sp. n.**
http://zoobank.org/771BE1DE-E369-4879-A4E0-08276BF30F46
http://species-id.net/wiki/Lumicella_rotundata
Figs 1–20

Description. Body length: Male 3.7–3.9mm.

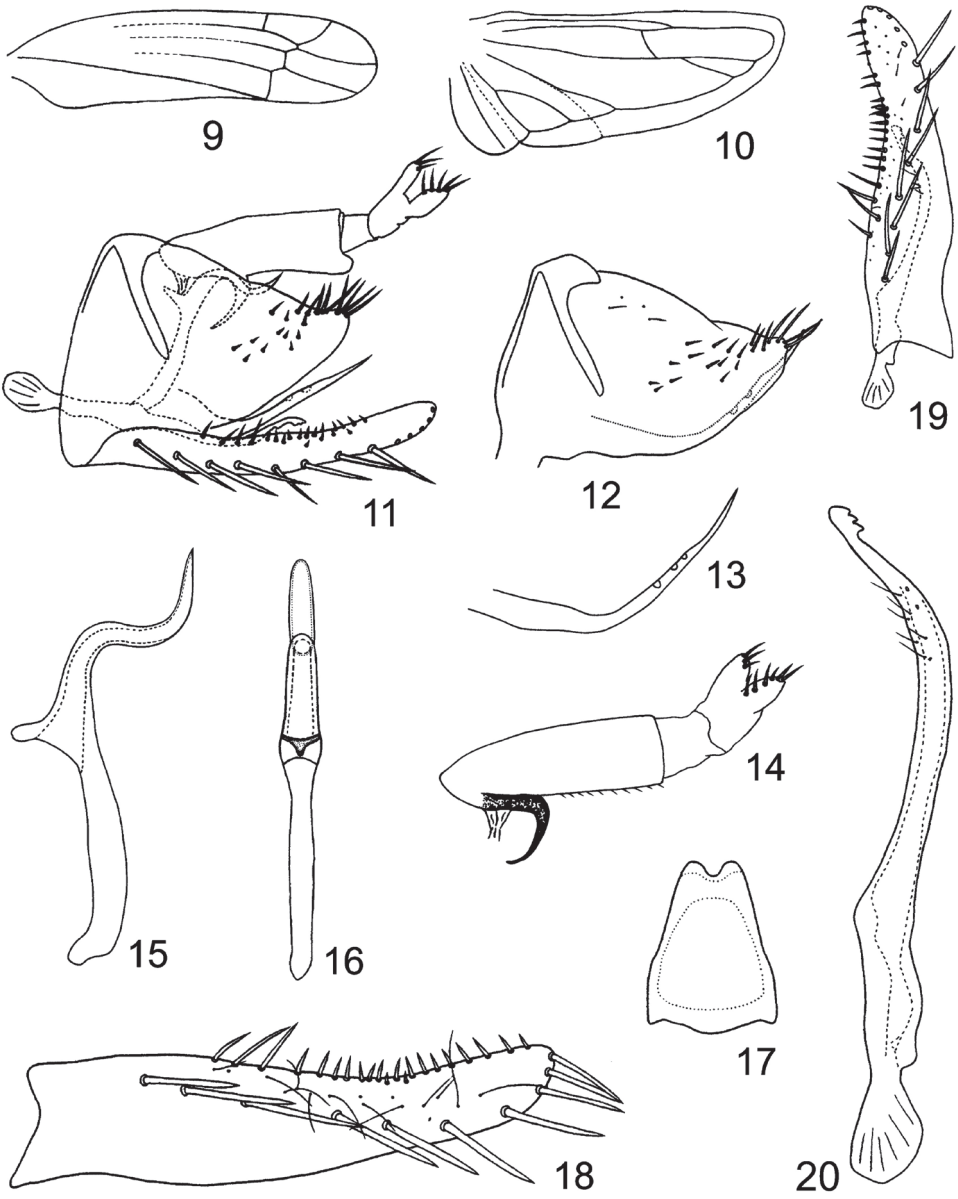
General colour variable: lighter coloured specimens yellow to ochre-yellow. Vertex with borders at eyes creamy-yellowish, semilunar patch mesocaudad of ocelli creamy. Face and basal antennal segments light yellow. Eyes blackish-brown. Disc of pronotum golden-yellow, irregular arch of hypodermal pattern light-yellow in addition to three large creamy patches along anterior margin. Centre of scutellum sordid cream, scutoscutellar sulcus beige. Darker specimens brown to sordid brown, semilunar patch



Figures 1–8. *Lumicella rotundata* sp. n. **1** male adult (abdomen removed), dorsal view **2** same, left lateral view **3** head and thorax, dorsal view **4** face **5** male genitalia, left lateral view **6** same, dorsal view **7** anal tube and anal styli, aedeagus, connective, paramere and subgenital plate, left lateral view **8** abdominal apodemes.

mesocaudad of ocelli, borders at eyes, genae, patches on pronotum and centrally on scutellum, sordid cream.

Male genitalia: Basal sternal abdominal apodemes exceeding half of segment 4 (Fig. 8). Male pygofer with about 16 rigid setae on outer and inner surface of lobe, ventral pygofer appendage slim and bent caudodorsad near base, surpassing caudal margin of lobe, tapering and sculptured with depressions subapically (Figs 5, 11–13). Subgenital plate with nearly same width in basal third, apical 2/3 gradually narrowing towards apex, A-group setae (3–4) rigid, B-group seate (15-17) small, roughly uniseriate along dorsal margin in apical half, C-group setae (13–14) arising near base of plate, sharply terminated, D-group setae roughly bi- or tri-seriate, starting caudad of C-group setae (Figs 5, 11, 18, 19). Paramere sinuate in caudal part, apically bearing 3 big teeth preceded by ca. 6 fine setae and few sensory pits (Figs 5, 11, 19, 20). Connective narrowing to deeply emarginate apex (Fig. 17). Aedeagal shaft tubular,



Figures 9–20. *Lumicella rotundata* sp. n. **9** forewing **10** hindwing **11** male genitalia, left lateral view **12**, pygofer side and ventral pygofer appendage, left lateral view **13** ventral pygofer appendage, left lateral view **14** anal tube and anal styli, left lateral view **15** aedeagus, left lateral view **16** same, dorsal view **17** connective **18** subgenital plate **19** subgenital plate and paramere, dorsal view **20** paramere.

longer than preatrium, in profile its middle part right-angled and curved caudoventrad followed by vertical apical region, gonopore large on ventral side, in ventral view aedeagus with rounded apex (Figs 5, 11, 15, 16). Anal tube process well sclerotized,

originating subapically from ventral margin of anal tube, nearly reaching 1/3 height of pygofer (Figs 5, 11, 14).

Type material. Holotype. ♂ (NWAFU), China, Fujian Province, Wuyi Mountain, 17 Aug 2008, coll. X. Gao and X. T. Li. **Paratypes.** 4♂♂(NWAFU), same data as holotype; 1♂(NWAFU), China, Fujian Province, Wuyi Mountain, 17 Sept 1980, coll. T. Chen; 10♂♂(NWAFU), China, Fujian Province, Wuyi Mountain, 17 Aug 1984, coll. Z. X. Cui.

Etymology. The name is derived from the Latin word "rotundus" (round), which refers to the rounded apex of the aedeagal shaft.

Distribution. Known only from the type locality in Fujian Province in southeastern China.

Host plant. Unknown.

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RESEARCH ARTICLE

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Are some prepupae and pupae of male mealybugs and root mealybugs (Hemiptera, Coccoidea, Pseudococcidae and Rhizoecidae) mobile?

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Abstract

It is hypothesised here that some mealybug (Pseudococcidae) and root mealybug (Rhizoecidae) prepupae and pupae are mobile. The prepupa and pupa of the mealybug *Promyrmococcus dilli* Williams and the prepupa of the root mealybug *Ripersiella malschae* (Williams) are described and illustrated and their probable mobility is discussed. It is also suggested that the prepupae and pupae of the mealybug *Macrocepicoccus loranthi* Morrison can move rapidly on the leaves when disturbed.

Keywords

Coccoidea, Pseudococcidae, Rhizoecidae

Introduction and discussion

Most male scale insects (Hemiptera: Coccoidea) only feed in their first and second instars and then pass through non-feeding prepupal and pupal instars before emerging as an adult that can be either wingless or alate. During development of what are now usually termed the archaeococcoid scale insects (Gullan and Cook 2007), the prepupa of alate species may be similar in morphology to the previous feeding stage except that they have no functional mouthparts, and the legs are sometimes reduced in size. Other

archaeococcoids that are alate may have a prepupa with developing wing pads and legs. The prepupae and pupae of the morphologically more derived families, the neococcoids, show some signs of legs and the alate species possess developing wing pads. For examples of the life cycles of some families see Danzig (1980) and Gill (1993). Before its moult to a prepupa, the second-instar male usually secretes a covering for the developing prepupa and pupa. It may be a fairly loose covering of wax, an intricate shelter of felted wax, a cocoon-like structure as in the Eriococcidae, or a pupal cell formed of translucent wax as in the Coccidae (Gill 1988). Adult males always emerge backwards from whatever covering protected the prepupal and pupal stages.

Because most male prepupae and pupae develop beneath this waxy cover, the legs are usually non-functional with the claw either showing no signs of development or reduced to a mere point. However, among male mealybugs (Pseudococcidae), there are some taxa in which the prepupa and pupa have relatively well-developed legs, including claws and digitules, that would appear to allow mobility of these insects (Figs 1–3).

Digitules in scale insects are modified setae. The term is usually used for a pair of setae at the base of the claw and a pair at the outer distal end of the tibia of all female instars and the male feeding stages of most taxa. Although sometimes these digitules are setose, many are so-called knobbed or actually spoon-shaped. The adult females of *Steingelia* Nasonov (Steingeliidae) have 12 such digitules on the claw (Morrison 1928), but this is not usual. The claw digitules are often longer than the claw and the tarsal digitules are usually longer than the claw digitules. As far as we know, digitules are present in all first-instar nymphs or crawlers, the main dispersal stage. It has been shown that first-instar nymphs of some armoured scale insects (Diaspididae) attach themselves to their phoretic hosts (Diptera and Coleoptera) by the digitules (Magsig-Castillo et al. 2010). These authors suggest that the swollen ends of each digitule can cling by suction. Regardless of the means by which digitules adhere to surfaces, they must be released rapidly as the nymphs walk. In most illustrations of scale insects that possess legs, the claw is a shown as curved inwards because it is in this position on slide-mounted specimens. In life, however, the claws point outwards so that the plantar surface and the digitules are in contact with a surface.

Digitules reach their fullest development in mealybugs of the tribe Allomyrmococcini (Pseudococcidae) as they are much larger than the claw and are widely expanded distally, sometimes wider than the width of the claw (Williams 1978; Dill et al. 2002). These well-developed digitules are used to climb onto herdsmen ants of the genus *Dolichoderus* when the colony is transported or even onto each other when the ants scoop up a few of the mealybugs when the colony is disturbed. Additional knobbed setae, similar to knobbed digitules, are present also on the lateral surface of the tibiae and tarsi of the adult females of the ant-attended mealybugs *Malaicoccus clavulatus* Williams, *M. pilulosus* Williams and *M. sumatranus* Williams, and, in addition to these setae, there are knobbed setae on the tibiae and tarsi and body margin in *Thaimyrmococcus daviesi* Williams (Dill et al. 2002; Williams 2004).

Allomyrmococcine mealybugs associated with herdsmen ants are either in the ant trails or in the ants' nests. It has been shown by Dill et al. (2002) that only about 30% of

the mealybugs from an ant-mealybug association is carried in the trail and the remainder are in the nest. Although some gravid females are in the trails, most are present in the ant nests. It was only at the end of the study of the mealybug *Promyrmococcus dilli* Williams by Dill et al. (2002) that adult males, prepupae and pupae were found and these were in nests that had been dropped whole into ethanol. There was no sign of any wax covering on the prepupae or pupae but all stages had well-developed claws and knobbed tarsal digitules (Figs 1, 2). It would be an advantage for prepupae and pupae of mealybugs associated with herdsmen ants to move in the nests and even attach themselves to ants if the nest was moved to another location and the inference is that they are mobile.

Female mealybugs of the genus *Leptococcus* Reyne and *Macrocepicoccus* Morrison have unusually long legs and long slender claws. These mealybugs live on leaf surfaces and are probably parenchyma feeders (Miller and Denno 1977; Williams 2004; Kondo and Gullan 2008). Adult females and nymphs are very active when disturbed. Claws in all of these stages are long and slender. The prepupa and pupa of *M. loranthi* Morrison and the prepupa of *L. metroxyli* Reyne possess well-developed tarsal digitules although the claw digitules are short (Miller and Denno 1977). *Macrocepicoccus loranthi* was described from Guyana on *Loranthus* sp. by Morrison (1919) and has been recorded from Colombia (also on *Loranthus* sp.) by Kondo et al. (2008). Recent observations of *M. loranthi* on *Oryctanthus amplexicatus* (Loranthaceae) show that aggregations of the mealybugs on the leaves are very active when disturbed (Takumasa Kondo, personal communication). This activity is not only confined to the feeding stages but also applies to the prepupae and pupae, which move rapidly. These prepupae and pupae are without any covering or cocoon-like structures and have little wax.

Among the group of root mealybugs based on the genus *Rhizoecus* Künckel d'Herculais, elevated recently to family level, the Rhizoecidae (Hodgson 2012), there are two subfamilies, the Rhizoecinae and the Xenococcinae. Prepupae and pupae of the Xenococcinae have legs without tarsal or claw digitules, and the claws are very poorly developed (Williams 1998). Within the Rhizoecinae, despite the large numbers of species, little is known of their life histories. Mukhopadhyay and Ghose (1996) report that prepupae and pupae of *Rhizoecus amorphophalli* Betrem are without any covering. Adult males of Rhizoecidae are either wingless, brachypterous or alate (Hodgson 2012). Prepupae and pupae of *Ripersiella malschae* (Williams) possess legs with well-developed claws and, although the claw digitules and tarsal digitules are short and setose (Fig. 3), they are similar to those of mobile female stages, as shown in Williams (2004). This species lives in close association with ants of the genus *Pseudolasius* (Malsch et al. 2001; their mealybug #21). The adult male is wingless. It would be an advantage for the prepupae and pupae living in ants' nests to move and the well-developed claws would help in this process.

It is clear that prepupae and pupae of some Pseudococcidae and probably of the Rhizoecidae can show mobility. Observations on other species of mealybugs and root mealybugs in which the prepupae and pupae have claw and tarsal digitules also may show that these stages can move.

We are taking the opportunity to describe and illustrate the prepupa and pupa of *Promyrmococcus dilli* and the prepupa of *Ripersiella malschae*.

Pseudococcidae

***Promyrmococcus dilli* Williams**
http://species-id.net/wiki/Promyrmococcus_dilli

Promyrmococcus dilli Williams (In Dill et al. 2002: 170).

Material studied. Sabah, Kinabalu, Poring, in nest of *Dolichodorus*, 18.vii.1991, M. Dill (BMNH): 7/2 prepupa (one pharate) + 5 pupae (3 pharate) (good – descriptions taken from non-pharate individuals, with details checked on others).

Prepupa (Fig. 1)

Mounted material. Moderate sized, body 1.23–1.38 mm long, 0.7–0.84 mm widest; oval. Body with numerous very long flagellate setae. Legs and antennae well developed; mouthparts present but lacking stylets; wing buds absent. Ostioles present both anteriorly and posteriorly.

Dorsum membranous, segmentation obvious, particularly on abdomen. Each segment densely covered in fine flagellate setae, each 40–50 μm long; also with frequent, extremely long setae with very flagellate apices, each up to about 350 μm long, distributed approximately as follows: with medial pairs on pro-, meso- and metathorax and on abdominal segments I–VI; with 1 long and 1 slightly shorter seta on each side of each segment but with more on abdominal segments VI and VII; with 1–4 slightly shorter setae anterior to each ostiole, and with intermediate fine flagellate setae (each about 100 or so μm long) sparsely throughout. Loculate pores absent but small simple pores frequent throughout, each about 2 μm wide. Ostioles each 90–95 μm wide. Anus about 45 μm wide, with two setae of intermediate length on either side and a pair on posterior body margin.

Margin not demarcated; without wing buds. Eyespot 33–35 μm wide.

Venter membranous. Circulus present medially between abdominal segments II and III. Fine, flagellate setae similar to those on dorsum, covering most of venter; extremely long flagellate setae only present submarginally on abdominal segments V and VI, and perhaps only marginally on VII; setae of intermediate length infrequent, but present sparsely on VII. Loculate pores, each 6–7 μm wide with an uncertain number of loculi, mainly present medially and submedially on thorax and anterior abdominal segments; simple pores frequent throughout.

Antennae about 515 μm long, 6 segmented but with segment II clearly partially divided and with a campaniform pore present distally on more proximal half of this segment; each segment with many flagellate setae similar to those covering most of body, but with fewest on distal half of segment II; subapical segment with 1 fleshy seta and apical segment with 3 or 4 fleshy setae. Mouthparts clearly present; tentorium barely sclerotized but quite large; labium perhaps 3 segmented, 50–65 μm long, with (on ventral surface) 2 pairs of setae on basal segment, 2 pairs on medial segment and 5 pairs on apical segment; also with 2 pairs on dorsal surface. Spiracles each with peritreme 20–24 μm wide. Legs particularly well developed, lengths (in μm for metathoracic leg): coxa about 120–135; trochanter+ femur 275–310; tibia

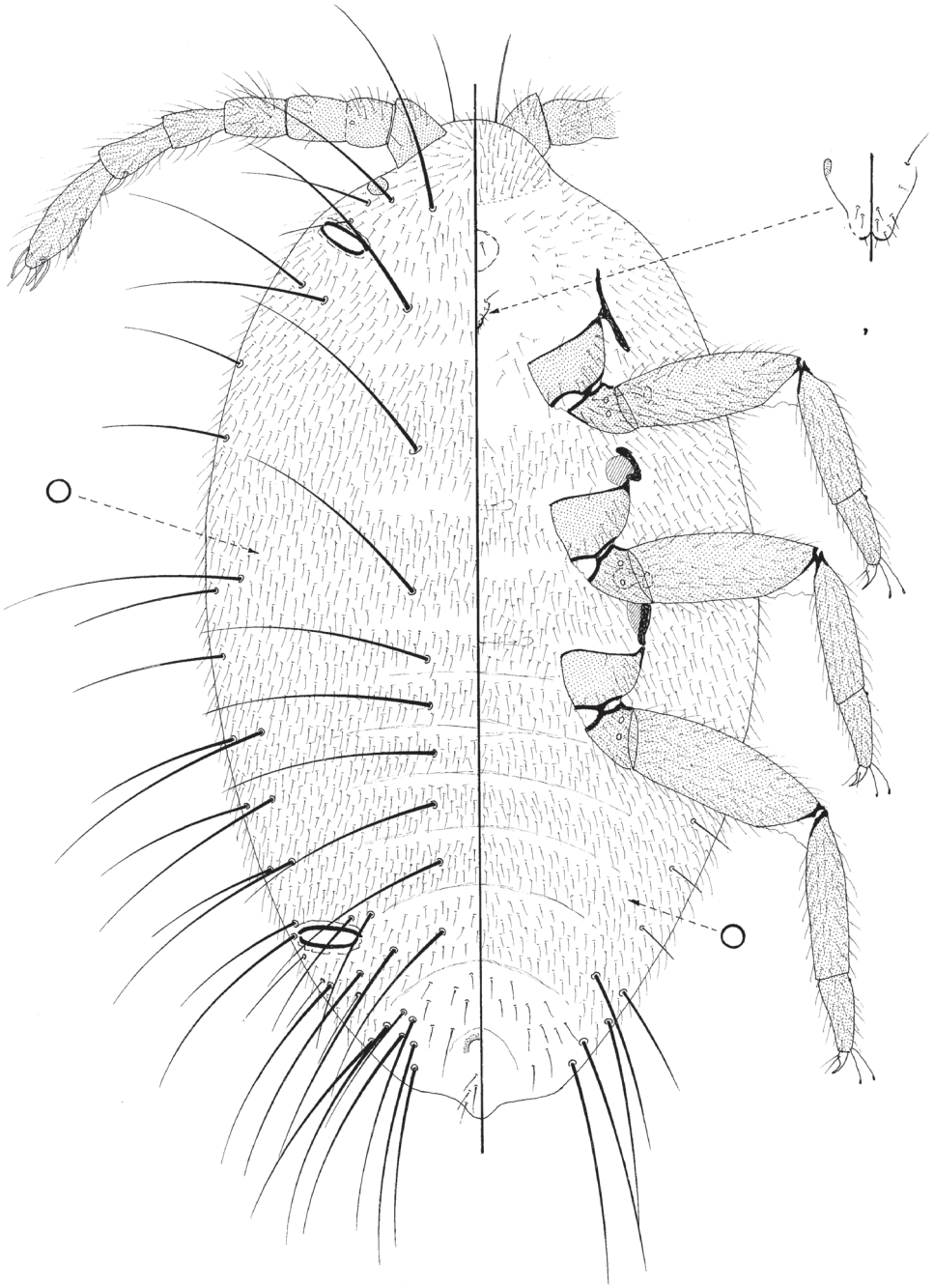

Figure 1. Prepupa of *Promyrmococcus dilli* Williams.

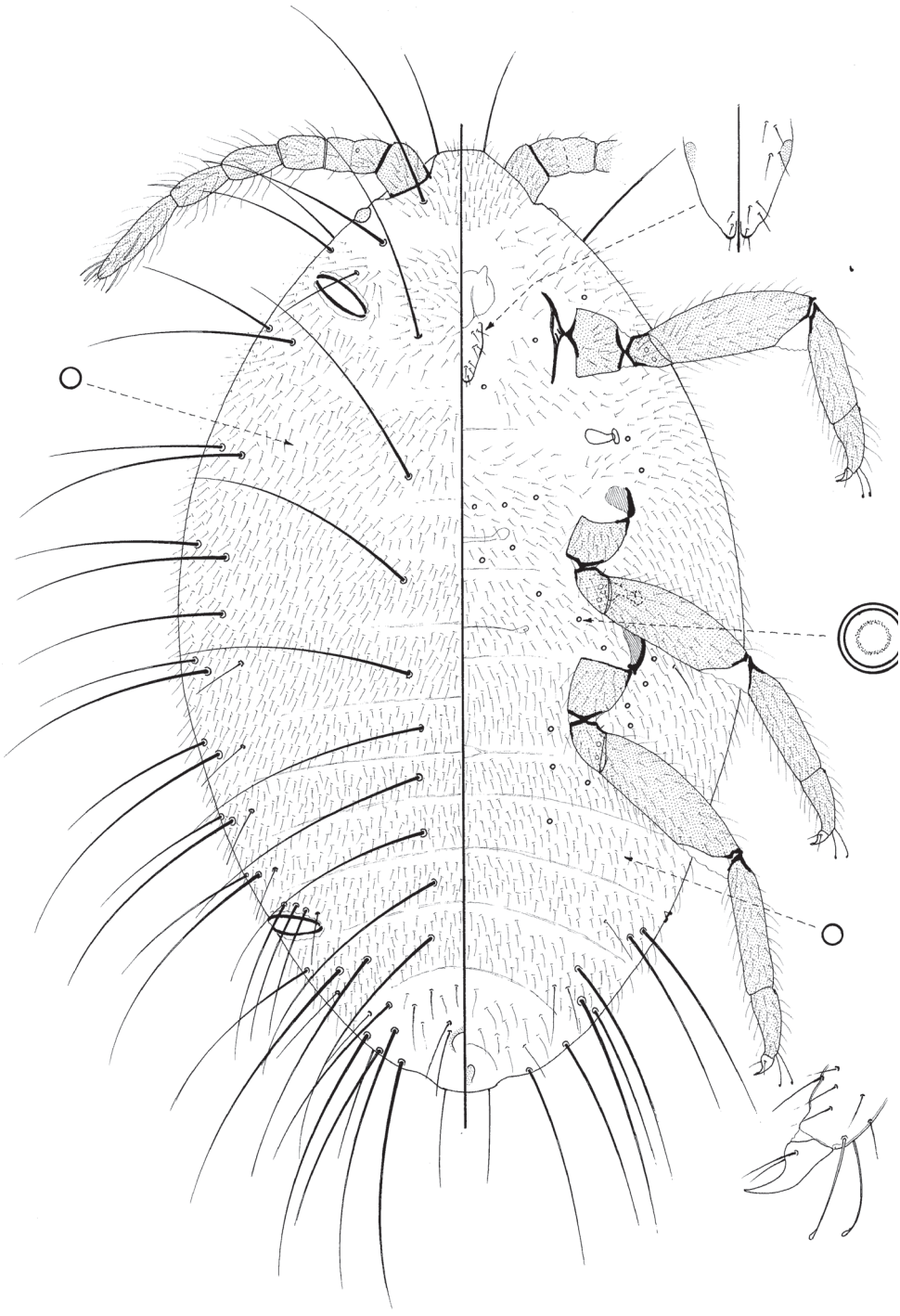

Figure 2. Pupa of *Promyrmococcus dilli* Williams.

190–210; tarsus 95–112; claw 35–38; each trochanter with 2 roundish campaniform pores on each side; each tibia and tarsus without tibial spurs; tarsi one-segmented; tarsi each with a tarsal campaniform pore; tarsal digitules long, extending as long as claw and capitate; claw digitules setose and barely reaching claw apex; claws without a denticle.

Pupa (Fig. 2)

Mounted material. Moderate sized, body 1.20–1.4 mm long, 0.63–0.73 mm widest; oval. Body with numerous very long flagellate setae. Legs and antennae well developed; mouthparts present but very reduced and without stylets; wing buds absent. Ostioles present both anteriorly and posteriorly.

Dorsum almost identical to that of the prepupa, and with 0–3 setae of intermediate length on either side of anus.

Margin not demarcated, without wing buds. Eyespot 33–35 μm wide.

Venter membranous. As for prepupa except loculate pores absent.

Antennae 6 segmented as in prepupa, length about 520–615 μm long. Mouthparts very reduced; tentorium a small, roundish membranous area and labium short, about 35 μm long, with a few setae both dorsally and ventrally. Spiracles each with peritreme 20–28 μm wide. Legs particularly well developed, lengths (in μm for metathoracic leg): coxa about 125–150; trochanter+ femur 295–330; tibia 200–225; tarsus 95–105; claw 35–38; legs otherwise as on prepupa.

Comment. The basic morphology of the prepupa and pupa of *P. dilli* is very similar to that of the adult male (Williams 2004; Hodgson 2012), except that they lack the genital structures, have (at most) 7-segmented antenna (9 segmented on the adult male) and their tarsi appear to be only one segmented. Reduced mouthparts lacking stylets are also present on the adult male.

Rhizoecidae

***Ripersiella malschae* (Williams)**
http://species-id.net/wiki/Ripersiella_malschae

Rhizoecus malschae Williams, 2004: 779.
Ripersiella malschae (Williams): Kozár and Konczné Benedicty 2007: 495.

Prepupa (Fig. 3)

Material studied. Paratype, Sabah, Kinabalu Park, Poring Hot Springs, with *Pseudolasius*, 28.iii.1998, A. Malsch (BMNH): 1/1 pharate prepupa (good, but distribution of pores and leg setae difficult to ascertain as pupa fairly-well developed).

Mounted material. Small, body 508 μm long, 286 μm widest, oval but rather pointed posteriorly. Legs and antennae well developed; mouthparts and wing buds absent.

Dorsum membranous, segmentation obvious, particularly on abdomen. Each segment with a dense band of short setae, each 5–7 μm long on a convex basal socket;

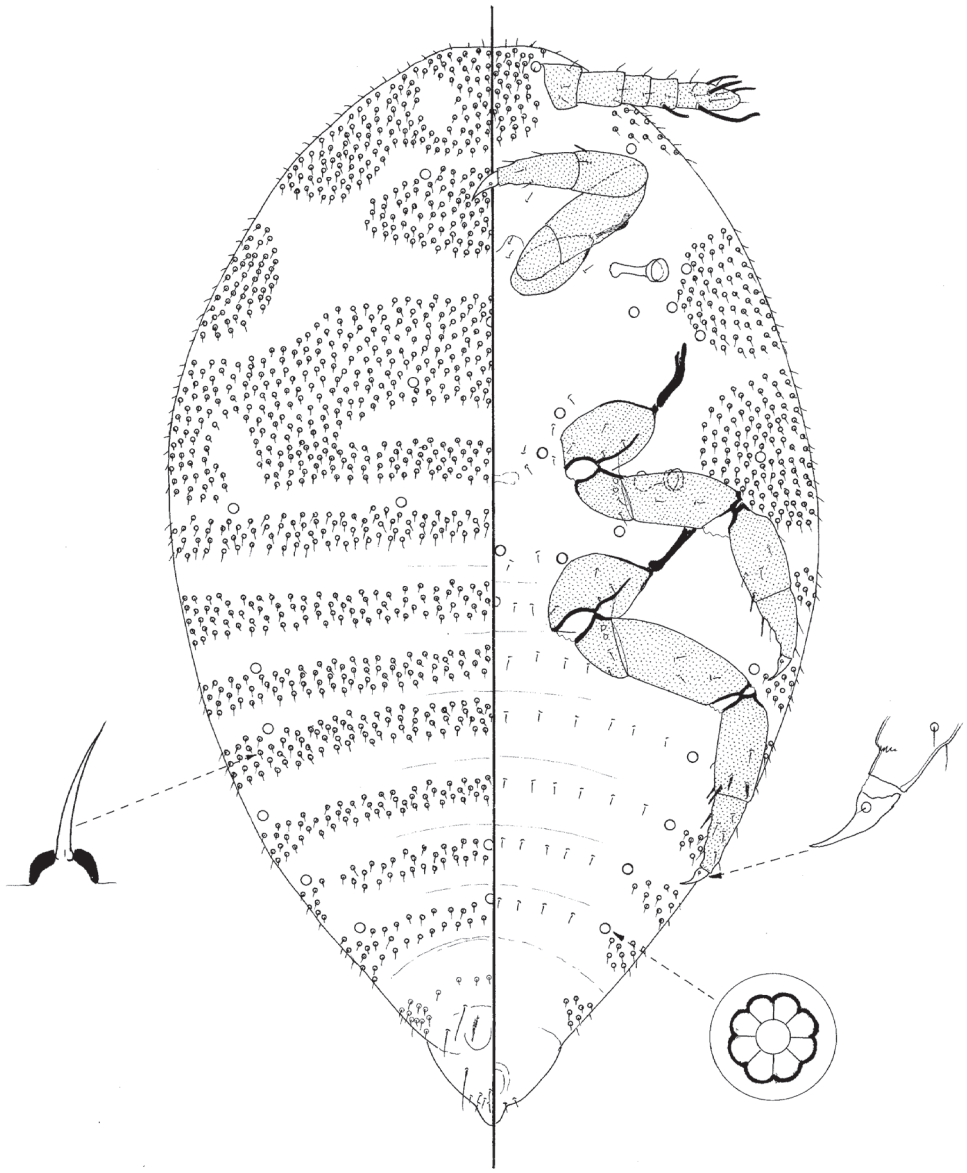

Figure 3. Prepupa of *Ripersiella malschae* (Williams).

bands narrowest on posterior segments. With 3 pairs of long setae on posterior-most segments, each 23–30 μm long; incipient penial sheath with a group of about 18 setae, similar to those on rest of dorsum. With loculate pores, each about 6 μm wide with mainly 8 loculi, near margins of abdominal segments II–VI and also on metathorax.

Margin not demarcated; without wing buds.

Venter membranous. Small setae, similar to those covering most of dorsum, present anteriorly and laterally on head, in large broad groups laterally on pro- and mesothorax, and in small groups laterally on metathorax and abdominal segments II–VII; somewhat similar setae also present very sparsely medially across all segments except perhaps prothorax. Loculate pores similar to those on dorsum present submarginally on abdominal segments and sparsely medially on all thoracic segments and head.

Antennae 6 segmented, about 100 μm long; pedicel very short, about 10 μm long; all segments with a few setose setae; subapical segment with a fleshy seta and apical segment with 3 or 4 fleshy setae. Mouthparts absent. Spiracles each with peritreme 16–18 μm wide. Legs particularly well developed, lengths (metathoracic leg in μm): coxa about 50; trochanter + femur 88; tibia + tarsus 85; claw 21; each trochanter with 2 roundish campaniform pores on each side; each tibia with 2 tibial spurs on distal ventral margin but also with perhaps 2 more laterally; tarsi with a spur-like seta on ventral margin near proximal end; tarsal campaniform pores present but tarsal digitules considered to be absent; claw digitules present but minute; claws without a denticle. Anus apparently on ventral surface.

Comment. The prepupa of *R. malschae* looks similar to the adult male (Hodgson 2012) but lacks the well-developed penial sheath. In addition, the loculate pores on the prepupa clearly have mainly 8 loculi whereas they appeared to have 5 or fewer on the adult male.

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RESEARCH ARTICLE

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Pseudouroctonus peccatum, a new scorpion from the Spring Mountains near "Sin City," Nevada (Scorpiones, Vaejovidae)

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Abstract

A new scorpion species is described from the Spring Mountain Range near Las Vegas, Nevada. The new species appears to be geographically isolated from other closely related species of *Uroctonites* Williams & Savary and *Pseudouroctonus* Stahnke. We tentatively place the new species in *Pseudouroctonus* and provide detailed descriptions and illustrations of type material. We compare the new species to 17 congeneric taxa, briefly discuss the taxonomic history of *Pseudouroctonus*, and provide DNA barcodes for two paratypes to assist ongoing research on the systematics of family Vaejovidae.

Keywords

Barcoding, COI, hemispermatophore, *Pseudouroctonus*, taxonomy, *Uroctonites*

Introduction

Low dispersal potential and ecological specialization (stenotopy) are thought to make certain groups of scorpions predisposed to accelerated diversification (Prendini 2005; Bryson et al. 2013a). Scorpions restricted to highland ecosystems are particularly diverse, perhaps resulting from allopatric divergence on small spatial scales (microallopatry, see Fitzpatrick et al. 2008) facilitated by historical changes in geomorphology and climate regimes (Bryson et al. 2013a, 2013b). Recently, this hypothesis has been repeatedly supported by the discovery of numerous new scorpion species from isolated mountain ecosystems, especially from the "sky islands" region of the North American aridlands (Graham 2007; Ayrey 2009, 2012; Graham and Bryson 2010; Ayrey and Soleglad 2011; Hughes 2011; Graham et al. 2012; Sissom et al. 2012; Ayrey and Webber 2013).

The Spring Mountain Range, located just outside of Las Vegas in southern Nevada, is among the most insular of the sky islands, reaching elevations more than 3,400 m above the Mojave Desert lowlands. While conducting diurnal surveys for myriapods in the Spring Mountains, we serendipitously discovered yet another new scorpion that appears to be restricted to a sky island ecosystem. After numerous diurnal and nocturnal (using UV light; Stahnke 1972) surveys, we only managed to collect a total of five individuals from mixed pine-oak woodlands in Kyle Canyon, one of the most heavily visited regions in the Spring Mountains. Unfortunately, forest fires ravaged the type locality shortly after we collected the type series and surveys in other areas of the mountains were unsuccessful.

The new species is clearly a member of family Vaejovidae, and is most similar to genera *Pseudouroctonus* and *Uroctonites*, both of which are stenotypic and contain species endemic to sky island ecosystems in southwestern North America. Interestingly, the Spring Mountains are situated in the middle of a substantial gap between the known distributions of these two genera (Fig. 1), so the new species could prove to be a missing link in our understanding of the biogeography of this group (Bryson et al. 2013a) and the southwestern sky islands. Herein, we tentatively place the new species in *Pseudouroctonus*, although we predict that the genus is polyphyletic and in need of a thorough systematic revision.

Since the population at the type locality may have been extirpated during recent fires, we provide DNA barcodes (COI) for two specimens (paratypes) to assist colleagues in their ongoing research on the biogeography and systematics of family Vaejovidae. Given that the species went undetected for so long despite occurring in a populated region near one of the most visited cities in the world, we suspect that similar new species may still await discovery in the more remote and less-explored sky islands of southern Nevada and California.

Brief taxonomic history. Of the 22 species and subspecies currently comprising genera *Pseudouroctonus* and *Uroctonites* (including the new species described herein), sixteen were described by Gertsch and Soleglad (1972). At that time, twelve of these species were placed in genus *Uroctonus* (now in family Chactidae) and the other four in genus *Vaejovis*. Stahnke (1974) moved most of the species placed in *Uroctonus* into genus *Vaejovis* and created the new genus *Pseudouroctonus* solely for *P. reddelli*. Stockwell (1992) reversed most of Stahnke's taxonomic acts by moving the species Stahnke placed in *Vaejovis* into *Pseudouroctonus*. In their important paper, Williams and Savary

(1991) defined the new genus *Uroctonites* comprised of a new species, *U. giulianii*, and three *Pseudouroctonus* species originally defined by Gertsch and Soleglad (1972). Finally, four other species have now been placed in *Pseudouroctonus*: *P. m. minimus* (Kraepelin, 1911), *P. glimmei* Hjelle, 1972, *P. sprousei* Francke & Savary, 2006, and *P. saavasi* Francke (2009). See Soleglad and Fet (2003:103–104) for a more detailed discussion on the taxonomic history of these interesting scorpions.

Material and methods

Measurements are as described by Sissom et al. (1990), trichobothrial patterns are as in Vachon (1974) and Soleglad and Fet (2003), and pedipalp finger dentition follows Soleglad and Sissom (2001).

Acronyms of depository.—AMNH, American Museum of Natural History, New York, New York, USA; MRG, personal collection of Matthew R. Graham, Willimantic, Connecticut, USA.

Molecular analysis

We extracted total genomic DNA from leg tissue from the left side of the two female paratypes using a DNeasy Extraction Kit (Qiagen Inc.). A portion of the cytochrome oxidase subunit I (COI) gene, which is used for barcoding, was amplified with forward primer COI_modF (5' - ATCATAAGGATATTGGGACTATGT - 3', Bryson et al. 2013) and reverse primer LE1r (5' - GTAGCAGCAGTAAARTARGCYCGAGTATC - 3', Esposito 2011). Double-stranded cycle sequencing was performed using the same primers and the Big Dye Terminator v. 3.1 Cycle 6 Sequencing Kit (Applied Biosystems). The COI sequences were submitted to the Barcode of Life Data system (BOLD, Ratnasingham and Hebert 2007), and GenBank (Accession Nos. KF841448 & KF841449).

Systematics

Family Vaejovidae Thorell, 1876
Subfamily Vaejovinae Thorell, 1876
Genus *Pseudouroctonus* Stahnke, 1974

***Pseudouroctonus peccatum* sp. n.**
http://zoobank.org/0392FDFD-1BC6-4D7D-B80A-C232C9BDEBCB
http://species-id.net/wiki/Pseudouroctonus_peccatum
Figs 1–17; Table 1–2

Type material. United States: *Nevada:* female holotype, Kyle Canyon Road, Spring Mountains, 36.2666°N, 115.5988°W, 10 May 2013, A.E. Tate, R.R. Riddle, and

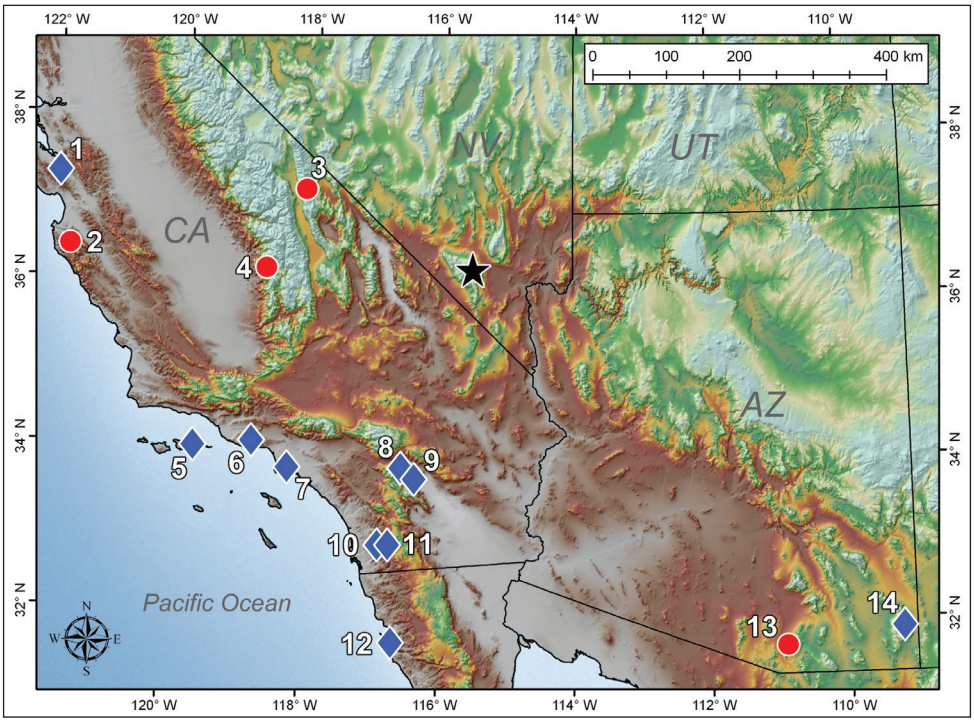


Figure 1. Map depicting the distribution of *P. peccatum* sp. n. (black star) and type localities of geographically proximate species in genus *Pseudouroctonus* (blue diamonds) and *Uroctonites* (red circles): **1** *P. glimmei* (Hjelle, 1972) **2** *U. montereus* (Gertsch & Soleglad, 1972) **3** *U. giulianii* William & Savary, 1991 **4** *U. sequoia* (Gertsch & Soleglad, 1972) **5** *P. minimus thompsoni* (Kraepelin, 1911) **6** *P. angelenus* (Gertsch & Soleglad, 1972) **7** *P. minimus minimus* (Kraepelin, 1911) **8** *P. bogerti* (Gertsch & Soleglad, 1972) **9** *P. andreas* (Gertsch & Soleglad, 1972) **10** *P. williamsi* (Gertsch & Soleglad, 1972) **11** *P. minimus castaneus* (Gertsch & Soleglad, 1972) **12** *P. rufulus* (Gertsch & Soleglad, 1972) **13** *U. huachuca* (Gertsch & Soleglad, 1972) **14** *P. apacheanus* (Gertsch & Soleglad, 1972).

M.R. Graham *leg.* (AMNH, MRG1251); paratype, mature male, same location as holotype, A.E. Tate and R.R. Riddle *leg.* (AMNH, MRG1252); paratype, juvenile female, collected with holotype (AMNH, MRG1222); paratype, juvenile female, same location as holotype, 23 April 2013, A.E. Tate, R.R. Riddle, and D.R. Tate *leg.* (AMNH, MRG1221); juvenile female, same location as holotype, 2 July 2013, R.R. Riddle *leg.* (AMNH, MRG1253).

Etymology. The specific epithet is Latin for "sin" in reference to the proximity of the type locality to Las Vegas, which is known informally as "Sin City."

Diagnosis. Medium to large sized species for the genus, females reaching 50 mm; pectinal tooth counts 13–14 for females and 15 for males. Hemispermatophore lacking secondary lamellar hook; lamellar hook protrudes somewhat from the lamina base creating a modest basal constriction; lamina terminus with a distinct distal crest. Chelicera movable finger ventral edge with low-profiled pigmented crenulated teeth; fixed

Figure 2–3. Dorsal view of male (**2**) and female (**3**) *Pseudouroctonus peccatum* sp. n. *in vivo*.

finger ventral edge with several small flat pigmented protuberances; movable finger dorsal edge with two subdistal (*sd*) denticles. Chelal movable finger with seven inner denticles (*ID*). Metasomal segment V somewhat stout in adults, length compared to width 1.908 in females and 1.953 in males.

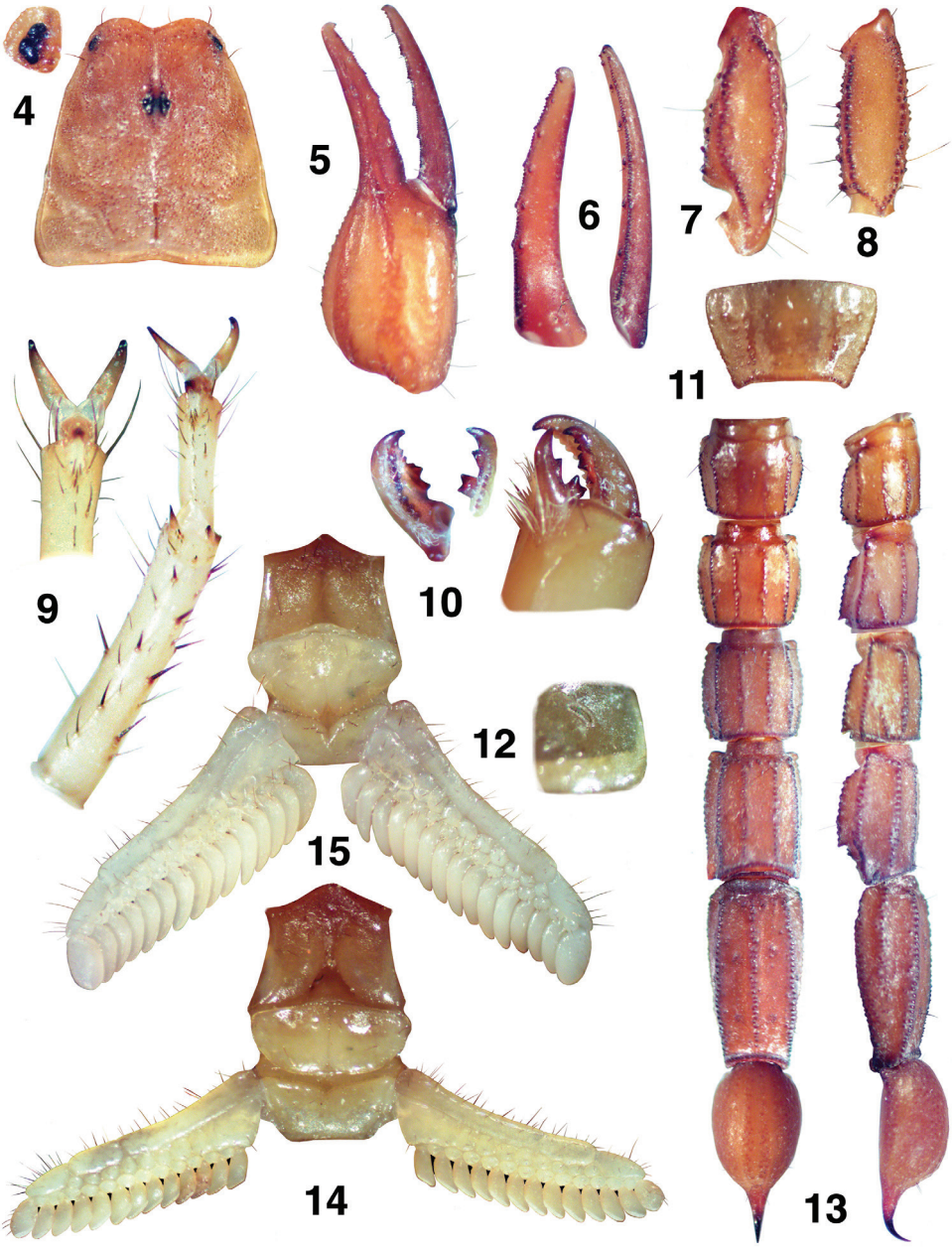
Description of holotype. *Color:* Carapace, trochanter, femur, patella, tergites, and metasoma have a yellow-orange base color with dark brown to black markings along the carinae of the pedipalp and metasoma. Legs are light yellow. Pedipalp chelae are dark brown in color with darker reddish-brown coloration at the anterior portion of the palm where the fixed finger and movable finger meet. Chelicerae are light yellow on proximal half with dark reddish-brown fingers. Vesicle portion of the telson is yellow-orange with a dark reddish-brown to black aculeus. Pectines and genital operculum are light yellow.

Morphology: Carapace: trapezoidal with noticeably emarginated anterior margin; surface finely granular with scattered small granules, with larger granules symmetrically flanking the lateral and median eyes; median furrow is slight and traverses length of carapace; ratio of location of median eyes location (from anterior edge)/carapace length = 0.338. Tergites: surface with small granules on distal 1/3–1/2 of tergites I–VI; tergite VII with two pairs of granular lateral carinae, and a slight median hump. Sternites: III–VI smooth to very finely granular and without

Table 1. Measurements (in millimeters) of all known specimens of *Pseudouroctonus peccatum*.

Scorpion ID	Holotype MRG1251	Female MRG1221	Female MRG1222	Male MRG1252	Female MRG1253
Total Length	52.45 mm	32.37 mm	27.71 mm	36.12 mm	39.83 mm
Carapace L	5.59 mm	4.37 mm	3.66 mm	4.53 mm	4.90 mm
Mesosoma L	19.89 mm	8.30 mm	8.67 mm	9.79 mm	13.94 mm
Metasoma L	20.8 mm	15.00 mm	11.70 mm	17.03 mm	16.36 mm
MET I L	3.15 mm	2.43 mm	1.98 mm	2.66 mm	2.53 mm
MET I W	3.45 mm	2.55 mm	2.12 mm	2.63 mm	2.81 mm
MET I D	2.65 mm	1.98 mm	1.62 mm	1.99 mm	2.20 mm
MET II L	3.43 mm	2.40 mm	1.79 mm	2.84 mm	2.69 mm
MET II W	3.42 mm	2.55 mm	2.12 mm	2.65 mm	2.79 mm
MET II D	2.91 mm	1.98 mm	1.55 mm	2.03 mm	2.31 mm
MET III L	3.59 mm	2.52 mm	1.90 mm	3.00 mm	2.82 mm
MET III W	3.39 mm	2.55 mm	2.06 mm	2.69 mm	2.69 mm
MET III D	2.62 mm	1.95 mm	1.62 mm	2.07 mm	2.22 mm
MET IV L	4.41 mm	3.06 mm	2.29 mm	3.61 mm	3.20 mm
MET IV W	3.25 mm	2.48 mm	1.98 mm	2.51 mm	2.63 mm
MET IV D	2.57 mm	1.95 mm	1.59 mm	2.15 mm	2.52 mm
MET V L	6.22 mm	4.59 mm	3.74 mm	4.98 mm	5.12 mm
MET V W	3.26 mm	2.48 mm	2.01 mm	2.55 mm	2.59 mm
MET V D	2.47 mm	1.77 mm	1.54 mm	1.95 mm	2.17 mm
Telson L	6.17 mm	4.47 mm	3.68 mm	4.77 mm	4.63 mm
Vesicle L	4.21 mm	3.02 mm	2.30 mm	3.39 mm	3.49 mm
Vesicle W	3.13 mm	2.31 mm	1.70 mm	2.33 mm	2.19 m
Vesicle D	2.41 mm	1.68 mm	1.49 mm	1.67 mm	2.62 mm
Aculeus L	1.96 mm	1.45 mm	1.38 mm	1.38 mm	1.14 mm
Pedipalp L	18.79 mm	15.09 mm	11.80 mm	16.08 mm	16.44 mm
Femur L	4.71 mm	3.74 mm	2.96 mm	3.88 mm	4.30 mm
Femur W	1.81 mm	1.26 mm	1.11 mm	1.39 mm	1.41 mm
Femur D	1.24 mm	0.95 mm	0.82 mm	1.00 mm	0.95 mm
Patella L	5.02 mm	3.80 mm	3.40 mm	4.05 mm	4.41 mm
Patella W	1.80 mm	1.43 mm	1.19 mm	1.42 mm	1.59 mm
Patella D	1.71 mm	1.38 mm	0.92 mm	1.34 mm	1.38 mm
Chela L	9.06 mm	7.55 mm	5.27 mm	8.15 mm	7.73 mm
Palm W	2.54 mm	1.81 mm	1.26 mm	2.10 mm	1.49 mm
Palm L	4.84 mm	2.85 mm	2.46 mm	3.79 mm	3.38 mm
Chela D	3.31 mm	2.23 mm	1.44 mm	2.84 mm	2.65 mm
MF L	5.55 mm	4.05 mm	3.33 mm	4.36 mm	4.35 mm
FF L	4.37 mm	3.32 mm	2.94 mm	3.25 mm	3.65 mm
Cara to eyes	1.89 mm	1.65 mm	1.20 mm	1.37 mm	1.67 mm
Pectine count	13/13	14/14	13/13	15/15	14/14

carinae; VII with granular ventral lateral carinae on posterior 4/5, median carinal pair essentially obsolete. Spiracles: ellipsoid and with median side rotated 40° away from posterior sternite margin. Genital Operculum: sclerites separated on posterior 1/5. Pectines: tooth count 13/13; middle lamellae 8/8; sensorial areas present on all



Figures 4–15. *Pseudouroctonus peccatum* sp. n., Spring Mountains, Clark Co., Nevada, USA. Female holotype. **4** Carapace and close-up of lateral eyes. **5–8** Right pedipalp **5** Chela, external view **6** Chelal finger dentition, fixed and movable fingers **7** Patella, dorsal view **8** Femur, dorsal view **9** Right leg II, tarsus close-up, partial ventral view. Right leg III, tarsus and basitarsus, ventral view **10** Cheliceral movable and fixed fingers, ventral view. Chelicera, dorsal view **11** Sternite VII **12** Left stigma I **13** Metasoma and telson, ventral and lateral views **14** Sternopectinal area **15** Male paratype, sternopectinal area.

pectine teeth. Metasoma: ratio of segment I length/width 0.91; segment II length/width 1.00; segment III length/width 1.06; segment IV length/width 1.36; segment V length/width 1.91. Segments I–IV: dorsal carinae are moderately denticulate on segments I–IV and have slightly enlarged distal denticles; dorsolateral carinae are moderately crenulate on segment I and moderately denticulate on segments II–IV with enlarged posterior denticles; posterior denticles are largest and most pronounced on dorsolateral carinae IV; ventrolateral carinae are moderately crenulate on segments I–IV; segments II and III have sparse and moderately crenulate intermediary carinae forming an approximately 30° angle with the dorsolateral carinae on the posterior 2/3 on segment II and posterior 1/3 segment III; ventromedian carinae are smooth to obsolete on segment I, and crenulate on segments II–IV; ventrolateral setae 2/2:2/2:2/2:2/2; ventral submedian setae 3/3:3/3:3/3:3/3. Segment V: dorsolateral carinae subtly denticulate; lateral carinae crenulate and obsolete on posterior 1/3; ventrolateral carinae crenulate; ventromedian carinae crenulate; intercarinal spaces finely granular; dorsolateral setation 3/3; lateral setation 3/3; ventrolateral setation 4/4; ventromedian setation 4/4. Telson: smooth to slightly granular with no subaculear tubercule and lacking LAS denticles (Fet et al. 2006). Chelicerae: dorsal edge of fixed finger with four teeth, one distal, one subdistal, one median, and one basal, the latter two denticles formed as a bicuspid; ventral edge with seven low-profile pigmented protuberances, the distal two smaller in size; dorsal edge of movable finger has five teeth total comprised of one distal, two subdistal, one median tooth, and one basal tooth; ventral edge of movable finger has seven small pigmented crenulated teeth; serrula with approximately 23 tines. Pedipalps: trichobothrial pattern type C, orthobothriotaxic: trichobothria *ib-it* positioned on extreme base of fixed finger, *Dt* located considerably basal of palm midpoint, *Db* ventral of digital carina, patellar trichobothria v_3 adjacent to et_3; ratio of chela length/width 3.57; femur length/width 2.60; patella length/width 2.79; fixed finger length/carapace length 0.78. Chela: median denticles (MD) of fixed finger aligned and divided into six subrows by five outer denticles (OD); flanked by six inner denticles (ID); movable finger with six subrows of MD, five OD and seven ID. Chela carinae: Digital carina rounded, rough, and somewhat flattened; subdigital essentially obsolete, formed by two granules; dorsosecondary flat, rounded, and roughly textured; dorsomarginal very rounded, with large scattered granules; dorsointernal rounded, with scattered granules; ventroexternal rounded, roughly textured, terminating at external condyle of movable finger; ventromedian flat, essentially obsolete; ventrointernal rounded, with small granules scattered distally; external carina very rounded, roughly textured. Femur: dorsointernal, and ventrointernal carinae denticulate and black in color, ventroexternal and dorsoexternal carinae denticulate with small granules throughout, internal surface has 6 prominent dentate granules. Patella: dorsointernal carinae are denticulate, dorsoexternal carinae are mildly crenate, ventral internal carinae are dentate, ventroexternal carinae are moderately denticulate, internal surface has 11 prominent granules. Legs: Ventral surface of tarsus with single median row of 7–17 spinules terminating distally with one to two

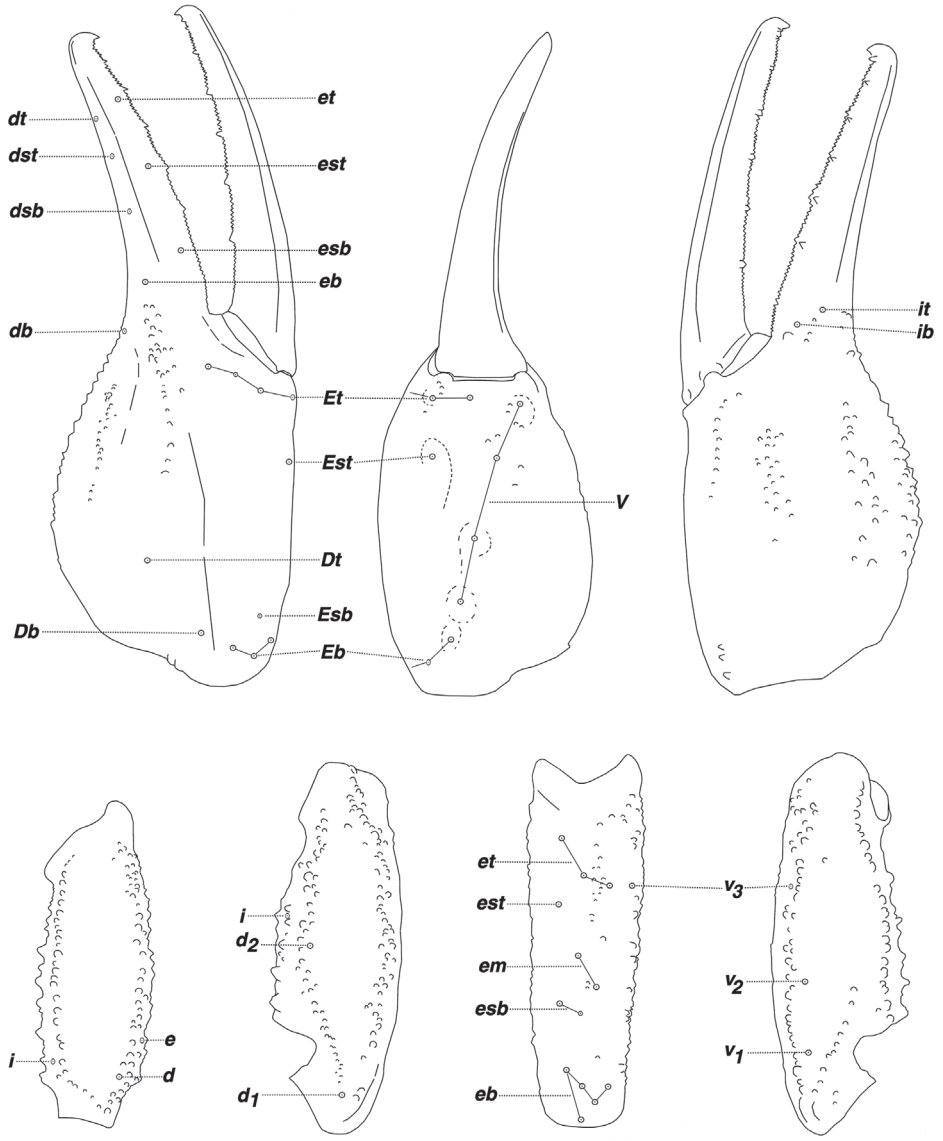


Figure 16. *Pseudouroctonus peccatum* sp. n. female holotype, Spring Mountains, Clark Co., Nevada, USA. Trichobothrial pattern.

pairs of spinules. The terminal spinules are slightly stouter than the those comprising the median row. Flanking setal pairs are essentially absent in legs I–II, with 2–3 irregularly positioned and sized pairs on legs III–IV. Basitarsus spinule rows are limited, 2 rows on leg I, 1 row on leg II, 1 weak row on III, and absent on IV. Basitarsus populated with large irregularly placed darkly pigmented setae. Hemispermatophore: Left hemispermatophore is 5.7 mm in length; lamina length 3.71,

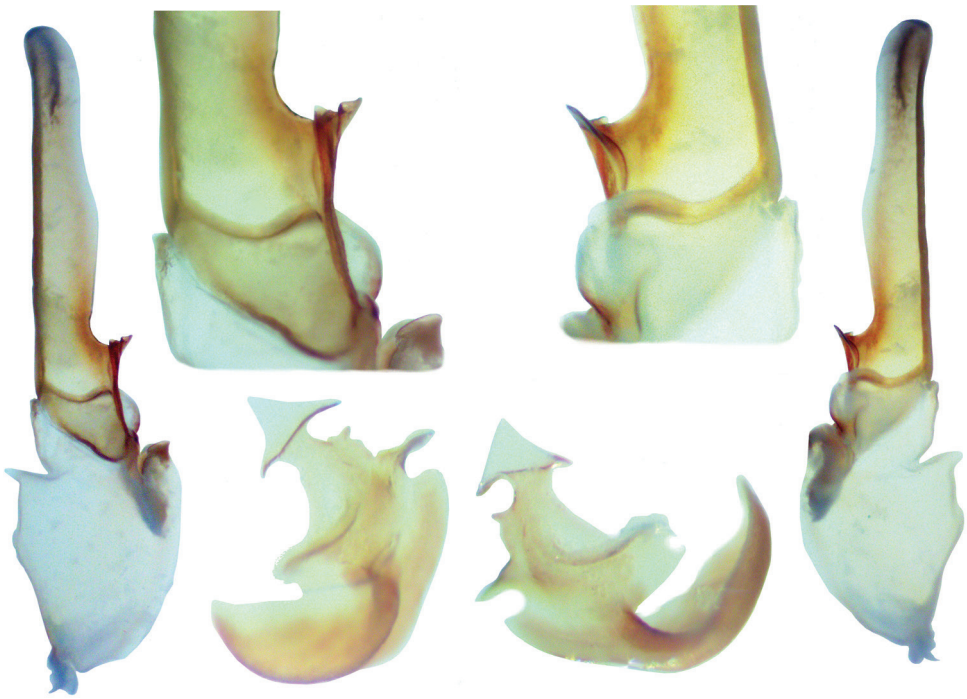


Figure 17. *Pseudouroctonus peccatum* sp. n., male paratype, Spring Mountains, Clark Co., Nevada, USA. Left hemispermatophore and mating plug (both reversed, photographed submerged in alcohol). **Outside** hemispermatophore, dorsal and ventral views. **Top** closeup of median area, dorsal and ventral views. Note, due to the translucency of the hemispermatophore, the sclerotized ventral trough is partially visible from the dorsal side. **Bottom** mating plug. dorsal and ventral views. Note, the sclerotized edge of the mating plug barb is located on the dorsal surface and is partially visible from the ventral side due to the translucency of the plug.

lamellar hook length 1.03, and trough different (vertical distance between ventral and dorsal troughs) 0.55. Lamellar edges sub-parallel, except for slight expansion on mid-area of the internal edge; terminus blunted with a distinct distal crest on the dorsal side. Lamellar hook extends somewhat from lamina base, is distinctly bifurcated, and is formed entirely from the dorsal trough. A modest basal constriction and a deep truncal flexure are present. Sclerotized mating plugs with smooth barbs were extracted from the ventrointernal aspect of both hemispermatophore median areas.

DNA barcodes (COI) – MRG1221 (paratype, GenBank no. KF841448):

CTCTAAGTTTAATGATTCGTGCGGAAATTGGTAGAC-
CTGGGTCTTTTATCGGGGATGATCAAATTTATAATGTTGTGGT-
TACTGCTCATGCTTTTGTCATAATTTTTTTTATGGTTATGCCAAT-
TATGATTGGGGGTTTTGGTAATTGGTTAGTTCCTTTGATGTTAGGT-
GCTCCTGATATGGCTTTTCCTCGTTTAAATAATATAAGATTTTGATT-
ATTACCACCTGCATTTTTTATGCTTTTAGGTTCGGCATCGTTGGAAA-
GAGGGGCAGGTACAGGCTGAACTGTGTACCCTCCTCTTTCCTCATA-

Figure 18. Type locality of *Pseudouroctonus peccatum* sp. n. on the eastern slope of the Spring Mountains, Nevada. Red arrow indicates where the holotype was discovered.

Table 2. Matrix showing character set that distinguishes *Pseudouroctonus peccatum* sp. n. from the other seventeen species in the genus. If a species differs significantly for a given data character, its state is shaded. Note, except for *P. peccatum* sp. n., most of the data contained in this table is based on published information (see reference list). *ID* = inner denticle.

	Hemispermatophore	Cheliceral Dentition	Chelal Movable Finger *ID*	Pectinal Tooth *Counts*	Metasoma Seg V (Length/Width)
P. peccatum	Distal crest present on lamina terminus; secondary lamellar hook absent	*va* on MF and FF crenulated; two subdistal denticles	Seven *ID*	15 ♂ 13–14 ♀	1.953 ♂ 1.908 ♀
P. andreas	Distal crest absent	smooth	Six *ID*	smaller 9–10 ♂ 7–9 ♀	- 1.933 ♂ 2.000 ♀
P. angelenus	Distal crest absent; secondary lamellar hook present	smooth	Seven *ID*	smaller 11 ♂ ? ♀	thinner 3.059 ♂ ? ♀
P. apacheanus	?	smooth	Seven *ID*	smaller 10–11 ♂ 9–10♀	- 2.412 ♂ 2.176 ♀
P. bogerti	Distal crest absent; secondary lamellar hook present	smooth	Seven *ID*	-? ♂ 12 ♀	thinner 3.444 ♂ 3.091 ♀
P. cazieri	Distal crest absent	*va* on MF and FF toothed	Six *ID*	- 13 ♂ 11–12 ♀	- 2.150 ♂ 2.217 ♀
P. chicano	?	smooth	Seven *ID*	smaller ? ♂ 9♀	- ? ♂ 2.067 ♀
P. glimmei	Distal crest absent; secondary lamellar hook present	smooth	Seven *ID*	smaller 10–12 ♂ 9–11♀	thinner 2.600 ♂ 2.609 ♀
P. iviei	Distal crest absent; secondary lamellar hook present	smooth	Seven *ID*	smaller 11 ♂ 10♀	- 2.191 ♂ 2.304 ♀
P. lindsayi	Distal crest absent	smooth	Six *ID*	- 14 ♂ 12 ♀	- 2.286 ♂ 2.286 ♀
P. m. castaneus	Distal crest absent	smooth	Seven *ID*	smaller 10–12 ♂ 10♀	wider 1.402 ♂ 1.364 ♀
P. m. minimus	Distal crest absent	smooth	Six *ID*	smaller 10–11 ♂ 9–10♀	wider 1.650 ♂ 1.565 ♀
P. m. thompsoni	?	smooth	Seven *ID*	smaller 10–11 ♂ 10♀	wider 1.667 ♂ 1.538 ♀
P. reddelli	Distal crest absent	*va* on MF and FF toothed	Seven *ID*	larger 18–19 ♂ 15–16♀	thinner 3.036 ♂ 2.871 ♀
P. rufulus	Distal crest absent	smooth	Seven *ID*	smaller 11 ♂ 9♀	- 2.400 ♂ 2.176 ♀
P. saavasi	Distal crest absent	Smooth, one subdistal (*sd*)	Seven *ID*	smaller 10–11 ♂ 9♀	thinner 2.810 ♂ 2.563 ♀
P. sprousei	Distal crest absent	*va* on MF and FF toothed	Seven *ID*	larger 17 ♂ ?♀	thinner 4.214 ♂ ? ♀
P. williamsi	Distal crest absent; secondary lamellar hook present	smooth	Seven *ID*	- 13 ♂ 11–12 ♀	thinner 3.400 ♂ 3.400 ♀

TATGTTTCATTCTGGGGGGTCTGTTGATATGACTATTTTTTCGT-
TACATTTGGCTGGTGTTTCTTCAATTTTAGGAGCTATTAATTTTAT-
TACTACTATTTTGAATATGCGAAGATCTGGAATATTGTTGGAGCGTG-
TGCCTTTATTTGTATGGTCTGTTAAGATTACTGCTATTCTTCTGTT-
GTTGTCTCTTCCAGTTCTTGCGGGTGCAATTACTATGCTATTAACA-
GATCGAAATTTTAATACTTCTTTTTTTGATCCAGCGGGTGGAGGG-
GATCCTATTTTGTACCAACATTTATTTTGATTTTTTGGTCATCCTGAG-
GTTTATATTTTAATTCTTCCGGGATTTGGAATGATTTCTCATATTATT-
AGTCATCATACTGGGAAGAGGGAACCTTTCGGAGCTTTAGGAATGATT-
TATGCTATGGTGGCTATTGGGTTTTTGGGTTTTGTGGTTTG.

MRG1222 (paratype, GenBank no. KF841449):

CTCTAAGTTTAATGATTCGTGCGGAAATTGGTAGAC-
CTGGGTCTTTTATCGGGGATGATCAAATTTATAATGTTGTGGT-
TACTGCTCATGCTTTTGTCATAATTTTTTTTATGGTTATGCCAAT-
TATGATTGGGGGTTTTGGTAATTGGTTAGTTCCTTTGATGTTAGGT-
GCTCCTGATATGGCTTTTCCTCGTTTAAATAATATAAGATTTTGATT-
ATTACCACCTGCATTTTTTATGCTTTTAGGTTCGGCATCGTTGGAAA-
GAGGGGCAGGTACAGGCTGAACTGTGTACCCTCCTCTTTCCTCATA-
TATGTTTCATTCTGGGGGGTCTGTTGATATGACTATTTTTTCGTTA-
CATTTGGCTGGTGTTTCTTCAATTTTAGGAGCTATTAATTTTATTAC-
TACTATTTTGAATATGCGAAGATCTGGAATATTGTTGGAGCGTGTGC-
CTTTATTTGTATGGTCTGTTAAGATTACTGCTATTCTTCTGTTGTT-
GTCTCTTCCAGTTCTTGCGGGTGCAATTACTATACTATTAACAGATC-
GAAATTTTAATACTTCTTTTTTTGATCCAGCGGGTGGAGGGGATC-
CTATTTTGTACCAACATTTATTTTGATTTTTTGGTCATCCTGAGGTT-
TATATTTTAATTCTTCCGGGATTTGGAATGATTTCTCATATTATTAGT-
CATCATACTGGGAAGAGGGAACCTTTCGGAGCTTTAGGAATGATTTAT-
GCTATGGTGGCTATTGGGTTTTTGGGTTTTGTGGTTTG.

Discussion

The adult male and female can be differentiated by the larger pectinal tooth counts in the male (15 versus 13–14), the completely separated genital operculum in the male and the presence of genital papillae (in the female the sclerites are only separated on the posterior one-quarter and papillae are absent). The adult female is larger in size, 52 mm versus 36 mm. The metasoma of the male is slightly thinner than in the female, exhibiting the following percentage differences for all five segments when the segment's length is compared to its width (2.4–10.8%).

On the dorsal edge of the right cheliceral movable finger of the female holotype, a small bifurcation is present on the distal aspect of the median (*m*) denticle (see Fig. 10). This bifurcation is not present on the left chelicerae, so we consider it an anomaly.

Distribution

Known only from the type locality in the Spring Mountains of Southern Nevada where it was collected at an elevation of 2,103 m.

Comparison to similar species

Soleglad and Fet (2008: fig. 196) considered *Uroctonites* and *Pseudouroctonus* to form a distinct clade within the vaejovid subfamily Vaejovinae. Pending results from detailed morphological and molecular analyses, we place *Pseudouroctonus peccatum* sp. n. in this group based on the following characters: (1) the chelae are thick with swollen palms that are somewhat flat in appearance due to the weak to obsolete development of the dorsosecondary and ventromedian carinae; (2) the carapace exhibits a conspicuous anterior emargination; (3) the median eyes are reduced in size and located considerably anterior of the carapace midpoint; (4) the ventral edges of the cheliceral movable finger and fixed finger are equipped with small crenulations and protuberances, respectively; these two characters are found in many species of this group and likewise generally absent in the other genera comprising the subfamily (*Vaejovis* and *Franckeus*); (5) the serrulae are well-developed; (6) the pectinal tooth counts as compared to the scorpion's adult size are relatively small.

Within the clade, *P. peccatum* clearly has a closer affinity to *Pseudouroctonus* than *Uroctonites*. The sides of the hemispermatophore lamina are sub-parallel (not tapered), the terminus is truncated (not pointed); lamellar hook is located distal of dorsal trough (not adjacent to), and the terminus is bifurcated (not intact). The mating plug is sclerotized (not partially gelatinous). Ventral setal pairs of the leg tarsus are irregularly positioned and sized, and of medium development (not aligned, the same size, and stout). Although the relative pectinal tooth counts in this species are somewhat small, they are not as small as seen in *Uroctonites*. For example, *P. peccatum* and *U. huachuca* are similarly sized species, but *P. peccatum* has 67% (females) and 76% (males) more teeth than *U. huachuca*. Pectinal tooth counts in the four species of *Uroctonites* range 7–10 in females and 8–10 in males.

Pseudouroctonus is comprised of eighteen species (including *P. peccatum*). The new species can be separated from the other species based on the structure of its hemispermatophore, cheliceral and chelal dentition, pectinal tooth counts, and morphometrics of the fifth metasomal segment (see Table 2 for character comparisons across all species). Before addressing each of the seventeen species, we must point out that *P. peccatum* has a distal crest on the hemispermatophore lamina terminus and its lamellar hook is somewhat removed from the lamina base, providing a small basal constriction. Both of these characters are unknown from any of the hemispermatophores so far reported for this genus [note: we have information on the hemispermatophore for fifteen *Pseudouroctonus* species, only three are unknown. See Williams and Savary (1991: figs 21–29), Francke and Savary (2006: figs 15–21), Soleglad and Fet (2008: figs 67–69),

and Francke (2009: figs 2–4)]. Although it is clear, based on Table 2, that more than one diagnostic character is available for separating *P. peccatum* from the other species, we will restrict our discussion to discrete characters thus minimizing the dependence on meristic and morphometric data. *Pseudouroctonus peccatum* does not have a secondary hook on the hemispermatophore as found in *P. williamsi*, *P. angelenus*, *P. bogerti*, *P. glimmei*, and *P. iviei*. Although *P. peccatum* is equipped with crenulated denticles on the ventral edge of the cheliceral movable finger, as well as small protuberances on the venter of the fixed finger, they are not enlarged and tooth-like as found in *P. reddelli*, *P. sprousei*, and *P. cazieri*. The lamellar hook on the hemispermatophore of *P. peccatum* is distal from the dorsal trough, whereas in *P. lindsayi* it is adjacent. *Pseudouroctonus peccatum* has two subdistal (*sd*) denticles on the cheliceral movable finger dorsal edge, whereas the cave adapted species *P. saavasi* has only one. *Pseudouroctonus peccatum* has seven inner denticles (*ID*) on the chelal movable finger, whereas *P. andreas* has only six. As expected given its somewhat large size, *P. peccatum* has a relatively large pectinal tooth count (i.e., 15 male and 13–14 female), separating it from *P. chicano*, *P. apacheanus*, and *P. rufulus* which range from 10–11 in males and 9–10 in females. Although *P. peccatum* has a relatively stout metasomal segment V, it is not as robust as that found in *P. minimus minimus*, *P. minimus castaneus*, and *P. minimus thompsoni*. Segment length/width ratios are 1.908 for the female holotype and 1.953 for the male paratype, compared to 1.364–1.565 in females and 1.402–1.667 males for the *P. minimus* subspecies.

Interestingly, *P. peccatum* was not found within the known range of any of the *Pseudouroctonus* species, and was discovered closer to two species of *Uroctonites*, *U. giulianii* and *U. sequoia*; the distances between the type localities are roughly 258 and 282 km, respectively. The most geographically proximate species of *Pseudouroctonus* are all found in southern California: all three subspecies of *P. minimus*, *P. angelenus*, *P. bogerti*, *P. williamsi*, and *P. andreas*. The distance from these type localities ranges 290–426 km. Based on Table 2, *P. peccatum* appears more closely related to *P. andreas,* the smallest species in the genus, and the three subspecies of *P. minimus.* The other *Pseudouroctonus* spp. (*P. angelenus*, *P. bogerti*, and *P. williamsi*) exhibit considerable differences in the hemispematophore structure.

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RESEARCH ARTICLE

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Integrative taxonomy and preliminary assessment of species limits in the *Liolaemus walkeri* complex (Squamata, Liolaemidae) with descriptions of three new species from Peru

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Abstract

Species delimitation studies based on integrative taxonomic approaches have received considerable attention in the last few years, and have provided the strongest hypotheses of species boundaries. We used three lines of evidence (molecular, morphological, and niche envelopes) to test for species boundaries in Peruvian populations of the *Liolaemus walkeri* complex. Our results show that different lines of evidence and analyses are congruent in different combinations, for unambiguous delimitation of three lineages that were "hidden" within known species, and now deserve species status. Our phylogenetic analysis shows that *L. walkeri*, *L. tacnae* and the three new species are strongly separated from other species assigned to the *alticolor-bibronii* group. Few conventional morphological characters distinguish the new species from closely related taxa and this highlights the need to integrate other sources of data to erect strong hypothesis of species limits. A taxonomic key for known Peruvian species of the subgenus *Lioalemus* is provided.

Resumen

Los estudios sobre delimitación de especies basados en un enfoque integral han recibido considerable atención en los últimos años, y proveen las hipótesis más robustas sobre límites de especies. Usamos tres líneas de evidencia (molecular, morfológica y modelos de nichos) para evaluar los límites de especies entre poblaciones peruanas del complejo *Liolaemus walkeri*. Nuestros resultados muestran que las diferentes líneas de evidencia y análisis en diferentes combinaciones son congruentes en el descubrimiento no ambiguo de tres linajes que estuvieron confundidos con especies ya conocidas y que ahora merecen reconocimiento específico. Nuestro análisis filogenético muestra que *L. walkeri*, *L. tacnae* y las tres nuevas especies están bien distanciadas de las otras especies asignadas al grupo *alticolor-bibronii*. Pocos caracteres morfológicos convencionales distinguen las nuevas especies de otras estrechamente relacionadas, y esto indica la necesidad de integración de diferentes fuentes de datos para elaborar hipótesis más sólidas sobre límites entre especies. Se proporciona una clave taxonómica para las especies peruanas conocidas del subgénero *Liolaemus*.

Keywords

Liolaemus walkeri complex, integrative taxonomy, new species, viviparity

Introduction

The issue of species delimitation (building explicit hypotheses about species lineages and their geographic boundaries) has received considerable attention in the last decade due in part to an emerging consensus about species concepts and new approaches for testing species boundaries (Sites and Marshall 2003, 2004, de Queiroz 2007, Knowles and Carstens 2007, Wiens 2007, Padial and De la Riva 2010, Padial et al. 2010, Hart 2011, Zapata and Jiménez 2012, Camargo and Sites 2013). The ontological General Lineage Concept (GLC) defines a species as a group of separately evolving meta-population lineages, originally proposed by Mayden (1997, 2002) and de Queiroz (1998, 2005). This definition is generally supported by a consensus view in evolutionary biology (Padial and De la Riva 2010, Padial et al. 2010, Hart 2011, Zapata and Jiménez 2012, but see Hausdorf 2011). The GLC distinguishes the primary property (species are separately evolving meta-population lineages) that is shared by most previous competing species concepts (e.g., biological, phylogenetic, ecological species concept, etc.), from secondary properties (e.g., reproductive isolation, character fixation, niche

differentiation, etc.) that arise at different times during the processes of speciation (de Queiroz 2007). These secondary properties are lines of evidence that are relevant to inferring the species boundaries (de Queiroz 2005, 2007).

In addition to this agreement with respect to GLC, there is a growing number of new empirical methods of species delimitation (SDL; Pons et al. 2006, Knowles and Carstens 2007, Kubatko et al. 2009, Carstens and Dewey 2010, Flot et al. 2010, Hausdorf and Hennig 2010, Martínez-Gordillo et al. 2010, Gurgel-Gonçalves et al. 2011). These new methods for testing hypotheses of species boundaries have been accommodated under the new term "integrative taxonomy" (IT; Dayrat 2005, Padial and De la Riva 2010, Padial et al. 2010). Methods such as the multi-locus coalescent to infer species limits without monophyletic lineages, ecological niche modeling (ENM) to assess spatial distributions of closely related species, and multivariate tolerance regions to test for discontinuities or gaps in morphology, have all been used in new integrative taxonomic studies (Omland et al. 2006, Knowles and Carstens 2007, Raxworthy et al. 2007, Rissler and Apodaca 2007, Vasconcelos et al. 2012, Zapata and Jiménez 2012).

Character fixation as well as discontinuities or gaps have been used as a SDL criterion to assess species limits based on genetic and morphological characters (Marshall et al. 2006, Zapata and Jiménez 2012). Fixed differences and gaps in morphology suggest that some evolutionary force (e.g., absence of gene flow, natural selection) prevent two putative taxa from homogenizing (Wiens and Servedio 2000, Zapata and Jiménez 2012). Often analysis of variance or discriminant analysis have been used to evaluate morphological differentiation in SDL studies, but these statistics, even if significant, evaluate central tendencies and not gaps in morphology, and the latter may be more relevant for testing species boundaries (Zapata and Jiménez 2012). In addition to character fixation and gaps in morphology, niche envelopes can be used to assess the status of uncertain populations which are separated from closely related species by areas that are outside of the climatic niche envelope, and where gene flow between these species is unlikely because it would involve crossing unsuitable habitat (Wiens and Graham 2005). Ecological niche modeling (ENM) can summarize niche envelopes and this approach has also been used in SDL studies (e.g., Raxworthy et al. 2007, Rissler and Apodaca 2007).

Well-supported hypotheses of species boundaries are essential because species are used as basic units of analysis in several areas of biogeography, ecology, and macroevolution, and from the broader perspective of evolutionary theory, delimiting species is important in the context of understanding many evolutionary mechanisms and processes (Sites and Marshall 2003, 2004, Wiens 2007). Among animal groups, lizards have been used extensively in evolutionary studies ranging from community ecology, behavioral ecology, multiple origins of body elongation coupled with limb reduction/loss, multiple origins of novel reproductive modes, including parthenogenesis and viviparity (Sites et al. 2011), as well as phylogeography and speciation studies (Camargo et al. 2010).

SDL studies in lizards have included molecular markers, morphological characters and/or models of species distributions (Camargo et al. 2010). In particular, several clades of the genus *Liolaemus* Wiegmann, 1834 have been studied intensively using

molecular and morphological data to delimit species and infer phylogeographic histories (Morando et al. 2008, Victoriano et al. 2008, Breitman et al. 2011a, 2012), and for testing hypotheses about evolutionary processes (Olave et al. 2011) and performance (in accuracy and precision) of different SDL methods (Camargo et al. 2012). This South American genus includes ~ 230 species (Breitman et al. 2011b), and extends from central Peru to Tierra del Fuego, and from sea level on both Atlantic and Pacific coasts to almost 5000 m in elevation. Species diversity is highest in the Andes and adjacent arid regions, and new species descriptions are published at a rate of 4–5/yr, from moderately well-known areas in Argentina and Chile.

In most cases these studies have demonstrated that populations assigned to single species based on generalized morphological features and limited field sampling, tend to under-represent biodiversity. Distinct lineages have been revealed by molecular data, many of which are later described as new species (e.g., Breitman et al. 2011a, b). The largest poorly-known areas for the genus are the Andean regions of Bolivia, Peru and northern Chile. Intensive fieldwork and molecular phylogenetic studies have never been systematically carried out in these regions, and species descriptions have traditionally been based on gross comparisons of morphological characters from small sample sizes and limited geographic sampling. So SDL studies are needed in the extreme northern range of *Liolaemus* (e.g., Peru) based on intensive geographic sampling and large series for collection of new molecular, coloration, and various classes of morphological data.

Currently, 14 species of *Liolaemus* are known from Peru (*L. montanus* group, 10 spp; *L. alticolor* group, 4 spp), but SDL studies based on an integrative approach have not been carried out in either of these groups. Moreover, several areas in the Peruvian Andes remain completely unexplored, and based on recent studies in the southern range of *Liolaemus*, it is highly probable that the Peruvian Andes harbor many undiscovered species. Here, we use new molecular, morphological, and geographic data from known Peruvian species (*L. alticolor* Barbour, 1909, *L. incaicus* Lobo, Quinteros & Gómez, 2007, *L. tacnae* (Shreve, 1941) and *L. walkeri* Shreve, 1938), assigned to the *L. alticolor* group, and three populations morphologically similar to *L. walkeri* (identified by their regions of occurrence: Ancash, Ayacucho and Cusco), to present the first SDL study based on an IT approach. Our results provide evidence that three new lineages deserve species status, and these are described herein.

Methods

Sampling and DNA extraction

Lizards were collected by hand, photographed and sacrificed with an injection of pentobarbital. After liver tissue was collected for DNA samples, whole specimens were fixed in formaldehyde at 10% and transferred to 70% ethanol for permanent storage in museum collections. Tissue samples were collected in duplicate, stored in 96% ethanol and deposited at the Bean Life Science museum at Brigham Young University (BYU)

and Museo de Historia Natural de San Marcos (MUSM) (see Data resources below). Total genomic DNA is extracted from liver/muscle tissue following the protocol of Fetzner (1999), and using Qiagen DNeasy kits (Qiagen, Inc., Valencia, CA).

Mitochondrial DNA amplification and sequencing

Forty-eight samples from 40 localities were sequenced for 669bp of the mtDNA cytochrome b (cyt-b) region, with LIO742F 5'–TCGACCTVCCYGCCCCATCA–3' and LIO742R 5'–GAGGGGTTACTAAGGGGTTGGC–3' primers (this study), and all unique cyt-b haplotypes were sequenced for a 12S region (752 bp) using primers 12Stphe 5'AAAGCACRGCACTGAAGATGC–3' and 12SE 5'–GTRCGCTTAC-CWTGTTACGACT–3' (Wiens et al. 1999). Double stranded polymerase chain reactions (PCR) were amplified under the following conditions: 1.0 µL of genomic DNA, 1.0 µL of light strand primer 1.0 of µL of heavy strand primer, 1.0 µL of dinucleotide pairs, 2.0 µL of 5x~ buffer, 1.0 µL of MgCl 10x~ buffer, 0.18 µL of Taq polymerase, and 7.5 µL of diH2O. PCR amplification was executed under the following conditions: initial denaturation at 95°C for 2 min, followed by a second denaturation at 95°C for 35 s, annealing at 52°C for 35 s, followed by a cycle extension at 72°C for 35 s, for 31 cycles. PCR products were visualized on a 10% agarose gels to ensure the targeted products were cleanly amplified, and then purified using a MultiScreen PCR (mu) 96 (Millipore Corp., Billerica, MA) and directly sequenced using the BigDye Terminator v 3.1 Cycle Sequencing Ready Reaction (Applied Biosystems, Foster City, CA). The cycle sequencing reactions were purified using Sephadex G-50 Fine (GE Healthcare) and MultiScreen HV plates (Millipore Corp.). Samples were then analyzed on a ABI3730xl DNA Analyzer in the BYU DNA Sequencing Center.

Phylogenetic reconstruction

All sequences were aligned in MUSCLE (Edgar 2004) plugin, and cyt-b sequences were translated to check for premature stop codons in GENEIOUS®PRO v5.6.6. Cyt b haplotype diversity was estimated using DnaSP (Librado and Rozas 2009), and concatenated cyt-b and 12S regions were edited using GENEIOUS®PRO (Drummond et al. 2011). For ingroups and outgroups we used selected species of the subgenus *Liolaemus* that are assigned to different species groups and for which cyt-b and 12S sequences are available in GenBank. Our ingroup samples included taxa that have been assigned to the same species group as *L. tacnae* and *L. walkeri* (*alticolor-bibronii* group), including: *L. abdalai* Quinteros, *L. bibronii* (Bell), *L. gracilis* (Bell), *L. ramirezae* Lobo & Espinoza and *L. saxatilis* Ávila & Cei (Lobo et al. 2010). To further test for monophyly of the *alticolor-bibroni* group, we sampled three species assigned to different species groups (*robertmertensi, pictus* and *monticola* groups), but nested within the subgenus *Liolaemus*; these include: *L. monticola* Müller & Hellmich, *L. pictus* Duméril & Bribon

and *L. robertmertensi* Hellmich (Lobo et al. 2010). We used *L. lineomaculatus* Boulenguer, a species belonging to the subgenus *Eulaemus* (Lobo et al. 2010, Fontanella et al. 2012) as the outgroup. All new sequences were deposited in GenBank (accession numbers KF923633–KF923660 and KF923661–KF923688 for cyt-b and 12s respectively) and a list of all haplotypes, GenBank accession and museum voucher numbers used for the phylogenetic analysis are provided as Supplementary file 1.

Bayesian Information Criteria in JMODELTEST (v 0.01; Posada 2005) identified the best-fit model of evolution for the complete data set of haplotypes as TPM2+ I + Γ. A Maximum-likelihood (ML) search in PHYML (Guindon and Gascuel 2003) was performed with 1000 replicates for bootstrap analyses; we consider strong nodal support for bootstrap values ≥ 70 (Hillis and Bull 1993; with caveats). Because the TPM2+ I + G model is not incorporated in the MRBAYES (Huelsenbeck and Ronquist 2001) plugin of GENEIOUS®PRO v5.6.6, we used a model with the closest likelihood available (GTR + I + Γ). Two parallel runs were performed in MRBAYES using four chains (one cold and three hot) for 1.1×10^6 generations and sampling every 200 generations from the Markov Chain Monte Carlo (MCMC). We determined stationarity by plotting the log likelihood scores of sample points against generation time; when the values reached a stable equilibrium and split frequencies fall below 0.01, stationarity was assumed. We discarded 100,000 samples and 10% of the trees as burn-in. A maximum clade credibility (MCC) tree was constructed using TREEANNOTATOR v1.7.5 (Drummond et al. 2012). We consider Bayesian Posterior Probabilities (BPP) >95% as evidence of significant support for a clade (Huelsenbeck and Ronquist 2001, Wilcox et al. 2002).

Morphological data and analyses

A total of 199 individuals (see species descriptions and Data resources below) representing three putative different populations and four Peruvian species (*L. alticolor*, *L. incaicus*, *L. tacnae* and *L. walkeri*) assigned to the *L. alticolor* group were scored for three classes of morphological characters. We performed a character analysis of 17 discrete binomial characters related to scalation, pattern of coloration and skin folds, including the following: presence/absence of smooth (1) temporal scales and (2) dorsal head scales, contact or not of (3) rostral to nasal scale, presence/absence of (4) mucronate dorsal scales and (5) precloacal pores, (6) preocular scale same or different color as loreal region, presence/absence of (7) spots on dorsal head scales, (8) black line surrounding the interparietal scale, regular spots or marks in (9) paravertebral field and (10) lateral field, presence/absence of dorsolateral stripes (11) and vertebral line (12), marks or spots on throat (13), melanistic belly (14), ringed pattern in ventral tail (15), and presence/absence of antehumeral (16) and neck folds (17). All characters were scored using a stereomicroscope and from photos of live animals taken in the field.

For statistical analyses of these discrete variables we used tolerance intervals as described in the tolerance package of Young (2010), which in a random sample of a univariate population, is an interval expected to contain a specified proportion or

more of the sampled population (Krishnamoorthy and Mathew 2009). We used binomial tolerance intervals to estimate the number of individuals that comprise 95% of the population expected to have one state with a 0.05 level of significance (following Wiens and Servedio 2000, Zapata and Jiménez 2012). One-sided binomial tolerance intervals were estimated using the Wilson method (WS), which is appropriate when the sample sizes are small ($n \leq 40$) (Young 2010).

We scored the following 11 morphometric characters: (SVL) snout-vent length, (AGL) axilla-groin length (between the posterior insertion of forelimb and anterior insertion of thigh), (HL) head length (from snout to anterior border of auditory meatus), (HW), head width (at widest point), (FOL) forelimb length (distance from the attachment of the limb to the body to the terminus of the fourth digit), (HIL) hindlimb length (distance from the attachment of the limb to the body, to the terminus of the fourth digit), (SL) snout length (from snout to anterior border of eye), (AMW) auditory meatus width, (AMH) auditory meatus height, (RW) rostral width, and (RL) rostral length. We also scored five meristic characters, including: (MBS) number of midbody scales (counted transversely at the middle of the body), (DTS) dorsal trunk scales (counted from the level of anterior border of ear to anterior border of thighs), (DHS) dorsal head scales (counted from the rostral scale to anterior border of ear), (VS) ventral scales (counted from the mental scales to the cloaca), and (SCI) number of scales in contact with the interparietal. Measurements and counts were taken from the right side of the animal using a stereomicroscope. Morphometric data were only taken for adult males and females.

After testing for normality in all morphometric and meristic characters with the Shapiro-Wilks test (Shapiro and Francia 1972), we summarized means and ranges for all population samples, and performed Principal Component Analyses (PCA) and Correspondence Analyses (CA) separately for each class of characters and by sex, to summarize patterns. Results of PCA and CA were then compared with the analysis of continuous characters by estimating normal tolerance intervals to find gaps or discontinuities in each class of morphological characters. We used normal tolerance intervals to estimate the lowest and highest values of a continuous character that is contained in 95% of the population with a 0.05 level of significance. Two-sided normal tolerance intervals were estimated using the Howe method (HE), which is considered to be extremely accurate, even for small sample sizes (Young 2010).

For comparison with normal tolerance intervals we assessed the morphometric and meristic characters with univariate ANOVA and Mann-Whitney *U* tests for parametric and non-parametric distributions, respectively. When the assumption of equal-variance was not met for an ANOVA test, the unequal- variance (Welch) version of ANOVA was performed. Each character was tested for intersexual differences, and if present, the sexes were analyzed separately. Results were considered significant when $p \leq 0.05$. However, we didn't use the results of the ANOVA and Mann-Whitney *U* tests in our taxonomic decisions (see Introduction and Discussion). Binomial and tolerance intervals were calculated with the package Tolerance (Young 2010) in R v3.0.1 (R core team 2013). Test of normality, PCA, CA and univariate tests were performed using PASTv. 2.08b, (Hammer et al. 2001).

Distributional models

We used the maximum entropy model implemented in the program MAXENT v3.3.3e (Phillips et al. 2006) to predict where the Peruvian lineages of *L. walkeri* complex are most likely to occur under current climatic conditions. MAXENT generates distributional models (or ecological niche models; ENMs) using presence-only records, contrasting them with background/pseudoabsence data sampled from the remainder of the study area. We chose this approach because of its overall better performance with presence-only data and with small sample sizes (Elith et al. 2006). ENMs were developed from occurrence points used in this study, and records without duplicates are: 22 for Ancash, 31 for Ayacucho, 16 for Cusco, 33 for *L. tacnae* and 52 for *L. walkeri* (see Data resources below). For niche predictions, we used the 19-bioclimatic variables from the WorldClim v1.4 dataset with a resolution of 2.5 min (Hijmans et al. 2005). Bioclimatic variables were derived from monthly temperature and precipitation layers, and represents biologically meaningful properties of climate variation (Hijmans et al. 2005). Layers were trimmed to the areas surrounding each species and populations that might represent new species, and then projected over a larger region (-9.828° to -17.839° and -77.486° to -69.811°).

For model calibration we used the default settings with 1000 iterations, and the minimum training value averaged over the 10 replicates as threshold with the default convergence threshold (10^{-5}). Due to our smaller samples sizes, we used for model calibration and performance the cross-validation option with 10 replicates, and average the results to estimate species niche and distributions. For model significance, 25% of localities were randomly set aside as test points and the area under the curve (AUC) was calculated, which summarizes the model's ability to rank presence localities higher than a sample of random pixels (Peterson et al. 2011). AUC values ≤ 0.5 correspond to predictions that are equal or worse than random. AUC values > 0.5 are generally classed into (1) poor predictions (0.5 to 0.7); (2) reasonable predictions (0.7 to 0.9); and (3) very good predictions (>0.90; but see Peterson et al. 2011, for caveats on use of AUC in presence/background data). Model clamping was checked with the "fade by clamping" option available in MAXENT v 3.3.3e. Estimates of bioclimatic variable importance was performed using the Jackknife test. We used the logistic output (probability values) and mapped the distributional models showing areas from the average minimum logistic values (threshold) to 1 as areas suitable for species.

Schoener's D metric was introduced as a measure of niche similarity between pairs of populations (or species) by Warren et al. (2010), and is calculated using the ENMTOOLS package. We calculated these values by comparing the climatic suitability of each grid cell in the projected area obtained with MAXENT. This similarity measure ranges from 0 (niche models have no overlap) to 1 (niche models identical; Warren et al. 2008). We estimated similarity measures and then tested whether the ENMs produced by two populations or species are identical using the niche identity test in ENMTOOLS. One hundred pseudoreplicate data sets were generated to obtain a distribution of D scores, and we reject the hypothesis of niche identity when the empirically observed value for D is significantly lower than the values expected from the pseudoreplicated data set (Warren et al. 2010).

Species descriptions

Species descriptions follow the terminology of Lobo and Espinoza (1999) and Quinteros (2013). For diagnosis, we selected the following non-Peruvian species assigned to the *L. alticolor* group: *L. aparicioi* Ocampo, Aguilar-Kirigin & Quinteros, *L. bitaeniatus* Laurent, *L. chaltin* Lobo & Espinoza, *L. pagaburoi* Lobo & Espinoza, *L. paulinae* Donoso-Barros, *L. puna* Lobo & Espinoza, *L. pyriphlogos* Quinteros, and *L. variegatus* Laurent. This selection is based on previous phylogenetic analyses (Espinoza et al. 2004, Díaz-Gómez and Lobo 2006, Schulte and Moreno-Roark 2010, Quinteros 2013), and taxonomic revisions and species descriptions of geographically proximate species (Donoso-Barros 1961, Laurent 1984, Lobo and Espinoza 1999, 2004, Quinteros 2012, Ocampo et al. 2012). We assumed that diagnostic characters are "fixed". Color descriptions are based on photographs of live animals taken in the field, and specimens examined are provided in Data resources.

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at http://doi.org/10.5061/dryad.0q7pc, and at GBIF, the Global Biodiversity Information Facility, http://ipt.pensoft.net/ipt/resource.do?r=ocurrence_records_liolaemus_walkeri_complex.

Results

Phylogenetic Analysis

A tree with maximum likelihood bootstrap values (logL = -8452.31415, MLB) and Bayesian posterior probabilities (BPP) based on 1421 aligned base pairs is shown in Fig. 1. Differences between both methods are mentioned below. Both ML and Bayesian analyses recovered Ancash, Ayacucho, Cusco, *L. tacnae* and *L. walkeri* haplotypes as monophyletic groups with high support. Both also showed a close relationship between Ayacucho and *L. walkeri* haplotypes, but relationships between Ancash, Cusco and the (*L. walkeri* + Ayacucho) clade were unresolved and with moderate support in the ML tree (MLB 65%). The Bayesian analysis recovers Ancash as the sister to the (*L. walkeri* + Ayacucho) clade with low support (BPP 0.5), and Cusco as the sister clade to the ((*L. walkeri* + Ayacucho) Ancash) clade with moderate support (BPP 0.9). In both analyses, *Liolaemus tacnae* is recovered as the sister group of the (Ancash + Cusco + (*L. walkeri* +Ayacucho)) clade with moderate support (MLB 65%, BPP 0.9). *Liolaemus tacnae* and *L. walkeri* are assigned to the *alticolor-bibronii* group, but the clade (*L. tacnae* (Ancash + Cusco + (*L. walkeri* + Ayacucho))) is strongly differentiated from the other species assigned to the *alticolor-bibronii* group (Fig. 1).

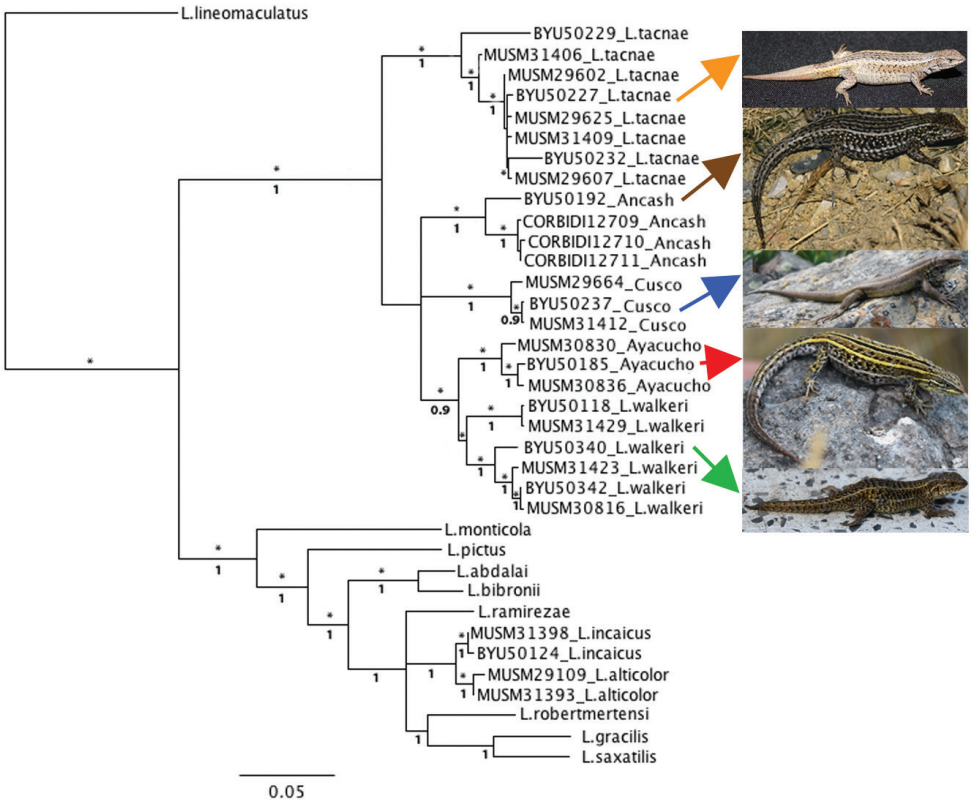

Figure 1. Concatenated maximum likelihood (-Log L = 8452.31415) tree based on cyt-b and 12S haplotypes of focal taxa (Ancash, Ayacucho Cusco) and species assigned to the *alticolor* group and outgroups. Bootstrap ≥ 70 (*) and posterior probabilities values are shown above and below branches respectively.

The monophyletic group (*L. tacnae* (Ancash + Cusco + (*L. walkeri* + Ayacucho))) is the sister group of a clade comprised of taxa assigned to different species groups in the subgenus *Liolaemus,* including species of the *alticolor-bibronii* group. The relationships of these two more inclusive clades showed high MLB, but low BPP values. In this clade, both ML and Bayesian analyses recovered *L. alticolor* and *L. incaicus* haplotypes as monophyletic groups with high support. In our ML analysis, the clade (*L. alticolor* + *L. incaicus*) has unresolved relationships with *L. ramirezae* and the clade (*L. robertmertensi* + (*L. gracilis* + *L. saxatilis*)), and this latter clade has high BPP but low MLB support (Fig. 1). *Liolaemus abdalai* and *L. bibronii* are recovered as sister taxa with high support, and this clade is sister to the clade (*L. ramirezae* + (*L. incaicus* + *L. alticolor*) + (*L. robertmertensi* + (*L. gracilis* + *L. saxatilis*))) also with high support (Fig. 1). *Liolaemus pictus* is sister to the clade ((*Liolaemus abdalai* and *L. bibronii*) + (*L. ramirezae* + (*L. incaicus* + *L. alticolor*) + (*L. robertmertensi* + (*L. gracilis* + *L. saxatilis*)))), and *L. monticola* is basal to a clade that includes *L. pictus* and its sister group.

Morphological analyses

Binomial discrete characters

Because our phylogenetic analysis did not show a close relationship between (*L. alticolor* + *L. incaicus*) and the (*L. tacnae* (Ancash + Cusco + (*L. walkeri* + Ayacucho))) clades, we focus our comparisons on these last five taxa. Of the 17 binomial characters, four were useful for species delimitation among these taxa (Table 1). One-sided binomial tolerance intervals (BTI) for 95% of the population with a 0.05 level of significance is indicated below for each of these four characters.

Ancash (n = 12) and *L. tacnae* (n = 18) males differed from Ayacucho, Cusco and *L. walkeri* males in lacking precloacal pores (Fig. 2A and D; vs. presence in panels B, C, and E). Although these differences are fixed in our samples, the BTI tests showed

Table 1. Binomial characters for females (F) and males (M) of focal populations of *Liolaemus* lizards sampled for this study. Character states useful for species discrimination are in bold, and states only assessed on adults are indicated with an asterisk.

	Ancash		**Ayacucho**		**Cusco**		***L. tacnae***		***L. walkeri***	
	F (n= 18)	M (n =12)	F (n=18)	M (n=10)	F (n=8)	M (n=8)	F (n=23)	M (n=18)	F (n=48)	M (n=21)
Temporal scales smooth	yes	yes/no	yes/no	yes/no	yes	yes	yes/no	yes	yes	yes/no
Dorsal surface of head completely smooth	yes/no	yes/no	yes/no	yes/no	yes	yes/no	yes/no	yes	yes/no	yes/no
Nasal contact rostral scale	yes/no	yes/no	yes/no	yes	yes/no	yes/no	yes/no	yes/no	yes	yes/no
Dorsal scales mucronate	no	no	yes/no	yes/no	no	no	no	no	no	no
Precloacal pores	no	**no**	no	**yes**	no	**yes**	no	**no**	no	**yes**
Sub and preoculars different in color from loreal region	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no
Dorsal surface of head with marks or dots	yes	yes	yes/no	yes/no	yes/no	yes	yes/no	yes/no	yes/no	yes/no
Black line surrounds interparietal scale	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no	yes/no
Regular marks or spots in paravertebral field	yes/no	no	yes	yes/no	yes/no	no	yes/no	yes/no	yes/no	yes/no
Regular marks or spots in lateral field	**yes**	**yes**	**yes**	**yes**	**no**	**no**	yes/no	yes/no	yes/no	yes/no
Dorsolateral stripes	yes	yes/no	yes	yes	yes	yes	yes	yes/no	yes	yes
Vertebral line	yes	yes	yes	yes	yes	yes	yes/no	yes/no	yes	yes/no
Throat not immaculate	yes/no	yes/no	yes/no	yes/no	yes	yes	yes/no	yes	no	yes
***Complete or partial melanistic belly**	yes/no	**yes**	no	yes/no	no	**yes**	no	no	no	**yes**
***Ventral tail with ringed pattern**	yes/no	yes/no	yes/no	**yes**	no	**no**	no/yes	yes	yes/no	**no**
Antehumeral fold	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Neck folds	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes

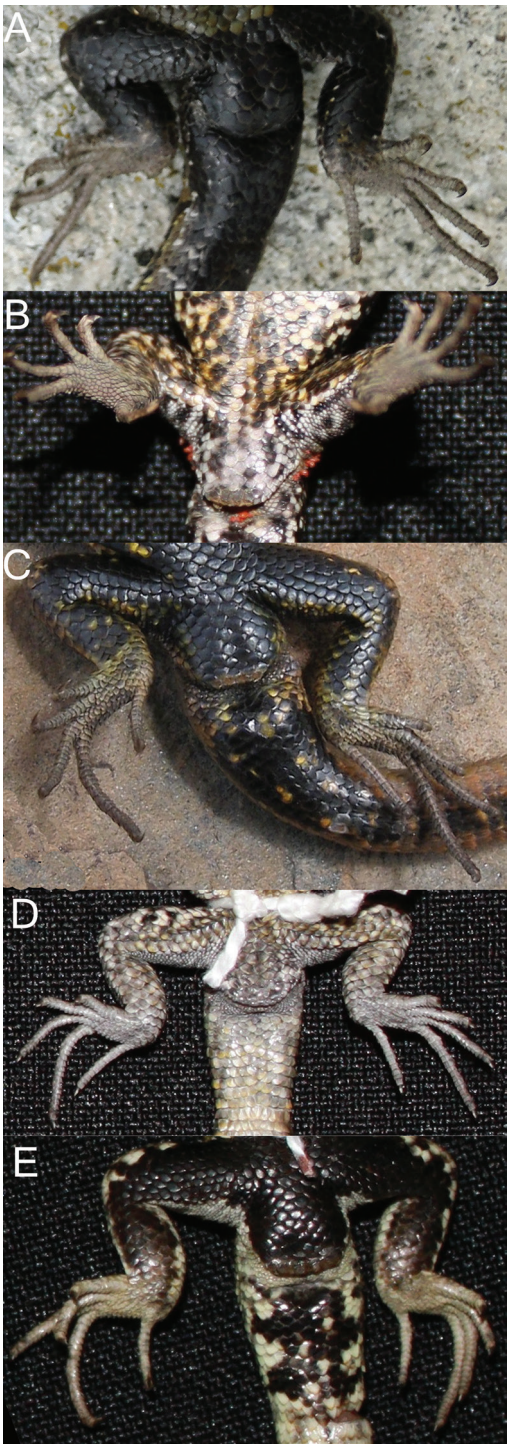

Figure 2. Detailed view of the cloaca region showing absence (**A, D**) or presence (**B, C, E**) of precloacal pores: **A** Ancash **B** Ayacucho **C** Cusco **D** *L. tacnae* and **E** *L. walkeri*.

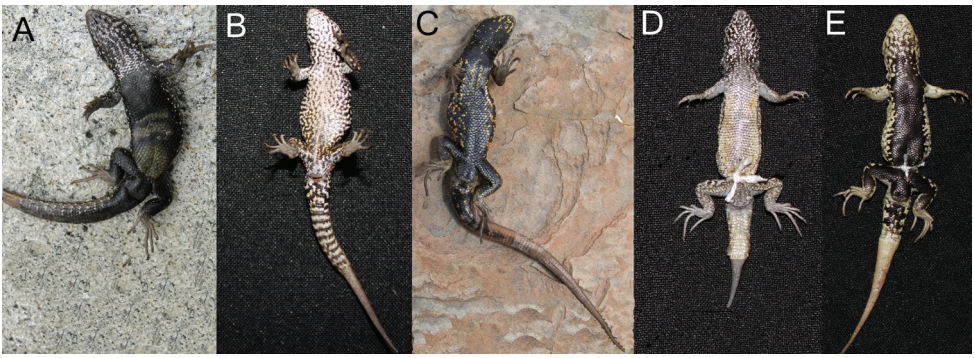

Figure 3. Ventral view showing the color patterns of the belly and tail: **A** Ancash **B** Ayacucho **C** Cusco **D** *L. tacnae* and **E** *L. walkeri*.

that up to 36% and 31% of the Ancash and *L. tacnae* populations, respectively, have a significant probability of possessing the alternative state ($P \leq 0.05$) in a larger sample.

Adult males of Ancash (Fig. 3A) differ from *L. tacnae* (Fig. 3D) in having a melanistic belly, and again while fixed in our samples, BTI showed that up to 36% of the population may have the alternative state ($P \leq 0.05$). Adult males of Ayacucho (Fig. 3B) can be diagnosed by their ringed ventral tail pattern, in contrast to the other four samples (Fig. 3A, C–E), but up to 44% of the population may have the alternative state ($P \leq 0.05$).

Both sexes of the Cusco sample (n = 16; Fig. 4B) differed from all Ayacucho (n = 28; Fig. 4A) and most individuals (90% of n = 69; Fig. 4C) of *L. walkeri*, in lacking regular spots or marks in lateral fields; but up to 33% of the population may have the alternative state ($P \leq 0.05$).

Morphometric and meristic characters

Our empirical results are summarized in Table 2, and tolerance intervals are given in Tables 3 and 4 for morphometric and meristic variables, respectively. Statistical tests rejected normality for HW, AMW, RW and all meristic characters, but we assumed normality because our sample sizes were too small to implement non-parametric tolerance interval tests. We did not find any diagnostic character or gaps in either data set (Tables 3 and 4).

Principal Component and Correspondence Analyses separated by sex or pooled together did not show any differences, so we present the results of the pooled analyses. Principal Component (PC) Analysis revealed that PC1 and PC2 explained 90% of the variance, and the Correspondence Analysis revealed that Correspondence Axis (CA) 1 and CA2 explained 66% of the similarity for morphometric and meristic data, respectively (see also Supplementary file 4 for corresponding eigenvalues, and percentages of variance and similarity accounted by principal components and correspondence axes). The bivari-

Figure 4. Lateral view showing the color patterns of **A** Ayacucho **B** Cusco, and **C** *L. walkeri*.

ate plot for the morphometric variables revealed extensive overlap of *L. walkeri* with the remaining four samples, but minimal overlap between the Ancash and Cusco samples, and little overlap between the Ayacucho and Cusco (Fig. 5A). Both of these pairs are differentiated primarily along PC1, for which SVL and AGL contributed the highest loadings (0.85 and 0.47 respectively). The Cusco samples are characterized by shorter SVL and axilla-groin lengths than the Ancash and Ayacucho samples. The PC analyses revealed extensive overlap among all samples along PC2, and the CA for the meristic variables (Fig. 5B) revealed extensive overlap among all five samples along both axes.

Only significant results of ANOVA are mentioned below and the sex of a particular species or population is indicated only if significantly different from the opposite sex. For SVL, there were significant differences between Ancash vs. Cusco, *L. tacnae* and *L. walkeri*; Ayacucho vs. Cusco and *L. tacnae*; Cusco vs. *L. tacnae* and *L. walkeri*.

For AGD, there were significant differences between Ancash males vs. Cusco males and *L. tacnae* males; Ancash females vs. *L. tacnae* females; Ayacucho females vs. Cusco females and *L. tacnae* females; Cusco males vs. *L. tacnae* males and *L. walkeri* males; Cusco females vs. *L. walkeri* females.

Table 2. Descriptive statistics of morphometric and meristic characters for three new species of *Liolaemus* described herein, and *L. tacnae* and *L. walkeri*. First rows show ranges and second rows show means and standard deviations. See methods for abbreviations.

	L. chavin **(Ancash, n=32)**	***L. pachacutec*** **(Cusco, n=18)**	***L. tacnae*** **(n=41)**	***L. walkeri*** **(n=78)**	***L. wari*** **(Ayacucho, n=30)**
SVL	51.0–66.5	33.4–52.0	42.6–56.6	41.5–64.4	50.0–61.4
	57.0±4.0	45.4±4.4	48.6±3.2	54.5±4.6	55.6±3.1
AGL	20.4–34.8	17.8–30.8	14.9–26.5	17.2–33.5	19.8–32.3
	26.5±3.4	22.6±3.6	21.8±2.7	25.3±3.4	25.9±3.9
HL	10.2–15.3	9.2–13.2	9.4–12.0	10.1–14.2	10.3–12.7
	12.4±1.1	10.6±1.0	10.7±0.7	12.2 ±0.9	11.4±0.8
HW	8.8–12.8	6.6–9.7	7.2–9.3	7.8–11.6	8.1–10.7
	10.3±1.1	8.2±0.7	9.6±0.8	9.6±0.9	9.4±0.8
SL	4.2–6.3	3.2–4.7	3.7–5.3	3.5–6.9	4.0–4.9
	5.2±0.5	4.0±0.5	4.5±0.4	5.1±0.5	4.4±0.3
FoL	14.1–19.1	12.9–17.4	13.1–8.3	13.5–21.5	13.9–18.3
	16.2±1.5	15.7±1.3	15.7±1.4	16.7±1.5	15.7±1.2
HiL	22.6–29.5	19.0–27.9	20.8–29.8	19.8–30.7	20.9–28.7
	25.8±1.8	23.4±2.1	24.6±2.2	25.2±2.5	24.2±2.5
AMH	1.7–2.9	1.3–2.4	1.5–2.5	1.4–2.6	1.7–2.5
	2.2±0.26	1.8±0.3	1.9±0.2	2.1±0.3	2.1±0.22
AMW	0.70–1.31	0.8–1.3	0.5–1.5	0.6–1.6	0.76–1.30
	1.0±0.2	1.0±0.1	1.2±0.1	1.2±0.2	1.1±0.1
RH	0.8–1.3	0.6–2.4	0.8–1.3	0.7–1.6	0.9–1.2
	1.0±0.1	1.0±0.3	1.0±0.1	1.1±0.2	1.0±0.1
RW	2.2–3.2	2.1–2.7	1.6 –2.8	1.9–3.1	2.0–2.9
	2.7±0.3	2.6±0.1	2.2±0.2	2.6±0.3	2.5±0.3
MBS	48–69	39–51	42–58	45–60	46–56
	56.8±6.1	46.5.6±3.4	48.1±4.1	53.8±3.6	50.6±3.0
DTS	43–72	42–57	40–55	42–66	40–55
	56.1±7.2	47.2±3.6	47.0±4.1	54.4±4.6	46.4±3.6
DHS	10–19	10–16	11–18	10–19	9–17
	14.6±2.1	13.5±1.5	14.0±1.7	13.7±1.7	12.7±1.8
VS	70–87	56–82	60–87	69–96	71–88
	79.6±4.5	72.8±6.4	76.3±6.5	80.7±5.2	77.7±4.1
SCI	5–12	4–8	5–10	5–9	5–13
	7.9±1.4	6.4±1.2	7.0±1.0	7.1±1.0	7.6±1.4

For HL, there were significant differences between Ancash males vs. Ayacucho males, Cusco, *L. tacnae* and *L. walkeri* males; Ancash females vs. Ayacucho females, Cusco, *L. tacnae* and *L. walkeri* females; Ayacucho males vs. Cusco and *L. tacnae*; Ayacucho females vs. *L. walkeri* females; Cusco vs. *L. walkeri* males and *L. walkeri* females.

For FoL, there were significant differences between Ancash males vs. Cusco and *L. tacnae*; Ancash females vs. *L. walkeri*; Ayacucho females vs. *L. walkeri*; Cusco vs. *L. walkeri*.

Table 3. Normal tolerance intervals for morphometric variables of three species of *Liolaemus* described herein, plus *L. tacnae* and *L. walkeri*; those identified with an asterisk were assumed to follow a normal distribution. See methods for abbreviations.

	Ancash (n=29)	**Ayacucho (n=16)**	**Cusco (n=17)**	***L. tacnae* (n=36)**	***L. walkeri* (n=74)**
SVL	46.7–67.3	46.5–64.6	32.7–58.1	40.5–56.8	44.0–65.1
AGD	17.7–35.3	14.4–37.3	12.3–32.9	15.1–28.6	17.5–33.0
HL	9.6–15.2	9.1–13.8	7.7–13.5	9.1–12.3	10.1–14.3
*HW	7.4–13.1	7.1–11.6	6.0–10.3	7.0–10.0	7.5–11.8
SL	4.0–6.4	3.6–5.2	2.6–5.3	3.5–5.6	3.9–6.4
FoL	12.4–19.9	12.2–19.2	11.9–19.5	12.8–19.0	13.4–20.3
HiL	21.2–30.4	17.1–31.4	17.5–29.3	19.2–30.0	19.5–31.0
AMH	1.6–2.9	1.5–2.7	0.9–2.6	1.4–2.4	1.4–2.8
*AMW	0.6–1.4	0.6–1.5	0.6–1.4	0.6–1.7	0.8–1.7
RH	0.7–1.4	0.8–1.3	0.2–2.1	0.7–1.3	0.7–1.6
*RW	1.9–3.5	1.8–3.3	1.8–2.9	1.6–2.8	2.0–3.2

Table 4. Normal tolerance intervals for meristic characters of three species of *Liolaemus* described herein, plus *L. tacnae* and *L. walkeri*; all variables were assumed to follow a normal distribution. See methods for abbreviations.

	Ancash (n=32)	**Ayacucho (n=30)**	**Cusco (n=18)**	***L.tacnae* (n=42)**	***L. walkeri* (n=79)**
MBS	41.4–72.3	43.1–58.2	36.9–56.1	38.0–58.2	45.6–62.0
DTS	38.0–74.2	37.2–55.7	36.9–57.5	37.0–57.0	43.8–65.3
DHS	9.2–20.0	8.3–17.4	9.2–17.9	9.8–18.3	9.9–17.5
VS	68.2–91.0	67.1–88.3	54.7–91.0	60.5–92.2	68.9–92.5
SCI	4.5–11.3	4.1–11.2	3.0–9.8	4.5–9.5	4.8–9.4

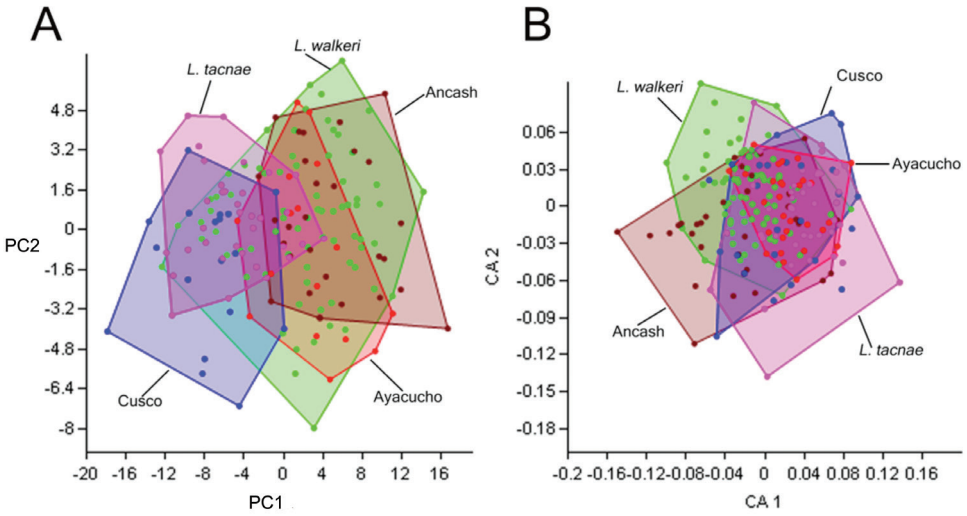

Figure 5. First and second principal components (PC) and correspondence axes (CA) of morphometric (**A**) and meristic (**B**) data of Ancash, Ayacucho, Cusco, *L. tacnae* and *L. walkeri* respectively.

For HiL, there were significant differences between Ancash males vs. Ayacucho, Cusco males and *L. tacnae*; Ancash females vs. Cusco females; Ayacucho vs. Cusco females and *L. walkeri* males; Cusco females vs. *L. tacnae* and *L. walkeri* females.

For SL, there were significant differences between Ancash males vs. Ayacucho males, Cusco, *L. tacnae* and *L. walkeri*; Ancash females vs. Ayacucho females and Cusco; Ayacucho males vs. Cusco; Ayacucho males and females vs. *L. walkeri*; Cusco vs. *L. tacnae* and *L. walkeri*;

For AMH, there were significant differences between Ancash vs. Cusco, *L. tacnae*, *L. walkeri*; Ancash vs. Ayacucho females; Ayacucho males vs. Cusco and *L. tacnae*; Ayacucho females vs. Cusco; Cusco vs. *L. tacnae* and *L. walkeri*.

For RH, there were significant differences between Ancash males vs. *L. tacnae*; Ancash females vs. *L. walkeri*; Ayacucho females vs. *L. walkeri*; Cusco vs. *L. walkeri*.

Only significant results of Mann-Whitney *U* are mentioned below and the sex of a particular species or population is indicated only if significantly different from the opposite sex. For HW, there were significant differences between Ancash males vs. Ayacucho, Cusco, *L. tacnae* and *L. walkeri*; Ancash females vs. Cusco and *L. tacnae*; Ayacucho vs. Cusco and *L. tacnae*; Cusco vs. *L. walkeri*.

For AMW, there were significant differences between Ancash vs. Cusco; Ayacucho vs. *L. tacnae*; Cusco vs. *L. tacnae* and *L. walkeri*.

For RW, there were significant differences between Ancash males vs. Cusco, *L. tacnae* males, and *L. walkeri*; Ancash females vs. Cusco and *L. tacnae* females; Ayacucho males vs. Cusco and *L. tacnae* males; Ayacucho females vs. *L. tacnae* females and *L. walkeri*; Cusco vs. *L. tacnae* females and *L. walkeri*.

For MBS, there were significant differences between Ancash vs. Ayacucho, Cusco, *L. tacnae* and *L. walkeri*; Ayacucho vs. Cusco, *L. tacnae* and *L. walkeri*; Cusco vs. *L. walkeri*.

For DTS, there were significant differences between Ancash vs. Ayacucho, Cusco, *L. tacnae*, *L. walkeri* males and *L. walkeri* females; Ayacucho vs. *L. walkeri* males and *L. walkeri* females; Cusco vs. *L. walkeri* males and *L. walkeri* females.

For DHS, there were significant differences between Ancash vs. Ayacucho females, Cusco females and *L. walkeri*; Ayacucho females vs. *L. tacnae* and *L. walkeri*; Cusco females vs. *L. tacnae*.

For VS, there were significant differences between Ancash vs. Cusco and *L. tacnae*; Ayacucho vs. Cusco and *L. walkeri* females; Cusco vs. *L. tacnae*, *L. walkeri* males and *L. walkeri* females.

For SCI, there were significant differences between Ancash males vs. Ayacucho, Cusco, *L. tacnae* and *L. walkeri*; Ancash females vs. Cusco; Ayacucho vs. Cusco and *L. tacnae*; Cusco vs. *L. walkeri*.

Distributional models

The predicted distribution in all cases matched the known range of each taxon, although some of these overlap. However, the distributional models of Ayacucho vs *L.*

tacnae (Fig. 6; C vs. E), as well as those for *L. walkeri* and *L. tacnae* (Fig. 6; E vs. F) are virtually mutually exclusive. All other combinations of distributional models overlapped, but differed in the contribution of bioclimatic variables to each niche envelope, and in predicting the known distribution of particular taxa (Table 5, Fig. 6). For example, the most important bioclimatic variables for the Ancash model were completely different from those for the *L. walkeri* and Ayacucho models (Table 5). In the same manner, the most important bioclimatic variables contributing to the Ayacucho model were completely different from those for the *L. walkeri* and Cusco models (Table 5). The most important bioclimatic variables for the Cusco model were completely different to those for *L. tacnae* (Table 5). Moreover results from the Niche Identity Test found all pairwise comparison between focal populations and species significantly different, except for Ancash and Cusco (Table 6).

The Ancash model (Fig. 6B) overlapped the known geographic distributions of Ayacucho, Cusco, *L. tacnae*, and partially with *L. walkeri*, but the two most important bioclimatic variables accounting for 94.3% of the contribution to this model were Precipitation of Warmest Quarter (63.3%) and Isothermality (31.0%; Table 5). These were also the most important variables in the permutation and jackknife tests. Thus the Ancash samples are characterized by a niche envelope with relative lower precipitation and more variation in annual temperature. The AUC score for this model = 0.87 (± 0.05), suggesting that the model prediction was reasonable (Fig. 7A).

The Ayacucho model did not overlap known distributions of Ancash, Cusco, *L. tacnae*, and only partially overlapped *L. walkeri* (Fig. 6C); the two most important bioclimatic variables accounting for 75.1% of the contribution to this model were Precipitation of Driest Quarter (64.4%) and Maximum Temperature of Warmest Period (10.7%; Table 5). In the permutation and jackknife tests, Precipitation of Driest Quarter was also the most important variable. In other words, the Ayacucho samples are characterized by a relatively wet and warm niche envelope, and the AUC score = 0.76 (± 0.06), suggesting that model prediction was reasonable (Fig. 7B).

The Cusco model did not overlap the known distribution of Ancash, overlapped most of Ayacucho and *L. walkeri*, and overlapped some of *L. tacnae* (Fig. 6D). The

Table 5. Percentage contributions of most important bioclimatic variables to the ecological niche envelopes for all population samples of three species of *Liolaemus* described herein, plus *L. tacnae* and *L. walkeri*.

	Ancash	**Ayacucho**	**Cusco**	***L. tacnae***	***L. walkeri***
Precipitation of the Warmest Quarter	63.3			30.0	
Isotermality	31.0		28.0		
Precipitation of the Driest Quarter		64.4		21.8	
Maximum Temperature of Warmest Period		10.7			
Precipitation of the Wettest Period			55.9		43.6
Precipitation of the Wettest Quarter				12.4	
Precipitation of the Driest Period					40.6

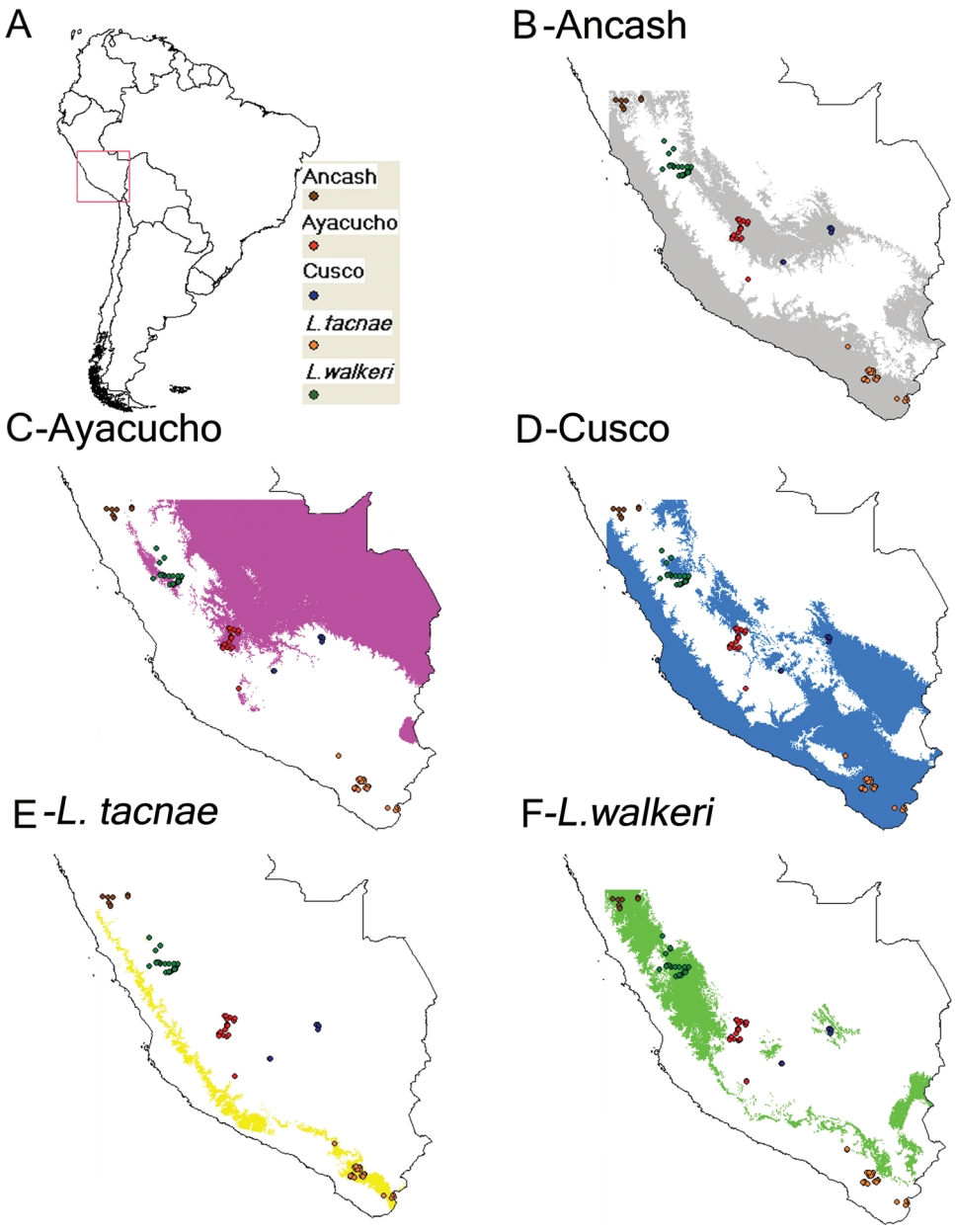

Figure 6. Predicted area and known geographic distribution (**A**) used to develop distributional models of Ancash (**B**) Ayacucho (**C**) Cusco (**D**) *L. tacnae* (**E**) and *L. walkeri* (**F**).

two most important bioclimatic variables accounting for 83.9% of the contribution to the model were Precipitation of the Wettest Period and Isothermality (Table 5). In the permutation and jackknife tests, Precipitation of the Wettest Period was also the most

Table 6. Schoener's D values and Niche Identity test results between focal populations and species. A value in bold denotes a pair of species that has statistically distinct ENMs.

	Ayacucho	Ancash	Cusco	*L. tacnae*	*L. walkeri*
Ayacucho	1	**0.167**	**0.100**	**0.004**	**0.108**
Ancash		1	0.670	**0.346**	**0.300**
Cusco			1	**0.328**	**0.356**
L. tacnae				1	**0.115**
L. walkeri					1

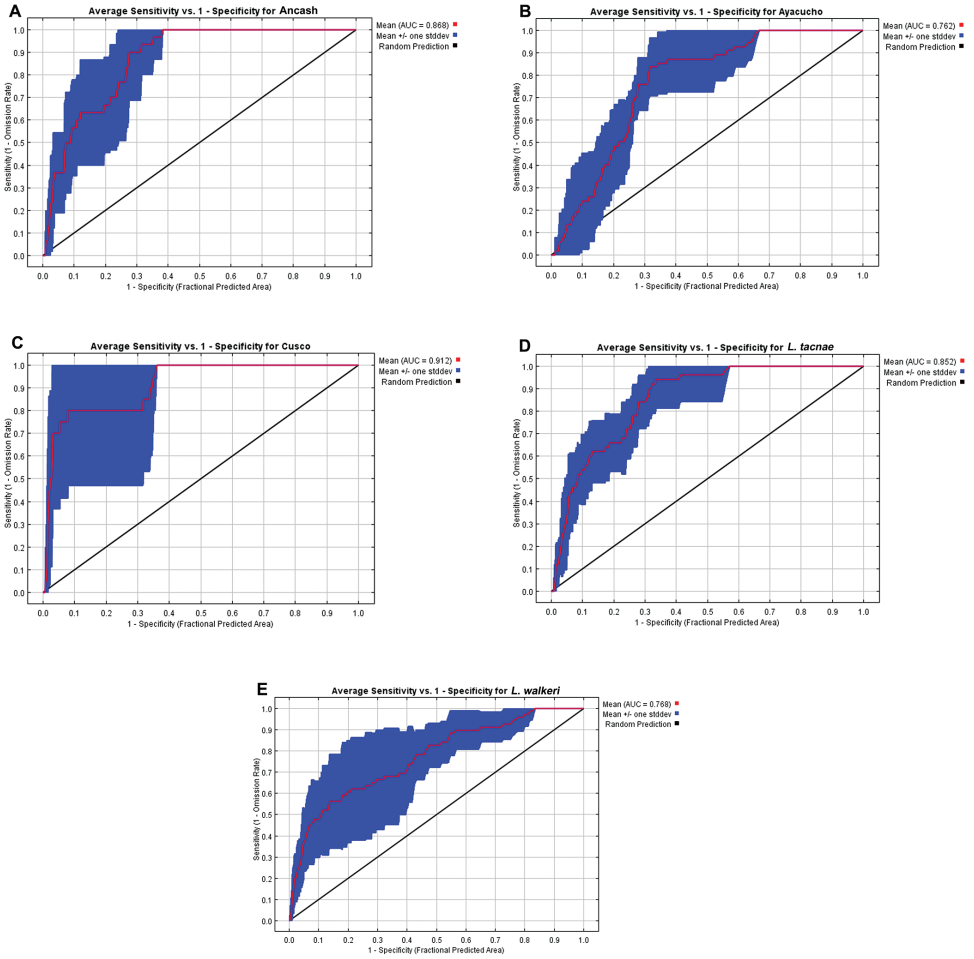

Figure 7. Receiver operating characteristic curves and AUC values for **A** Ancash **B** Ayacucho **C** Cusco **D** *L. tacnae* and **E** *L. walkeri*.

important variable indicating a niche envelope with relative more precipitation in the wettest period of the year. The AUC score = 0.91 (± 0.03), suggesting that model prediction was reasonable (Fig. 7C).

The *L. tacnae* model did not overlap the known distributions of any of the remaining taxa (Fig. 6E); the three most important bioclimatic variables accounting for 64.2% of the contribution to the model are Precipitation of Warmest Quarter, Precipitation of Driest Quarter, and Precipitation of Wettest Quarter (Table 5). In the permutation test, the most important variable was Precipitation of the Coldest Quarter, but in the jackknife tests Precipitation of Warmest Quarter, Precipitation of Wettest Quarter and Annual Precipitation were the most important variables. This indicates that *L. tacnae* samples are characterized by a drier niche envelope relative to all other populations and the AUC score (0.85 ± 0.06) suggests that this model prediction was reasonable (Fig. 7D).

The *L. walkeri* model overlaps the known distribution of the Ancash and partially that of the Cusco samples (Fig. 6F); the two most important bioclimatic variables accounting for 84.2% of the contribution to the model are Precipitation of Driest Period and Precipitation of Wettest Period (Table 5). In the permutation and jackknife tests, Precipitation of Wettest Period also was the most important variable. This suggests a relative wetter niche envelope relative to all other populations, and the AUC score (0.77 ± 0.08) suggests that the model prediction was reasonable (Fig. 7E).

The niche identity test results showed that observed values of Schoener's D between all populations and species were significantly lower than null distribution of pseudoreplicates except for Ancash and Cusco (Table 6).

Integrative taxonomy

Results of mitochondrial haplotypes, binary (presence/absence of precloacal pores, spots or regular marks in lateral field, melanistic belly in adult males, ringed ventral tail pattern), morphometric (snout-vent length, axila-groin length and hindlimb length) characters and niche identity tests in various combinations, differentiated Ancash, Ayacucho and Cusco samples from each other, and from *L. tacnae* and *L. walkeri*. Despite the fact that binomial tolerance intervals showed the possible presence of polymorphisms even at a frequency cut off of 0.5% in discrete characters, we hypothesize that increasing samples sizes will lower the hypothesized frequencies of the alternative states for each taxon. Normal tolerance intervals and distributional models showed overlap between all paired combinations of samples except for the Ayacucho vs. *L. tacnae* distributional models and niche identity tests showed statistical differences between all pairwise comparisons but Ancash vs. Cusco. Note that this is an extremely conservative approach; if we simply look at the data and count the number of "fixed" differences between all combinations of samples, we would conclude that the following pairs are unambiguously diagnosed: Ayacucho, Cusco and *L. walkeri* vs. Ancash (precloacal pores or not), Ancash vs. *L. tacnae* (melanistic belly or not), Ayacucho vs. Cusco and *L. walkeri* (ringed pattern in ventral tail or not), Cusco vs. most *L. walkeri* (lateral markings or not). Based on the integration of molecular, different classes of morphological data, and niche identity test results, we conclude that *Liolaemus* populations from Ancash, Ayacucho, and Cusco can be delimited as separate species, and we describe these new species below.

Species descriptions

***Liolaemus chavin* sp. n.**
http://zoobank.org/47B7926F-7D66-4C0B-9F25-9696C916E6C2
http://species-id.net/wiki/Liolaemus_chavin
Figure 8

2002 *Liolaemus alticolor* Lehr
2007 *Liolaemus incaicus* Lobo, Quinteros and Díaz Gómez
2011 *Liolaemus* aff. *walkeri* Langstroth

Holotype. MUSM 25417, adult male collected at Conococha, Recuay Province, Ancash Department, Peru, -10.123S, -77.293W, elevation 4100 m, on 31 March 2006 by Mikael Lundberg.

Paratypes. Three males (MUSM 20141, 20143, 20146) and twelve females (MUSM 25324, 25327, 25328, 25331, 25333, 25334, 25340, 25423, 25412, 30812, 30813, BYU 50192) from the same locality as the holotype. One male (MUSM 20147) from Carpa, Recuay Province, Ancash Department, on 28 February 2001 by Edgar Lehr and César Aguilar (see Data resources for elevation and coordinates). One female (MUSM 20201) from La Unión, Huánuco Department, on 3 March 1997 by Edgar Lehr (see Data resources for elevation and coordinates). Seven males (CORBIDI 10439, 10450, 10452, 10442, 10441, 10443, 10437) and six females (CORBIDI 10444, 10451, 10440, 10438, 10445, 10449) from Pampas de Huamani, San Marcos District, Huari Province, Ancash Department, on 12 February 2012 by Pablo J. Venegas (see Data resources for elevation and coordinates).

Diagnosis. Small (61.7 mm maximum SVL), slender *Liolaemus* closely related to *L. walkeri*, *L. tacnae, L. pachacutec* sp. n. and *L. wari* sp. n. (described below) (Fig. 1). It differs from *L. walkeri*, *L. pachacutec* sp. n. and *L. wari* sp. n. in the absence of precloacal pores in males. It differs from *L. tacnae* in having a melanistic belly in adult males (not melanistic in adult *L. tacnae* males). In comparison with other species assigned to the *L. alticolor* group, *L. chavin* sp. n. differs from *L. bitaeniatus* and *L. pagaburoi* in having a smooth dorsal surface of the head (rough to slightly rough dorsal surface). It differs from *L. alticolor*, *L. aparicioi*, *L. incaicus*, *L. paulinae*, *L. pyriphlogos, L. puna,* and *L. variegatus* in the absence of precloacal pores in males. *Liolaemus chaltin* also lacks precloacal pores in males, but *L. chavin* sp. n. differs in having also a melanistic belly in adult males.

Description of holotype. Adult male; SVL 56.8 mm; head length 13.7 mm; head width 11.3 mm; head height 7.7 mm; axilla-groin 21.0 mm (37% of SVL); foot length 10.3 mm (18.3% of SVL); tail length (regenerated) 35.2 mm (0.6 times SVL).

Fifteen dorsal head scales (from a line drawn horizontally between anterior edges of external auditory meatus to anterior border of rostral). Dorsal head scales smooth except for the interparietal and surrounding scales, scale organs more abundant in prefrontal, internasal, and supralabial regions. Five scale organs on postrostral. Nasal scale in contact

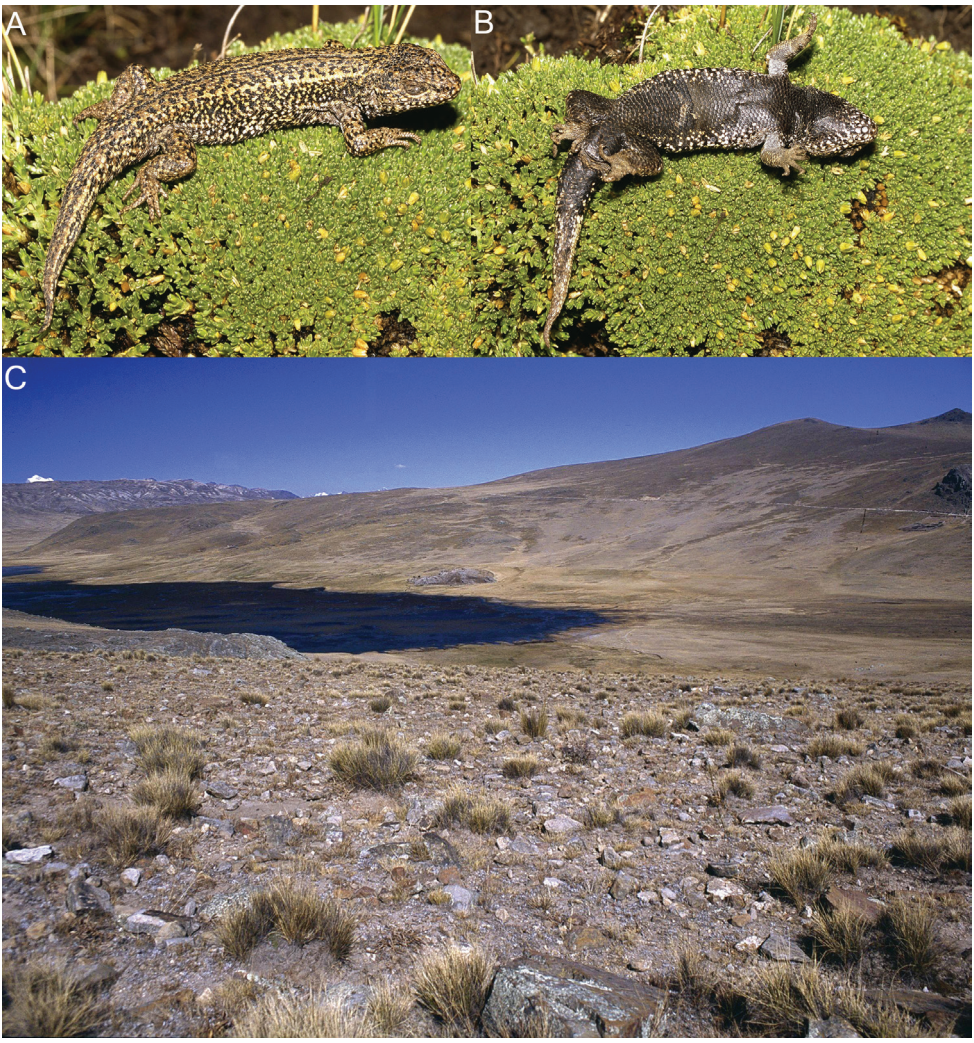

Figure 8. Dorsal (**A**) and ventral (**B**) views of the holotype of *Liolaemus chavin* sp. n. (**C**) Type locality.

with rostral, separated from first supralabial by one scale, nasal bordered by eight scales; canthus separated from nasal by one scale. Six supralabials. Six lorilabial scales, three in contact with the subocular. Six infralabials. Auditory meatus oval (height 2.3 mm, width 1.2 mm), with three small, projecting scales on anterior margin. Seven convex, smooth temporals. Orbit–auditory meatus distance 4.9 mm. Orbit–anterior margin of rostral distance 6.3 mm. Rostral almost three times wider than high (width 2.9 mm; height 1.2 mm). Mental subpentagonal, about two times as wide as high (width 3.2 mm; height 1.7 mm). Interparietal pentagonal with an elongated posterior apex, bordered by eight scales, the parietal slightly smaller. Frontal quadrangular. Supraorbital semicircles complete on both sides. Semicircles formed by 6 scales. Four enlarged supraoculars. Six distinctly imbricate superciliaries on both sides. Eleven upper and ten lower ciliaries.

Subocular elongate, 3.8 mm, longer than eye diameter (2.9 mm), separated from supralabials by a single, but interrupted row of lorilabials. Second supralabial elongate, 1.9 mm. Six lorilabials with single and double rows of scale organs. Sixth, fifth and fourth lorilabials contacting subocular. Preocular small, separated from lorilabial row by one scale. Postocular as large as preocular. Mental in contact with four scales: first infralabials (on each side) and two enlarged chin shields. Chin shields forming a longitudinal row of three enlarged scales separated one from the other by seven smaller scales. Scales of throat round, flat, and imbricate. Twenty-four gulars between auditory meatus. Longitudinal neck fold without keeled scales, that are similar to dorsal in size scales. Antehumeral pocket and antehumeral neck fold well developed. Forty-two scales between auditory meatus and shoulder (counting along postauricular and longitudinal neck fold), thirty-two scales between auditory meatus and antehumeral neck fold. Gular folds absent.

Dorsal scales rhomboidal, keeled, and imbricate. Sixty-six dorsal scales between occiput and level of groin. Sixty-two scales around midbody. Thirty rows of keeled scales on dorsum at midtrunk. Scales become smooth along flank and toward belly. Ventral scales slightly wider than dorsals. Eighty-two ventral scales between mental and cloaca; no precloacal pores. Supracarpals laminar, round, and smooth. Subdigital lamellae of fingers with three keels, in number I: 6; II: 11; III: 14; IV: 15; V: 10 (right hand). Claws moderately long. Supradigital lamellae convex, smooth, and imbricate. Infracarpals and infratarsals keeled, distinctly imbricate. Supratarsals smooth. Subdigital lamellae of toes I: 13; II: 13; III: 13; IV: 12; V: 6 (right foot).

Color pattern in preservation. Dorsal background color from occiput to base of tail greenish brown. Black continuous vertebral stripe present. Dark paravertebral marks. Paravertebral and vertebral fields of same background color. Dorsolateral stripes distinctly cream-color. Small dark cream-colored markings scattered in lateral field. Cream ventrolateral stripe, beginning on the upper auricular meatus, continuing across the longitudinal neck fold, through the shoulders, ending in the groin. Dark and small cream-colored marks in the ventral field. Black ventral color from about second third of head to femur, tibia and first third of tail. Dark and cream-colored small markings in first third of ventral head and two posterior thirds of tail.

Color pattern in life. Head dorsally brown with black and light brown dots. Subocular cream colored, dorsum bisected by a dark vertebral line. Vertebral field not conspicuous, bordering the vertebral line with a tenuous yellowish line. Paravertebral field with dark marks, bordered dorsally by a yellowish cream dorsolateral stripe. Lateral field with black and yellow reticulated pattern and white dots. Inconspicuous ventrolateral stripe, beginning on upper margin of auricular meatus, continuing from the longitudinal neck fold, through the shoulders, ending in the groin. Ventrolateral similar to lateral field but with more white dots. Fore and hind limbs same color as the paravertebral field, with diffuse dorsal markings. Dark, melanistic ventral color from about second third of head to femur, tibia and first third of tail. Dark and white dots in first third of ventral head and two posterior thirds of tail.

Variation. Variation in characters is summarized in Tables 1–4. There is sexual dichromatism. Adult males exhibit melanistic belly, cloacal region and throat, or mela-

nistic belly only; adult females exhibit black and white spots on belly, cloacal region and throat, or yellowish belly and tail.

Etymology. The specific epithet *chavin* refers to the pre-Inca culture Chavin, which had its center close to the type locality and frequently depicted reptile figures on some of its most remarkable sculptures. The species name is in the nominative singular.

Distribution and natural history. *Liolaemus chavin* sp. n. is known from four localities in the central Andes, at elevations of 3535–4450 m in Ancash and Huánuco Departments in western central Peru (Fig. 11). It is the northernmost species of the subgenus *Liolaemus*.

Liolaemus chavin sp. n. was found active and under rocks in grassland and shrubland habitats at higher and lower elevations respectively (Fig. 8). In Pampas de Huamani the new species was usually found basking on grass up to 60 cm above the ground, and when they were disturbed they escaped into the base of grass clumps. Individuals basking on rocks were very rare in all localities. On cloudy days we found this species inactive hidden in the base of grass clumps, although some individuals were also found inactive under rocks. This species is viviparous; one female showed two uterine chambers per side with developed embryos, yolk and no visible shell in either chamber, and three females showed two uterine chambers per side with yolk, without developed embryos and no visible shell in each chamber. At the type locality no sympatric species of reptiles were found, but four amphibians are known: *Pleurodema marmoratum* (Duméril & Bibron, 1840), *Telmatobius mayoloi* Salas & Sinsch, 1996, *Gastrotheca peruana* and *Rhinella (Bufo) spinulosa* (Wiegmann, 1834) (Lehr, 2002; personal observations). Sympatric species at Catac include the anurans *G. peruana*, *R. (Bufo) spinulosa*, *Telmatobius rimac* Schmidt, 1954, *T. mayoloi*, and the lizard *Stenocercus chrysopygus* Boulenger, 1900; at Carpa, *G. peruana* (Boulenger, 1900), *R. (Bufo) spinulosa* and *P. marmoratum*; at Pampas de Huamani, *G. peruana, P. marmoratum* and *R. (Bufo) spinulosa*; and at La Unión, *Gastrotheca griswoldi* Shreve, 1941, *G. peruana*, *R. (Bufo) spinulosa* and *S. chrysopygus* (Lehr, 2002).

***Liolaemus pachacutec* sp. n.**

http://zoobank.org/A979BB00-3CA1-47C9-8EB0-F605166FBF1A
http://species-id.net/wiki/Liolaemus_pachacutec
Figure 9

Holotype. MUSM 29683, adult male collected at Challabamba, Paucartambo Province, Cusco Department, Peru, -13.254S, -71.838W, elevation 4364 m, on 1 April 2009 by César Ramírez.

Paratypes. Three males (MUSM 29681, 29687, 29678) and four females (MUSM 29679, 29689, 29680, 29682) from the same locality as the holotype. Two males MUSM (29665, 29668) and one female (MUSM 29669) from Lamay, Calca Province, Cusco Department, on 12 October 2009 by César Ramírez (see Data resources for elevations and coordinates). One male (MUSM 29664), two females (MUSM

29688, BYU 50237) and one juvenile (MUSM 31412) from Pisac, Calca Province, Cusco Department, on 4 July and 11 October 2009 by César Ramírez, and on 28 June 2012 by César Aguilar, Perry Wood and Juan Carlos Cusi (see Data resources for elevations and coordinates). One male (MUSM 31540), two females (MUSM 31538-39) and one juvenile (MUSM 31537) from Tiaparo, Pocohuanca District, Aymaraes Province, Apurímac Department, on 11 June 2013 by Alfredo Guzmán (see Data resources for elevations and coordinates).

Diagnosis. Small (51.9 mm maximum SVL) *Liolaemus* closely related to *L. chavin* sp. n., *L. tacnae, L. walkeri,* and *L. wari* sp. n. (described below) (Fig. 1). It differs from *L. chavin* sp. n. and *L. tacnae* in having precloacal pores (males). *Liolaemus pachacutec* differs from *L. wari* sp. n. in having a partial or complete melanistic belly in adult males and in lacking a ringed pattern in ventral tail. *Liolaemus pachacutec* differs from most individuals (90%) of *L. walkeri* in lacking spots in the lateral field. In comparison with other species assigned to the *L. alticolor* group, *L. pachacutec* differs from *L. chaltin* in having precloacal pores in males. It differs from *L. paulinae* in the presence of a vertebral line and smooth neck scales. It differs from *L. puna, L. alticolor* and *L. incaicus* in having a partial or complete melanistic belly in adult males. It differs from *L. aparicioi* in lacking keeled temporal scales. It differs from *L. bitaeniatus* and *L. pagaburoi* in having a smooth dorsal surface of the head. It differs from *L. pyriphlogos* in the absence of red marks in lateral fields. It differs from *L. variegatus* in lacking keeled temporal scales, rugose dorsal head scales, and precloacal pores in females.

Description of holotype. Adult male; SVL 44.8 mm; head length 11.0 mm; head width 8.2 mm; head height 6.2 mm; axilla-groin distance 18.4 mm (41.1% of SVL); foot length 13.6 mm (30.4% of SVL); tail length 74.9 mm. (1.7 times SVL).

Dorsal head scales 16, dorsal head scales smooth, scale organs more abundant in loreal and supralabial regions. Two scale organs on postrostral. Nasal scale in contact with rostral, separated from first supralabial by one scale, nasal bordered by six scales; canthus separated from nasal by one scale. Four supralabials. Four lorilabials scales and one in contact with the subocular. Five infralabials. Auditory meatus oval (height 2.0 mm, width 1.0 mm), with two small, projecting scales on anterior margin. Six convex, smooth temporals (counting vertically from buccal commissure to posterior corner of orbit). Orbit–auditory meatus distance 3.9 mm. Orbit–anterior margin of rostral distance 4.3 mm. Rostral about two times wider than high (width 2.3 mm; height 1.0 mm). Mental subpentagonal, about two times as wide as high (width 2.5 mm; height 1.0 mm). Interparietal pentagonal with an elongated posterior apex, bordered by five scales, the parietal of similar size. Frontal trapezoidal.

Supraorbital semicircles complete on both sides. Semicircles formed by six scales. Five enlarged supraoculars. Six distinctly imbricate superciliaries on both sides. Eleven upper and lower ciliaries. Subocular elongate, 2.8 mm, longer than eye diameter (2.1 mm; measured between anterior and posterior commissure of ciliaries), separated from supralabials by a single, but interrupted row of lorilabials. Fourth supralabial elongate, 2.0 mm. Four lorilabials with single row of scale organs. Fourth lorilabial contacting subocular. Preocular small, separated from lorilabial row by one scale. Postocular as

Figure 9. Lateral (**A**) dorsal (**B**) and ventral (**C**) views of the holotype of *Liolaemus pachacutec* sp. n. (**D**) Habitat of *L. pachacutec*

large as preocular. Mental in contact with four scales: first infralabials (on each side) and two enlarged chin shields. Chin shields forming a longitudinal row of four enlarged scales separated one from the other by six smaller scales. Scales of throat round, flat, and imbricate. Twenty-two gulars between auditory meatus. Longitudinal neck

fold without keeled scales and smaller in size than dorsal scales. Antehumeral pocket and antehumeral neck fold well developed. Thirty-six scales between auditory meatus and shoulder (counting along postauricular and longitudinal neck fold), twenty-six scales between auditory meatus and antehumeral neck fold. Gular folds absent.

Dorsal scales rhomboidal, keeled, and imbricate. Forty-two dorsal scales between occiput and level of groin. Forty-five scales around midbody. Nineteen rows of keeled scales on dorsum at midtrunk. Scales becoming smooth along flank and toward belly. Ventral scales slightly wider than dorsals. Seventy-seven ventral scales between mental and precloacal pores. Five precloacal pores. Supracarpals laminar, round, and smooth. Subdigital lamellae of fingers with three keels, in number I: 8; II: 12; III: 16; IV: 18; V: 12 (right fingers). Claws moderately long. Supradigital lamellae convex, smooth, and imbricate. Infracarpals and infratarsals keeled, distinctly imbricate. Supratarsals smooth. Subdigital lamellae of toes I: 10; II: 14; III: 18; IV: 22; V: 15 (right toes).

Color in preservation. Dorsal background color from occiput to base of tail brownish-green. Black thin continuous vertebral line present. No dark paravertebral marks. Paravertebral and vertebral fields with same background color. Distinct cream dorsalateral stripes. No marks in lateral field. Cream ventrolateral stripes, beginning on the posterior corner of the eye, continuing across the upper auricular meatus, the longitudinal neck fold, through the shoulders, ending in the groin. No marks in the ventral field. Melanistic venter on throat, femur, tibia, and belly. Small and scattered dark marks in chin area and ventrolateraly. Ventral tail melanistic near the cloaca, with a thin longitudinal stripe, first half with small marks lateral to the stripe.

Color pattern in life. Head dorsally brown with scattered black dots. Subocular white. Thin and faint black vertebral line. Paravertebral field without dark marks. Creamy dorsolateral stripes. Lateral field without marks. Faint cream-white ventrolateral stripe, beginning on upper margin of eye, continuing from auricular meatus, the longitudinal neck fold, through the shoulders, ending in the groin. Ventral field yellow. Forelimbs and chin scales white with scattered black dots. Melanistic belly, hind limbs, posterior two thirds of throat. Belly with scattered yellow dots laterally. Tail with a black region close to the cloaca, black longitudinal stripe and dots at each side of the stripe.

Variation. Variation in characters is summarized in Table 1–4. There is sexual dichromatism. Males have a complete or partial melanistic belly and throat, while females have a white or yellow belly and black spots on throat. Some males have orange and yellow dots on lateral belly and yellow dots on chin scales, and ventral field with orange and black dots.

Etymology. The specific epithet *pachacutec* refers to one of most important Inca rulers, Pachacutec, who built the best known Inca ruins, including Machu Picchu and Pisac, this last site at a higher elevation just above the type locality. The species name is in the nominative singular.

Distribution and natural history. *Liolaemus pachacutec* sp. n. is known from four localities in the central Andes, at elevations of 4023–4972 m in the departments of Cusco and Apurímac in southeastern Peru (Fig. 11). The species was found under

rocks in grassland habitats (Fig. 9). It was found in sympatry at similar elevations with *Liolaemus ortizi* Laurent, 1982 and *Tachymenis peruviana* Wiegmann, 1835. This species is probably viviparous; two females showed one or two uterine chambers per side, with an embryo and abundant yolk in each chamber, but without a visible shell.

***Liolaemus wari* sp. n.**
http://zoobank.org/67A997B8-5854-4D0D-B1E0-77680FF47512
http://species-id.net/wiki/Liolaemus_wari
Figure 10

1999 *Liolaemus walkeri* Lobo and Espinoza
2002 *Liolaemus walkeri* Martínez Oliver and Lobo
2007 *Liolaemus walkeri* Lobo, Quinteros and Díaz Gómez
2012 *Liolaemus walkeri* Quinteros
2012 *Liolaemus walkeri* Ocampo, Aguilar-Kirigin and Quinteros

Holotype. MUSM 30837, adult male collected at Abra Toccto, Huamanga Province, Ayacucho Department, Peru, -13.345S, -74.167W, elevation 4231 m, on 4 June 2012 by César Aguilar and Víctor Vargas.

Paratypes. Three males (MUSM 30823, BYU 50184, 50185) and ten females (MUSM 30824, 30825, 30826, 30827, 30828, 30831, BYU 50186, 50187, 50191, 50243) from the same locality as the holotype. Two males (MUSM 30830, 30834) and three females (MUSM 30829, BYU 50188, 50190) from high area above the Historic Sanctuary Pampas, Huamanga Province, Ayacucho Department, on 3 June 2012 by César Aguilar and Víctor Vargas (see Data resources for elevations and coordinates). Two males (MUSM 25703, 25704) and one female (MUSM 25702) from Yanacocha Lake, La Mar Province, Ayacucho Department, on 24 November 2010 by Margarita Medina (see Data resources for elevations and coordinates). Two females (MUSM 25719, BYU 50189) from Huaychao, Huamanga Province, Ayacucho Department, on 1 December 2010 by Margarita Medina (see Data resources for elevations and coordinates). Two females (MUSM 30243, 30244) from Tambo, San Miguel Province, Ayacucho Department, by Michael Harvey. One male (MUSM 31411) and two juveniles (BYU 50235-36) from about 45 Km west Puquio-Cusco roadway, Lucanas Province, Ayacucho Department, on 11 June 2012 by César Aguilar and Víctor Vargas (see Data resources for elevations and coordinates).

Diagnosis. Small (61.4 mm maximum SVL), slender *Liolaemus*, closely related to *L. chavin* sp. n., *L. pachacutec* sp. n., *L. tacnae* and *L. walkeri* (Fig. 1). It differs from *L. chavin* sp. n., *L. pachacutec* sp. n. and *L. walkeri* in having a ringed pattern on the ventral tail of adult males. It differs from *L. pachacutec* sp. n. in having spots in the lateral fields. *Liolaemus wari* differs from *L. tacnae* and *L. chavin* in having precloacal pores in males. In comparison with other species assigned to the *L. alticolor* group, *L. wari* sp. n. differs from *L. chaltin* in having precloacal pores in males. It differs from

L. paulinae in lacking keeled neck scales. It differs from *L. puna, L. alticolor* and *L. incaicus* in having black spots on belly of adult males. It differs from *L. aparicioi* in lacking keeled temporal scales. It differs from *L. bitaeniatus* and *L. pagaburoi* in having a smooth dorsal surface of the head (rough to slightly dorsal surface of the head). It differs from *L. pyriphlogos* in the absence of red marks in the lateral field (red marks in the lateral fields present). It differs from *L. variegatus* in the absence of keeled temporal scales, rugose dorsal head scales and precloacal pores in females.

Description of holotype. Adult male; SVL 55.4 mm; head length 11.4 mm; head width 9.8 mm; head height 6.2 mm; axilla–groin distance 23.3 mm (42% of SVL); foot length 15.0 mm. (27.1% of SVL); tail length 83.7 mm. (1.5 times SVL).

Dorsal head scales 14, dorsal head scales smooth, scale organs more abundant in loreal and supralabial regions. Five scale organs on postrostral. Nasal scale in contact with rostral, separated from first supralabial by one scale, nasal bordered by seven scales; canthus separated from nasal by one scale. Four supralabials. Five lorilabials scales and two in contact with the subocular. Four infralabials. Auditory meatus oval (height 2.0 mm, width 1.9 mm), with two small, projecting scales on anterior margin. Seven convex, smooth temporals (counting vertically from buccal commissure to posterior corner of orbit). Orbit–auditory meatus distance 4.6 mm. Orbit–anterior margin of rostral distance 7.9 mm. Rostral almost three times wider than high (width 2.7 mm; height 1.0 mm). Mental subpentagonal, about two times as wide as high (width 2.6 mm; height 1.2 mm). Interparietal pentagonal with an elongated posterior apex, bordered by seven scales, the parietal slightly smaller. Frontal trapezoidal. Supraorbital semicircles complete on both sides. Semicircles formed by 6 scales. Four enlarged supraoculars. Five distinctly imbricate superciliaries on both sides. Eleven upper and lower ciliaries. Subocular elongate, 3.2 mm, longer than eye diameter (2.3 mm; measured between anterior and posterior commissure of ciliaries), separated from supralabials by a single, but interrupted row of lorilabials. Second supralabial elongate, 1.6 mm. Five lorilabials with single and double rows of scale organs. Fifth and fourth lorilabials contacting subocular. Preocular small, separated from lorilabial row by one scale. Postocular as large as preocular. Mental in contact with four scales: first infralabials (on each side) and two enlarged chin shields. Chin shields forming a longitudinal row of three enlarged scales separated one from the other by six smaller scales. Scales of throat round, flat, and imbricate. Twenty-one gulars between auditory meatus. Longitudinal neck fold without keeled scales and smaller in size than dorsal scales. Antehumeral pocket and antehumeral neck fold well developed. Twenty-nine scales between auditory meatus and shoulder (counting along postauricular and longitudinal neck fold), 21 scales between auditory meatus and antehumeral neck fold. Gular folds absent.

Dorsal scales rhomboidal, keeled, and imbricate. Forty-four dorsal scales between occiput and level of groin. Fifty-three scales around midbody. Twenty-two rows of keeled scales on dorsum at midtrunk. Scales becoming smooth along flank and toward belly. Ventral scales slightly wider than dorsals. Seventy-three ventral scales between mental and precloacal pores. Five precloacal pores. Supracarpals laminar, round, and smooth. Subdigital lamellae of fingers with three keels, in number I: 8; II: 12; III: 16;

Figure 10. Lateral (**A**) dorsal (**B**) and ventral (**C**) views of the holotype of *Liolaemus wari* sp. n. (**D**) Type locality.

IV: 16; V: 10 (right fingers). Claws moderately long. Supradigital lamellae convex, smooth, and imbricate. Infracarpals and infratarsals keeled, distinctly imbricate. Supratarsals smooth. Subdigital lamellae of toes I: 8; II: 12; III: 16; IV: 20; V: 13 (left toes).

Color pattern in preservation. Dorsal background color from occiput to base of tail brownish-green. Black continuous vertebral line present. Dark paravertebral marks. Paravertebral and vertebral fields with same background color. Highly distinct creamy-yellow dorsalateral stripes. Large dark and small cream marks in lateral field. Cream ventrolateral stripe, beginning on the posterior corner of the eye, continuing across the upper auricular meatus, the longitudinal neck fold, through the shoulders, ending in the groin. Dark and cream small marks in the ventral field. Black spots

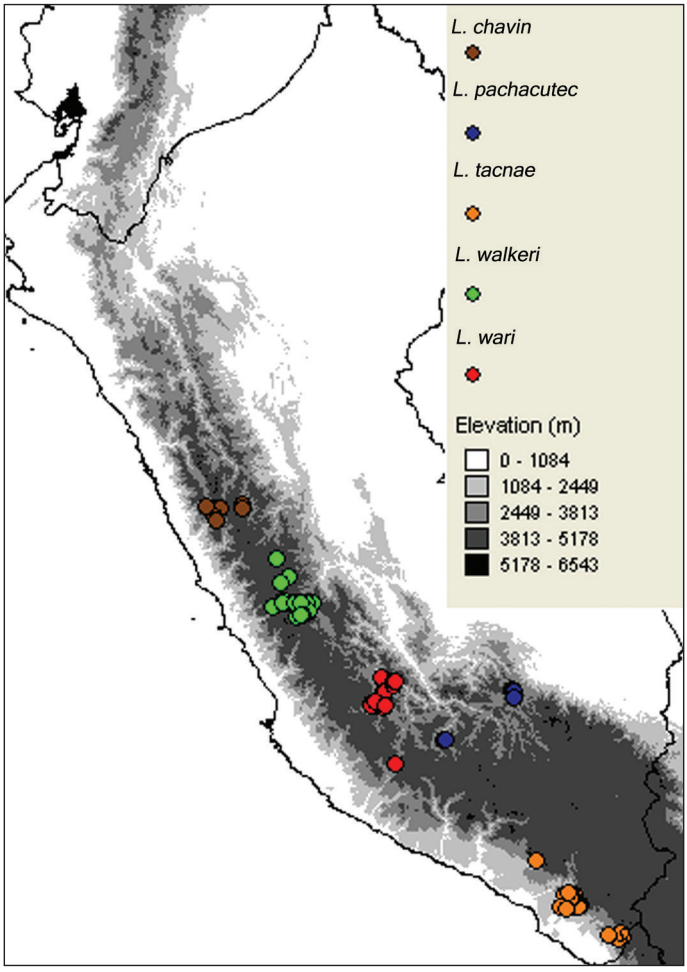

Figure 11. Geographic distribution of *L. chavin, L. pachacutec, L. tacnae, L. walkeri,* and *L. wari.*

on throat, femur, tibia, posterior third of belly and laterally in anterior two thirds of belly. Small and scattered dark marks in chest and anterior two thirds of belly. Tail with dark horizontal rows.

Color pattern in life. Head dorsally brown with black dots. Subocular cream. A black vertebral band with a thin yellow stripe on the middle. The vertebral band has a thin white stripe on each side. Paravertebral field with dark marks with posterior white dots. Creamy-yellow dorsolateral stripes. Lateral field with black marks separated by cream diagonal stripes. Yellowish-white ventrolateral stripe, beginning on upper margin of eye, continuing from auricular meatus, the longitudinal neck fold, through the shoulders, ending in the groin. Ventrolateral similar to lateral field and same color as the paravertebral field, with diffuse dorsal markings. Forelimbs, chest and belly yellowish-white with scattered and diffuse black dots. Black marks on hind

limbs, throat, and posterior third of belly. Tail with black horizontal bands separated by white bands.

Variation. The variation in morphological characters is shown in Tables 1–4. There is sexual dichromatism. Males have white or yellow belly and throat covered completely with black spots, yellowish belly and throat with black spots on posterior third of belly, or a melanistic belly on posterior third and cloacal region, with black dots on a white throat; females have white belly and yellowish throat with faint black dots, yellowish belly and throat with faint black spots, or yellowish belly and throat without spots. Adult males have white, yellowish and yellow tails with a conspicuous ringed pattern; adult females have white, yellowish or reddish ventral tails with or without a faint ringed pattern.

Etymology. The specific epithet *wari* refers to the pre-Inca culture Wari (600–850 AD), which had its center close to the type locality. The species name is in the nominative singular.

Distribution and natural history. *Liolaemus wari* sp. n. is known from seven localities in the central Andes, at elevations of 3768–4246 m in Ayacucho Department in eastern southern Peru (Fig. 11).

Liolaemus wari sp. n. was active on the ground or found under rocks in grassland (Fig. 10) and shrubland habitats. It was found in sympatry with another *Liolaemus* species belonging to the *L. montanus* series and the snake *Tachymenis peruviana*. This species is probably viviparous; three females each showed three uterine chambers per side; each chamber showed yolk, but with no developed embryos or visible shell.

Discussion

Phylogenetic relationships

Surprisingly, our phylogenetic analysis showed that the three new species described herein plus *L. tacnae* and *L. walkeri,* assigned to *alticolor-bibronii* group, are strongly separated from the other members of this species group included in this study. Specifically, the species *L. alticolor* and *L. incaicus* assigned to the *alticolor-bibronii* group (Lobo et al. 2010, Quinteros 2013) were not recovered with *L. tacnae*, *L. walkeri*, and the three new species.

Previous molecular based phylogenies did not include *L. alticolor*, *L. tacnae* and/or *L. walkeri* (Espinoza et al. 2004, Morando et al. 2007, Schulte and Moreno-Roark 2010) and much of what these different topologies show (including ours) is probably an artifact of incomplete taxon/population sampling. Previous morphology-based phylogenies included better taxon sampling, but all of them recovered clades with low or no statistical support, and relationships of *L. tacnae*, *L. walkeri*, *L. alticolor,* and *L. incaicus* with each other and other species assigned to the *alticolor-bibronii* group are ambiguous.

Species delimitation and integrative taxonomy

We take our results based the mtDNA gene tree as a first step in species "discovery" (Carstens et al. 2013), and identify the Ancash, Ayacucho, and Cusco clades as "candidate species" (Morando et al. 2003, Avila et al. 2004). Comparative morphological and niche envelope assessments of these three clades revealed combinations of characters from three different lines of evidence, that unambiguously diagnose these groups as distinct from each other and from *L. tacnae* and *L. walkeri* (this is the second step of species delimitation – "validation" – following Carstens et al. 2013). This result highlights the need for using an integrative approach rather than a single line of evidence (e.g. morphology, usually meristic data only) to delimit species.

Our results show that normal tolerance intervals of continuous morphometric and meristic characters could not discriminate between any of these new species nor between *L. tacnae* and *L. walkeri*. On the other hand, discrete character analysis revealed some diagnostic characters, including: (1) the presence/absence of pre-cloacal pores in males distinguishing *L. chavin* and *L. tacnae* from *L. pachacutec*, *L. walkeri*, and *L. wari*; (2) the presence/absence of a complete or partial melanistic belly in adult males distinguishing *L. chavin* from *L. tacnae*; (3) the presence/absence of a ringed ventral tail pattern of adult males distinguishing *L. wari* from *L. pachacutec* and *L. walkeri*; and (4) the presence/absence of regular marks or spots in lateral fields distinguishing *L. pachacutec* from *L. wari* and from most (90%) individuals of *L. walkeri*. However, binomial tolerance intervals showed that all these "fixed" character states in our samples have a high probably of non-fixation when statistical inference is extended to consider large sample sizes. Despite these findings, we encourage the use of these binomial tests to place empirical evidence into a broader context, and to make investigators aware that tolerance intervals will become narrower as sample sizes increase, and that taxonomic decisions should be based on statistical populations not on samples (Zapata and Jiménez 2012). Moreover, samples taken at random are important for strong statistical inferences, but obtaining random samples in observational studies (such as in most taxonomic studies) is often impractical or impossible, and thus potential for bias is a serious concern (Ramsey and Schafer 2002). Besides this limitation, a statistical inference (such as those based on tolerance intervals) is better than no inference at all. However, statistic tests that evaluate differences in central tendencies (e.g., the ANOVA and Mann-Whitney *U* tests we used here) do not seem relevant as SDL criteria or for practical taxonomic purposes. For instance, most pairwise comparisons of SVL between focal populations (Ancash, Ayacucho, Cusco) and species (*L. tacnae* and *L. walkeri*) are significant in an ANOVA test at a confidence level of 0.05, giving the false impression that this character is useful for species delimitation or taxonomic identification, but tolerance intervals indicate that these populations and species completely overlap with respect to this character (Table 2).

Molecular analysis and, in most cases, niche identity tests, support our species units based on these few morphological characters, and in combination provide more

robust hypotheses. Our model-based molecular phylogenetic analysis provided the basis for our "candidate species" hypotheses, but molecular phylogenetic analysis relies on the assumption that a chosen evolutionary model is a correct one (Posada 2009), and we recognize that in the absence of corroboration from independent data sets, mtDNA may often over-split species (Miralles and Vences 2013). However, assumptions are also pervasive in morphological and ENM analyses. Discovery of gaps in morphology assumes that discontinuities are not due polymorphisms, ontogenetic variation or phenotypic plasticity (Wiens and Servedio 2000, Zapata and Jiménez 2012), and ENM (especially those models based on background data and not true absence records) assumes that occupied distribution of a species is not reduced by biotic interactions and dispersal limitations (Peterson et al. 2011). Despite these assumptions, we think that robust hypotheses of species delimitation based on different data sets give stability to scientific names, provide the strongest inference about species boundaries, overcome overlapping character variation in any particular character system, and should be a prioritized research theme in systematics (Balakrishnan 2005, Will et al. 2005, Padial and De la Riva 2006, Padial et al. 2010). In addition, we expect more exciting results when new molecular coalescent-based multi-locus and morphological multivariate methods can be applied to our data (Zapata and Jiménez 2012, Camargo and Sites 2013).

Northern limits of squamate viviparity in the high Andes

Liolaemus chavin is the northernmost viviparous species of the subgenus *Liolaemus.* Two recognized *Liolaemus* species present in the extreme northern range of the genus are *L. robustus* Laurent, 1992 and *L. disjunctus* Laurent, 1990 (subgenus *Eulaemus*). In the case of *L. disjunctus*, our recent fieldwork in the area of the species' type locality did not locate any specimen. The same result was found when we revisited localities near the type locality of *L. disjunctus* in 2012, and to our knowledge this species has not been collected at least since its original description and data on its reproductive mode are still lacking (Laurent 1990). On the other hand, the colubrid snake *Tachymenis peruviana* is another viviparous squamate widely distributed in the high Andes of Argentina, Bolivia, Chile and Peru. Its northern limits are in the department of La Libertad, Peru at about latitude 7°S, and no other viviparous squamate species are present in the high Andes of northernmost Peru, Ecuador, and Colombia.

What selective pressures might have limited the distribution of viviparous squamates in the high Andes? Although there are no field or experimental studies that have addressed this question in particular, one distributional pattern seems to be evident in the northern distributional limit of *Liolaemus*. For instance, on the Pacific Andean slopes at about latitude 15°S and south in Peru, viviparous *Liolaemus* species are present in lower, middle and higher elevations (C. Aguilar, personal observations), and oviparous lizards (genera *Phyllodactylus*, *Ctenoblepharys* and *Microlophus* but not *Stenocercus*) are only present at lower and middle elevations. However, on the Pacific

Andean slopes at about latitude 12°S, *Liolaemus* species are only present at higher elevations and oviparous *Stenocercus* (Tropiduridae) species become common at lower and middle elevations, together with the above-mentioned oviparous genera. If we consider the actual northern limits of *Liolaemus* as represented by *L. chavin*, viviparous lizards in the high Andes do not extend north beyond about latitude 8–9°S. North of latitude 8°S, oviparous *Stenocercus, Petracola* and *Riama* (Gymnophthalmidae) species are the only lizard genera present in the high Andes of Peru and Ecuador. One interesting distributional and reproductive pattern that matches this change in reproductive mode in lizards is the distribution pattern of amphibians with direct development (genus *Pristimantis*). No *Pristimantis* species have been found in sympatry with northernmost *Liolaemus* species. At high elevations on the Pacific slopes, the northernmost *Liolaemus* species (*L. chavin* and *L. robustus*) have always been found with anurans having complete (genera *Rhinella*, *Pleurodema* and *Telmatobius*) or partial (*Gastrotheca*) indirect development.

Direct-development *Pristimantis* rely on high humidity substrates for egg development (Duellman and Lehr 2009), and what may have limited the distribution of direct-development frogs in the Pacific basin of southern Peru and northern Chile, and the Andean Plateau, is the formation of an Arid Diagonal area due to the interaction of the Humboldt Current and uplift of the Andes. If so, then a working hypothesis for the evolution of viviparity and placentation in some clades of *Liolaemus* is their relationship to the presence of these arid and hypoxic conditions. Arid environments in hypoxic middle and high elevations might be lethal to the development of oviparous lizard eggs. However, origins of viviparity in *Liolaemus* seem to be associated with shifts to cold climates (e.g., in the Oligocene; Schulte and Moreno-Roark 2010), thus supporting the cold climate hypothesis (CCH; Tinkle and Gibbons 1977). According to this hypothesis, viviparity has evolved to avoid lethal ambient temperatures in high elevations and latitudes, and through retention of eggs in the uterus coupled with female behavioral thermoregulation, this mode accelerates embryonic development (for a recent review see Sites et al. 2011). The CCH is a special case of a more general maternal manipulation hypothesis (MMH) where females can enhance fitness-related phenotypic attributes in offspring by manipulating thermal conditions during embryogenesis (Shine 1995). However, arid environments may be more important with increasing hypoxic conditions in high altitudes for the evolution of viviparity than cold climates, as has been suggested for *Phrynosoma* lizards (Hodges 2004, but see Lambert and Wiens 2013). In other words, altitude may be a surrogate of other selective factors important for the evolution of viviparity, not only cold climates (Hodges 2004). High altitude environments tend to be drier and have low oxygen conditions, and viviparous species may be able to provide a better oxygen environment for developing embryos via placental structures (Hodges 2004). Whether shifts in cold climates and/or appearance of arid zones along with Andean uplift are correlated with the origin of viviparity in *Liolaemus* should be tested with coalescent based multi-locus phylogenetic studies and a time-calibrated hypothesis of species relationships.

Key to Peruvian species of the subgenus *Liolaemus*

1a Dorsal body with mucronated scales, no melanistic or without black spots on throat or belly in males ..... ***Liolaemus alticolor* group**
1b Dorsal body usually without mucronated scales, melanistic or with spots on throat or belly in males **3**
2a Dorsal pattern without spots..... ***Liolaemus alticolor***
2b Dorsal pattern with spots..... ***Liolaemus incaicus***
3a Males without precloacal pores **4**
3b Males with precloacal pores **5**
4a Males with black spots on throat, no melanistic belly..... ***Liolaemus tacnae***
4b Males with melanistic belly..... ***Liolaemus chavin***
5a Males with ringed pattern in ventral tail, mucronated scales present or absent..... ***Liolaemus wari***
5b Males without ringed pattern in ventral tail, mucronated scales absent **6**
6a Spots absent in the lateral fields ..... ***Liolaemus pachacutec***
6b Spots present in the lateral fields (most individuals)..... ***Liolaemus walkeri***

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Appendix 1

Supplementary file 1. (doi: 10.3897/zookeys.364.6109.app1) File format: Microsoft Excel (xls).

Explanation note: GenBank accesion and museum voucher numbers of haplotypes used in this study.

Citation: Aguilar C, Wood PL Jr, Cusi JC, Guzmán A, Huari F, Lundberg M, Mortensen E, Ramírez C, Robles D, Suárez J, Ticona A, Vargas VJ, Venegas PJ, Sites JW Jr (2013) Integrative taxonomy and preliminary assessment of species limits in the *Liolaemus walkeri* complex (Squamata, Liolaemidae) with descriptions of three new species from Peru. ZooKeys 364: 47–91. doi: 10.3897/zookeys.364.6109 Supplementary file 1. doi: 10.3897/zookeys.364.6109.app1

Appendix 2

Supplementary file 2. (doi: 10.3897/zookeys.364.6109.app2) File format: Microsoft Excel (xls).

Explanation note: Museum voucher data of specimens used in the morphological analyses.

Citation: Aguilar C, Wood PL Jr, Cusi JC, Guzmán A, Huari F, Lundberg M, Mortensen E, Ramírez C, Robles D, Suárez J, Ticona A, Vargas VJ, Venegas PJ, Sites JW Jr (2013) Integrative taxonomy and preliminary assessment of species limits in the *Liolaemus walkeri* complex (Squamata, Liolaemidae) with descriptions of three new species from Peru. ZooKeys 364: 47–91. doi: 10.3897/zookeys.364.6109 Supplementary file 2. doi: 10.3897/zookeys.364.6109.app2

Appendix 3

Supplementary file 3. (doi: 10.3897/zookeys.364.6109.app3) File format: Microsoft Excel (xls).

Explanation note: Occurrence records and locality information of *Liolaemus tacnae, L. walkeri* and the three new species described in this study, and used to develop ecological niche models.

Citation: Aguilar C, Wood PL Jr, Cusi JC, Guzmán A, Huari F, Lundberg M, Mortensen E, Ramírez C, Robles D, Suárez J, Ticona A, Vargas VJ, Venegas PJ, Sites JW Jr (2013) Integrative taxonomy and preliminary assessment of species limits in the *Liolaemus walkeri* complex (Squamata, Liolaemidae) with descriptions of three new species from Peru. ZooKeys 364: 47–91. doi: 10.3897/zookeys.364.6109 Supplementary file 3. doi: 10.3897/zookeys.364.6109.app3

Appendix 4

Supplementary file 4. (doi: 10.3897/zookeys.364.6109.app4) File format: Microsoft Excel (xls).

Explanation note: Eigenvalues, percentage of variance and similarity accounted by principal components and correspondence axes 1 and 2 respectively.

Citation: Aguilar C, Wood PL Jr, Cusi JC, Guzmán A, Huari F, Lundberg M, Mortensen E, Ramírez C, Robles D, Suárez J, Ticona A, Vargas VJ, Venegas PJ, Sites JW Jr (2013) Integrative taxonomy and preliminary assessment of species limits in the *Liolaemus walkeri* complex (Squamata, Liolaemidae) with descriptions of three new species from Peru. ZooKeys 364: 47–91. doi: 10.3897/zookeys.364.6109 Supplementary file 4. doi: 10.3897/zookeys.364.6109.app4

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DATA PAPER

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A Tenebrionid beetle's dataset (Coleoptera, Tenebrionidae) from Peninsula Valdés (Chubut, Argentina)

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Resource citation: Centro Nacional Patagónico (CENPAT) (2013), A Tenebrionid beetle's dataset (Coleoptera, Tenebrionidae) from Peninsula Valdés (Chubut, Argentina), 118 records, Contributed by Cheli GH, Flores GE, Martínez Román N, Podestá D, Mazzanti R and Miyashido L. Available online at http://data.gbif.org/datasets/resource/14669/ and http://datos.sndb.mincyt.gob.ar/portal/datasets/resource/162, Version 9 (last updated on 2013-09-17), Resource ID: GBIF Key: http://www.gbif.org/dataset/0549aec6-5e7b-46a8-af80-76a3712c0ef6, Data Paper ID: doi: 10.3897/zookeys.364.4761

Abstract

The Natural Protected Area Peninsula Valdés, located in Northeastern Patagonia, is one of the largest conservation units of arid lands in Argentina. Although this area has been in the UNESCO World Heritage List since 1999, it has been continually exposed to sheep grazing and cattle farming for more than a century which have had a negative impact on the local environment. Our aim is to describe the first dataset of tenebrionid beetle species living in Peninsula Valdés and their relationship to sheep grazing. The dataset contains 118 records on 11 species and 198 adult individuals collected. Beetles were collected using pitfall traps in the two major environmental units of Peninsula Valdés, taking into account grazing intensities over a three year time frame from 2005–2007. The Data quality was enhanced following the best practices suggested in the literature during the digitalization and geo-referencing processes. Moreover, identification of specimens and current accurate spelling of scientific names were reviewed. Finally, post-validation

processes using DarwinTest software were applied. Specimens have been deposited at Entomological Collection of the Centro Nacional Patagónico (CENPAT-CONICET). The dataset is part of the database of this collection and has been published on the internet through GBIF Integrated Publishing Toolkit (IPT) (http://data.gbif.org/datasets/resource/14669/). Furthermore, it is the first dataset for tenebrionid beetles of arid Patagonia available in GBIF database, and it is the first one based on a previously designed and standardized sampling to assess the interaction between these beetles and grazing in the area. The main purposes of this dataset are to ensure accessibility to data associated with Tenebrionidae specimens from Peninsula Valdés (Chubut, Argentina), also to contribute to GBIF with primary data about Patagonian tenebrionids and finally, to promote the Entomological Collection of Centro Nacional Patagónico (CENPAT-CONICET) and its associated biodiversity data. For these reasons, we believe that this information will certainly be useful for future faunistic, ecological, conservational and biogeographical studies.

Keywords

Patagonia, Peninsula Valdés, Tenebrionidae, Pimeliinae, Tenebrioninae, Lagriinae, Edrotini, Nycteliini, Epitragini, Stenosini, Scotobiini, Opatrini, Belopini, *Blapstinus punctulatus*, *Ecnomoderes bruchi*, *Emmallodera hirtipes*, *Epipedonota cristallisata*, *Hylithus tentyroides*, *Leptynoderes strangulata*, *Leptynoderes tuberculata*, *Mitragenius araneiformis*, *Nyctelia nodosa*, *Rhypasma quadricollis*, Epitragus spp.

General description

Purpose: The general purpose of this dataset is to ensure accessibility to data associated with Tenebrionidae specimens from Peninsula Valdés (Chubut, Argentina) deposited in the Entomological Collection of Centro Nacional Patagónico (CENPAT-CONICET), Argentina. At present, datasets about Tenebrionidae beetles in GBIF portal contains only two records of Tenebrionids for whole Patagonia (accessed 04/13/2013), one of these is a fossil record, interpreted as Tenebrionidae indet (Locality: Rio Pichileufu, Rio Negro; Data Publisher: Marine Science Institute, UCSB; Dataset: Paleobiology Database; http://data.gbif.org/occurrences/40876235/). Taking into account this scenario, the dataset presented here makes a significant contribution of primary data about Patagonian tenebrionids. In addition, this information could be useful for future faunistic, ecological and conservation studies. Finally, through this dataset we intend to promote the Entomological Collection of Centro Nacional Patagónico (CENPAT-CONICET) and their associated biodiversity data.

Project details

Project title: A Tenebrionid beetle's dataset (Coleoptera, Tenebrionidae) from Peninsula Valdés (Chubut, Argentina)

Personnel: Germán H. Cheli (Resource creator, Collector, Tenebrionid identification, Curator, Metadata provider, Content provider); Gustavo E. Flores (Content provider, Tenebrionid identification); Nicolás Martínez Román (Collector, Processor, Data digitizer, Colection assistant); Darío Podestá (Processor, Collection assistant,

Data digitizer); Renato Mazzanti (Programmer, Data base manager); Lidia Miyashiro (Programmer, Data base assistant).

Funding: This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina (CONICET), including a PhD fellowship and the project: "*Estudios sistemáticos y biogeográficos de coleópteros epígeos de la estepa patagónica, con énfasis en la influencia de factores ambientales, linajes filogenéticos y patrones de especiación para ser aplicados en la conservación de su diversidad*" (grant ref. PIP 112-201101-00987). Digitalization of this biological collection is supported by *Sistema Nacional de Datos Biológicos* (SNDB, MINCyT, Argentina) by the project "*Informatización, Conservación y Fortalecimiento de las Colecciones del Centro Nacional Patagónico-CONICET*" (grant ref. SNDB-F9).

Study area descriptions/descriptor: Peninsula Valdés is a wide plateau, extending 4,000 km^2 in the NE of Chubut Province (42°05'–42°53'S; 63°35'–65°04'W). It is considered part of different biogeographic provinces by different authors, thus some include it in Patagonia (Soriano 1956, Morrone 2001, Morrone et al. 2002) while other authors consider it is in the Monte Phytogeographic Province (Cabrera and Willink 1973, Roig-Juñent and Flores 2001, Roig et al. 2009). The mean annual temperature in this area is 13.4°C, showing wide range during summer (Labraga et al. 2008). Predominant winds are from the western quadrant (Barros and Rodríguez Seró 1981) and annual rainfall ranges from 175 to 225 mm (Súnico et al. 1994).

Despite Peninsula Valdés is one of the largest arid areas included in Argentinian conservation programs, at present there is a fragmented knowledge of terrestrial arthropods (Cheli et al. 2010). Coleopterans are the most abundant and diverse non-social insects of Peninsula Valdés, and Tenebrionidae is the most numerous family among them (Cheli et al. 2010). These beetles play an important role as decomposers in arid lands (Flores 1998) and some species are omnivorous (Cheli et al. 2009). Moreover, tenebrionid beetles are sensitive indicators of biodiversity and habitat change (Cheli 2009).

Design description: Samples were processed in the laboratory and adult tenebrionid specimens were obtained (Figure 1). Preservation status of individuals was examined and those showing original good curatorial condition were housed in the collection. Species determination was done following reviews and keys (Kulzer 1955, 1963, Flores 1997, 1999) and comparing the collected material with specimens housed at CENPAT-CONICET and IADIZA-CONICET entomological collections. The classification of Tenebrionidae to tribes and subfamilies was based on the one proposed by Bouchard et al. (2005). Taxonomical determination of problematic specimens was verified by PhD Gustavo Flores (IADIZA-CONICET), a taxonomist specialized in South American tenebrionid beetles. Thereafter, data associated with specimens were digitized using ZOORBAR software (http://www.gbif.es/zoorbar/zoorbar.php). Geo-referencing details and current accurate spelling of scientific names are fully described in the "Quality control description" section. The dataset was exported on DarwinCore v.1.4 (http://www.gbif.es/Recursos2.php), postvalidation was applied using DARWINTEST software (http://www.gbif.es/darwin_test/Darwin_Test_in.php) and the metadata was integrated to the dataset in DarwinCore Archive format. Finally, the dataset was provided to Sistema Nacional de Datos

Biológicos, Ministerio de Ciencia, Tecnología e Innovación Productiva (SNDB, MinCyT, Argentina) and to the Global Biodiversity Information Facility (GBIF), by means of their Integrated Publishing Toolkit (IPT) (Figure 1).

Data published through GBIF: http://www.cenpat-conicet.gov.ar:8080/ipt-2.0.3/resource.do?r=cnp-e

Taxonomic coverage

General taxonomic coverage description: Dataset comprise 3 subfamilies, 7 tribes and 11 species. The most representative subfamilies are Pimeliinae and Tenebrioninae, each depicting half of the records. At tribal taxonomical level, Pimeliinae is the richest one, including Edrotini (21.2%), Nycteliini (12.7%), Epitragini (9.3%) and Stenosini (2.5%). Tenebrioninae comprises only two tribes, Scotobiini (5.9%) and Opatrini (47.5%). While Lagriinae, the third subfamily found, has only one record (*Rhypasma quadricollis* Fairmaire, Belopini tribe (0.8%)). *Blapstinus punctulatus* Solier is the most common species of the dataset, including more than 30% of the records in each year and more than 50% considering the period sampled; follow in importance by *Hylithus tentyroides* Lacordaire (16% in 2005 and 2006) and *Emmallodera hirtipes* Kulzer (16% in 2007) (Figures 2 and 3).

Taxonomic ranks

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Hexapoda
Class: Insecta
Order: Coleoptera
Suborder: Polyphaga
Infraorder: Cucujiformia
Superfamily: Tenebrionoidea
Family: Tenebrionidae
Subfamily: Lagriinae, Pimeliinae, Tenebrioninae
Tribe: Belopini, Edrotini, Epitragini, Nycteliini, Stenosini, Opatrini
Genus: Epitragus, Rhypasma, Hylithus, Epipedonota, Mitragenius, Nyctelia, Ecnomoderes, Blapstinus, Emmallodera, Leptynoderes
Species: *Rhypasma quadricollis*, *Hylithus tentyroides*, *Epipedonota cristallisata*, *Mitragenius araneifoirmis*, *Nyctelia nodosa*, *Ecnomoderes bruchi*, *Blapstinus punctulatus*, *Emmallodera hirtipes*, *Leptynoderes strangulata*, *Leptynoderes tuberculata*
Common names: darkling beetles, insect, beetles

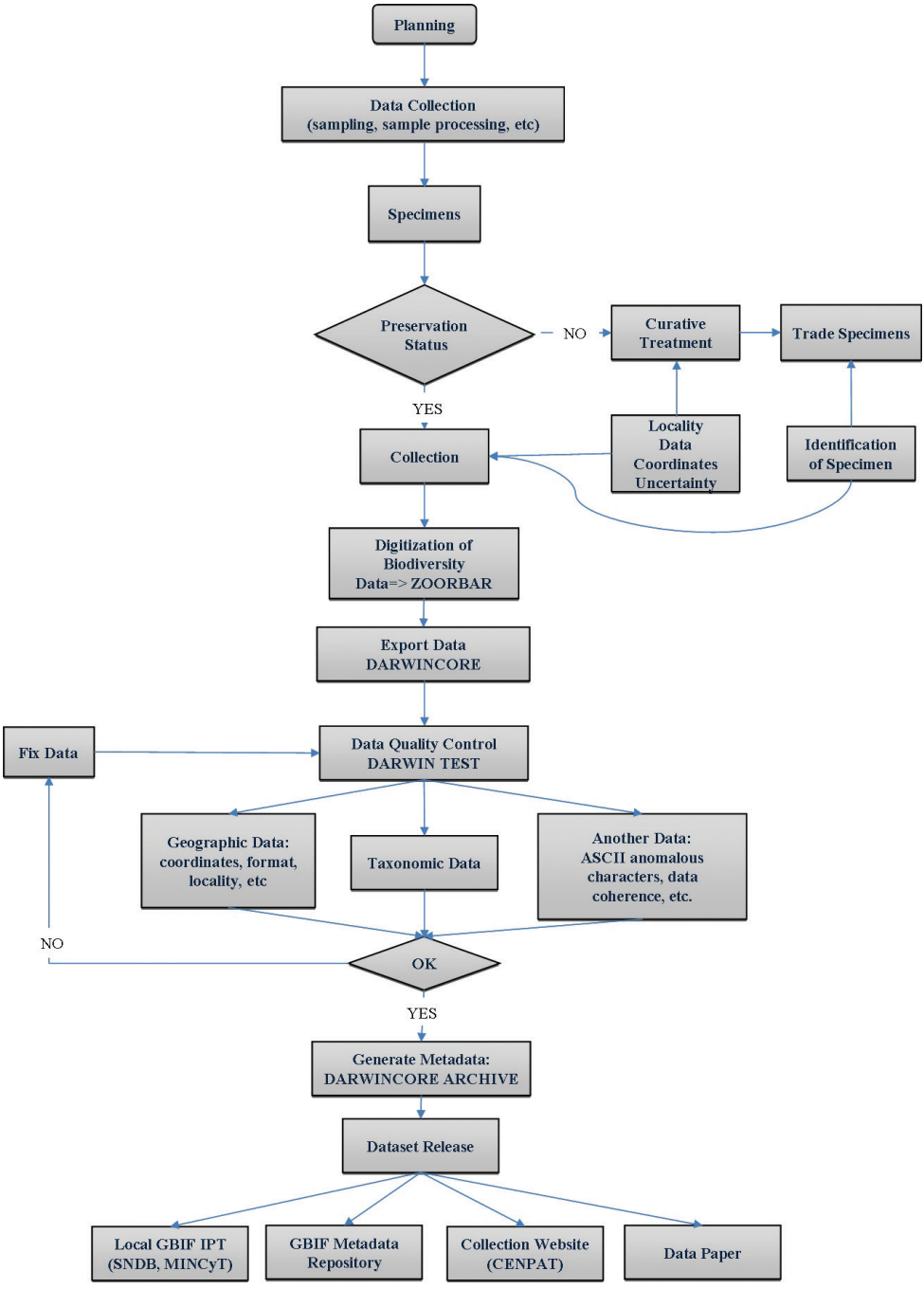

Figure 1. Flow chart describing the methods procedure: collection, digitalization and data publishing.

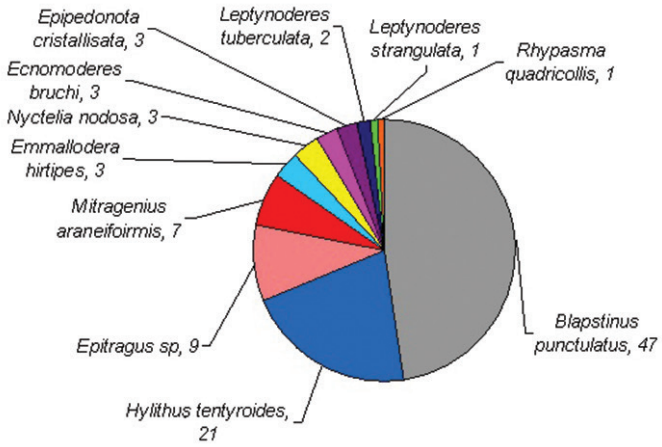

Figure 2. Distribution of tenebrionid species from Peninsula Valdés included in the dataset. The number next to the specific name indicates its percentage.

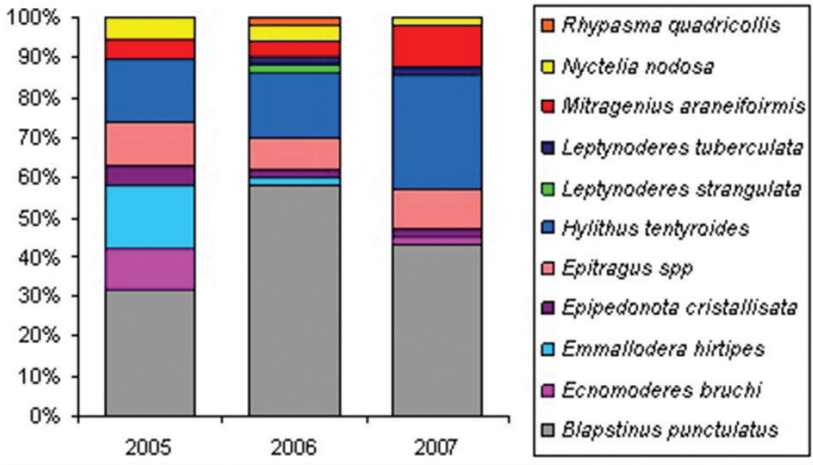

Figure 3. Distribution of tenebrionid species from Peninsula Valdés included in the dataset among the sampling years (2005 to 2007).

Spatial coverage

General spatial coverage: The Natural Protected Area Peninsula Valdés (Figure 4) is located on the Atlantic coast of Chubut province (Argentina) and was declared a World Heritage site by UNESCO in 1999.

Physiographycally the peninsula is characterized by a flat landscape with three endorheic depressions (Salina Grande, Salina Chica, and Gran Salitral) with ephemeral hypersaline lakes. There are no permanent watercourses in the area and due to the narrow isthmus connecting peninsula and continent, allochthonous courses cannot gain access (Beltramone 1983, Alvarez et al. 2010). Geologically, Peninsula Valdés is formed

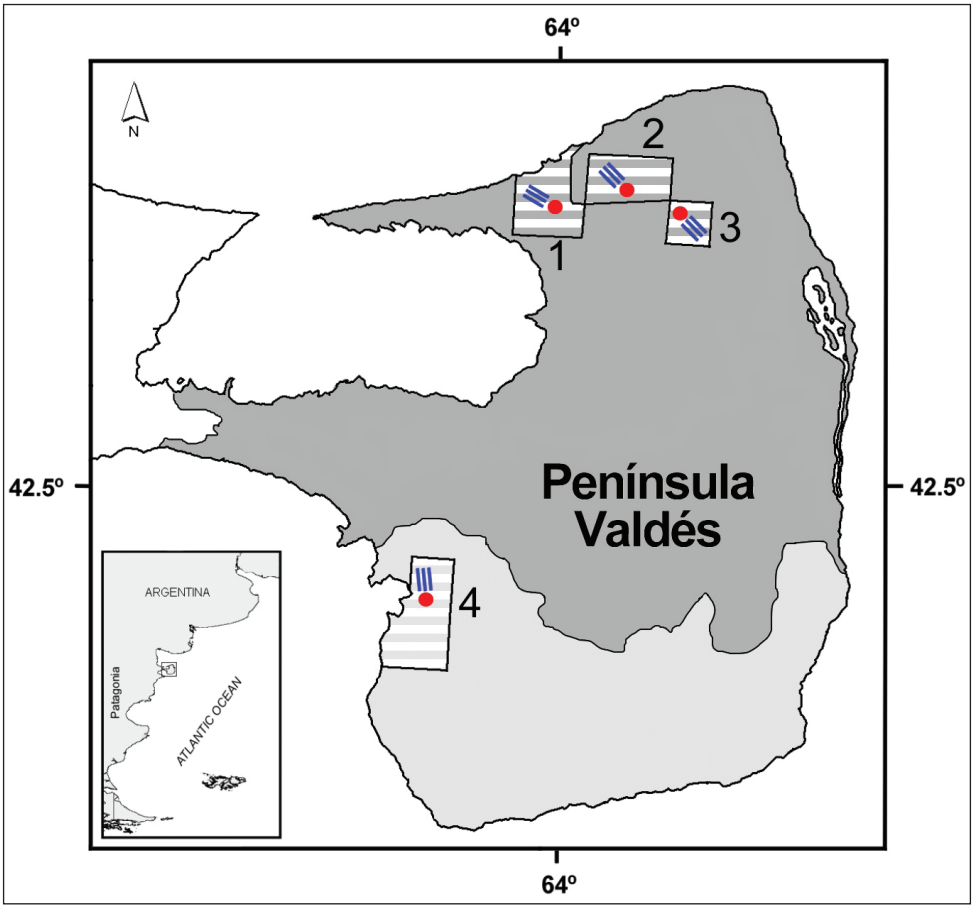

Figure 4. Location of the sampled farms (striped squares: **1** El Progreso **2** El Centro **3** La Falsa **4** San Pablo de Valdés). Colored areas show the main physiognomy units of Peninsula Valdés, shrub steppe (gray) and herbaceous steppe (white). Blue lines and red circles indicate sampling transects and water wells, respectively.

by Oligo-Miocenic marine sediments and exhibits a continuous cover of aeolian sediments intermingled with quaternary gravels (Súnico et al. 1994, Haller et al. 2001). The actual landscape configuration of the region was caused by Pre-Quaternary intense tectonic movements and strong periglacial winds during Pleistocene period (~1myrs). In general, soils correspond to the Aridisol and Entisol orders (Rostagno 1981).

Peninsula Valdés entails great importance from a biological perspective (UNESCO 1999, Yorio et al. 2005, Cheli et al. 2010). Floristically about 130 species of plants are found in the region, while faunistically it supports an important vertebrate biodiversity: 13 species of reptiles, 108 of terrestrial birds (Plan de Manejo del Área Protegida Sistema Península Valdés 1998) and 28 of terrestrial mammals (Nabte et al. 2009). It is interersting to point out that terrestrial arthropods show the greatest diversity, with about 160 species included in 18 orders and 52 families (Cheli et al. 2010).

Nevertheless, the knowledge of terrestrial fauna is still fragmentary for this area (Nabte et al. 2009, Cheli et al. 2010).

Nowadays, human population in Peninsula Valdés is scarce, including Puerto Pirámides as the only urban center, a few settlers dispersed among farms and temporary artisanal fishing camps. Since 1882 the economy of the region has been based on sheep livestock (Barba Ruíz 2003). In general, grazing is practiced extensively in big paddocks (more than 2,500 ha) with a single permanent water point. At present, there are an estimated number of 90 sheep farms and 80,000 sheep in Peninsula Valdés (Baldi et al. 1997). Furthermore, during the last two decades, tourism activity has increased significantly, with 250,000 tourists visiting the area each year (Nabte et al. 2009).

Peninsula Valdés shows serious signs of deterioration caused by human activities. Nearly 90% of its natural grasslands are in a poor state of conservation with soils and vegetation severely degraded by overgrazing. Even though the impact that land use and touristic activities caused on terrestrial vertebrates has not been evaluated (Nabte et al. 2009), it is known that terrestrial arthropods have shown significant changes as a consequence of sheep overgrazing (Cheli 2009). This feature allowed considering them as biological indicators of natural environment disturbance (Cheli et al. 2010).

Finally, even though Peninsula Valdés has been the target of several scientific contributions, their biogeographical identity is still a conflictive issue. Therefore, this data set improves the knowledge of the tenebrionids of the area and it could be useful to clarify the biogeographical identity of the peninsula.

Coordinates

43°5'24"S and 41°55'48"S Latitude; 64°52'12'"W and 63°23'60"W Longitude.

Temporal coverage

February (mid-summer in the Southern hemisphere), years 2005-2006-2007.

Natural collections description

Parent collection identifier: CNP

Collection name: Colección Entomológica del Centro Nacional Patagónico "Francisco Pascasio Moreno"

Collection identifier: CNP-CE

Specimen preservation method: All specimens are preserved in 70% ethyl alcohol. Individuals were stored in eppendorfs (1.5ml) or jars (20ml) full of alcohol (70%). All specimens belonging to the same species, in good curatorial conditions and found in the same sample (same date and site), were considered as a lot. Lots are the curatorial

units of the collection. Each one contains among 1 to 10 specimens and have a unique collections' number assigned (catalog number). In those cases where the lot had more than one eppendorf or jar, all of them were kept into a Ziploc© plastic bag and then located into a hermetic bigger jar filled with alcohol (70%). Each specimen was accompanied by its original label and a new one stating their unique catalog number, both labels were placed within the eppendorf or jar. If the genitalia of some specimen was studied, it was conserved into a different eppendorf inside the Ziploc© bag that contains the exemplar. All jars are kept in a room without windows at a relatively constant temperature (18°C).

Fluctuations in the temperature and relative humidity levels can be the biggest cause of environmental damage to biological collections (Alten 1999). In this sense, the use of alcohol for conserving entomological material helps to control the harmful effects of the factors mentioned above. Moreover, the best preservative for alcoholic collection of small invertebrates is 70% ethyl alcohol (Levi 1966). In addition, for insect DNA preservation the highest yields and least sheared DNA were obtained from specimens preserved in ethanol. Whereas DNA from individuals conserved in other type of alcohol was degraded to small fragments and dried pinned specimens gave undetectable yields of DNA (Post et al. 1993). Finally, when specimens are preserved in alcohol, they conserve their joints soft, thus greatly reducing the likelihood of damage during handling.

Curatorial unit: 118 (with an uncertainty of 0).

Methods

Method step description: Figure 1 summarizes the methodological procedure. *Planning and data collection:* The dataset was obtained from PhD thesis of G.H. Cheli (2009) whose main objectives were to improve the knowledge of the epigeal arthropods living in Peninsula Valdés and to study the effect of grazing on this group of animals in the region. This was the first study carried out in the area that used pitfall traps, for this reason the art of capture should be optimized (see Cheli and Corley 2010). Due to strong water limitations in Peninsula Valdés, grazing intensity varies in relation to the water well proximity (Lange 1969). This gradient of disturbance offers an experimental opportunity to study the effects of grazing over artropodofauna avoiding the methodological problems associated with other experimental designs (see Andrew 1988, James et al. 1999). Therefore, the grazing impact on terrestrial arthropods of Peninsula Valdés was assessed through transects related to water wells (Figures 5 and 6) (see "Sampling description"). *Data curation:* Damaged specimens were excluded from the dataset. When necessary, curative treatment was provided and these individuals were reserved like trade specimens. *Identification:* The taxonomic identification was carried out in the laboratory using suitable literature (see details in the "Design description" section). *Data management*: Biodiversity data existing on the specimens' labels (i.e. collection code, catalog number, species identification, name of determiner, locality, collection date, habitat, altitude, GPS coordinates, collector, ecological observations

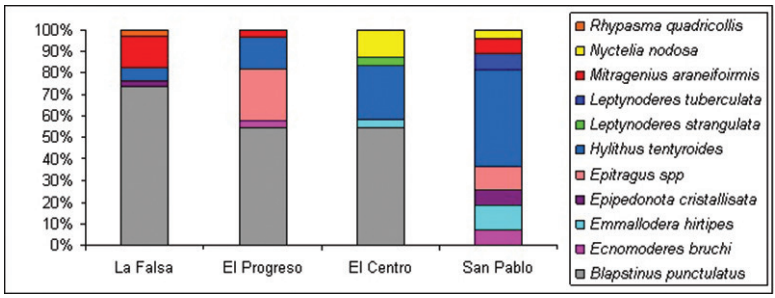

Figure 5. Distribution of Tenebrionid species among the sampled farms. Note that El Progreso, El Centro and La Falsa, belong to the shrub steppe physiognomy unit while San Pablo de Valdés, to the herbaceous steppe.

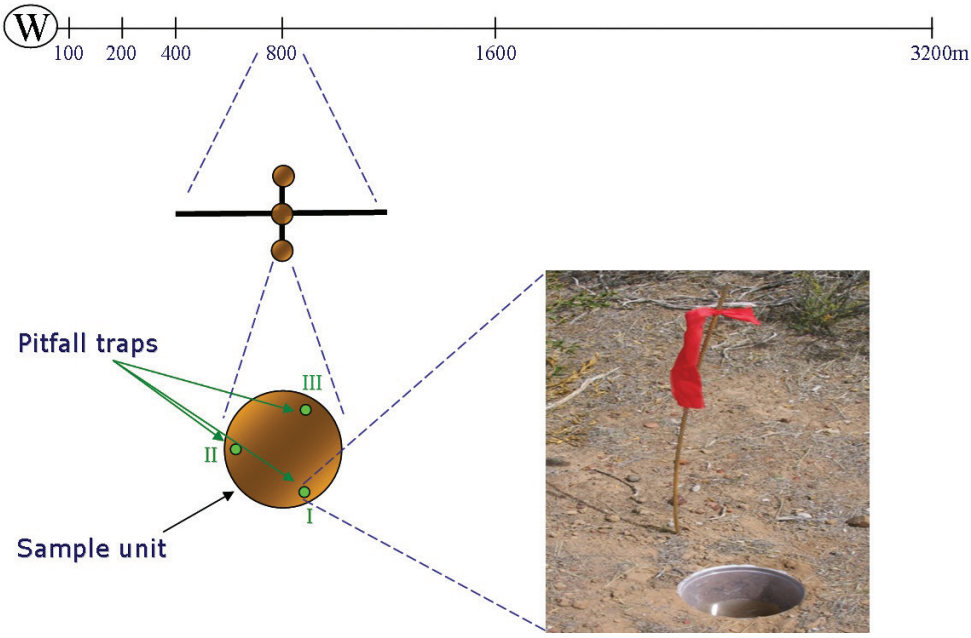

Figure 6. Design of sampling method. Each transect (3 per farm) consist of 6 sampling sites along a gradient of grazing disturbance (100, 200, 400, 800, 1600, and 3200m from water well). Each sample unit consists of 3 pitfall traps.

and notes) were included in a digital database using ZOORBAR software (http://www.gbif.es/zoorbar/zoorbar.php). Data were exported in Darwin Core (v1.4) format. *Data quality enhancement:* see details in the section on quality control. *Data publishing:* Once postvalidation was applied, dataset was transformed into DarwinCore Archive format associating their metadata. Finally, the dataset was published into the Global Biodiversity Information Facility (GBIF) portal, by means of their Integrated Publishing Toolkit (IPT) and provided to Sistema Nacional de Datos Biológicos, Ministerio de Ciencia, Tecnología e Innovación Productiva (SNDB, MinCyT, Argentina).

Study extent description: The variety of soils and plant communities living in the region determines the presence of several types of habitats in Peninsula Valdés. In the north portion, the dominant physiognomy is a shrub steppe of *Chuquiraga avellanedae*, *C. histrix*, *Condalia microphylla*, *Lycium chilense*, *Schinus polygamous* and *Prosopidastrum globosum*, accompanied by the grasses *Nassella tenuis*, *Piptochaetium napostaense* and *Poa ligularis* (Bertiller et al. 1981) (Figure 4). In the south, the shrub steppe is replaced by a herbaceous steppe where *Sporobolus rigens* becomes the most important species along with patches of *C. avellanedae* and *Hyalis argentea* (Bertiller et al. 1981) (Figure 4). The dataset presented here comprise tenebrionid beetles sampled in both physiognomy units, with three sampling sites (farms) in the shrub steppe and one in the herbaceous steppe (Figure 4). Sampling was made during the middle austral summer (February) of 2005, 2006 and 2007. Dataset include specimens from sampling sites along a gradient of grazing disturbance. This dataset also shows that several entomofaunal differences between these two main ecological areas of Peninsula Valdés are evident when the North and South collecting sites are taken into account separately. The main variation is observed in dominant tenebrionid species: *Blapstinus punctulatus* is the most common species in the northern part of Peninsula Valdés, while *Hylithus tentyroides* dominates in the southern one (Figure 5).

Sampling description: The specimens composing this dataset were collected using pitfall traps. This trapping technique was selected for several reasons: 1- it is the most frequently used method for sampling ground-dwelling arthropods (Niemelä et al. 1992, Pekár 2002, Phillips and Cobb 2005); 2- pitfall traps serve to evaluate the distribution of macroinvertebrates in diverse ecosystems at different scales, also to describe activity patterns and habitat associations, as well as establishing the effects of disturbances on biodiversity (Niemelä et al. 1992, Pekár 2002, Mazía et al. 2006); 3- in some cases, pitfall traps are the only alternative for sampling arthropods (Niemelä et al. 1993, Pearsal 2007); 4- their objectivity is a crucial feature that allows better comparisons (Vennila and Rajagopal 1999); 5- pitfall traps are a quick and cheap method to capture arthropods.

Four sheep farms, with a single well per fenced plot, were selected for conducting the study (three in the northern shrub steppe and one in the southern herbaceous steppe) (Figure 4). The sampling design consisted on 3 transects per farm covering different grazing intensities in relation to the position of the water well (Figures 4 and 6). At each transect, six sampling sites varying in distance to the water well were established (100, 200, 400, 800, 1600 and 3200m) (Figure 6). Three pitfall traps were placed at each sampling site and then treated as a sample unit. A total of 12 transects with 216 traps per year were established (making 648 traps in three years).

In order to enhance catches, each trap was placed in vegetation patches and neatly buried in the soil near bushes. Traps consisted of plastic jars of 12cm in diameter at the opening and 12cm deep. The quantity of traps used guaranteed capturing almost all taxa dwelling in the area (Cheli and Corley 2010). Besides, the type of traps employed has proved to be the most efficient pitfall configuration for this region. Each trap was filled with 300ml of a 30% solution of ethylene glycol used as preservative and opened on-site for two weeks (Cheli and Corley 2010).

Quality control description: Following Wieczorek (2001) and Chapman and Wieczorek (2006), validation of geographic, taxonomic and additional data was incorporated in the digitalization process at several steps (Figure 1), as well as the geo-referencing of all specimens. Therefore, the geographic coordinates were recorded in decimal degrees using a Garmin eTrex Legend GPS (WGS84 Datum) with an accuracy of less than 10 m and with at least 5 satellites. The calculated uncertainty was 2.83 meters (Wieczorek 2001). In addition, the geo-coordinates of each specimen were verified using digital cartography (satellite images; Quantum GIS v1.7; Google Earth). The taxonomical identification of specimens, scientific names and their current accurate spelling were reviewed using suitable literature (Kulzer 1955, 1963, Flores 1997, 1999) and verified by a tenebrionid's specialist (G. E. Flores). Other postvalidation procedures (including geographic coordinate format, coordinates within country/provincial boundaries, congruence between collection and identification dates absence of ASCII anomalous characters) were checked by use of the Darwin test software (http://www.gbif.es/darwin_test/Darwin_Test_in.php).

Dataset

Dataset description

Object name: Darwin Core Archive A Tenebrionid beetle's dataset (Coleoptera, Tenebrionidae) from Peninsula Valdés (Chubut, Argentina)
Character encoding: UTF-8
Format name: Darwin Core Archive format
Format version: 1.0
Distribution: http://www.cenpat-conicet.gov.ar:8080/ipt-2.0.3/archive.do?r=cnp-e
Publication date of data: 2013-01-09
Language: English
Licenses of use: This work is licensed under a Creative Commons CCZero 1.0 License http://creativecommons.org/publicdomain/zero/1.0/legalcode

External datasets
Dataset description

Object name: Centro Nacional Patagónico (CENPAT-CONICET)
Distribution: http://www.cenpat-conicet.gov.ar:8080/ipt-2.0.3/archive.do?r=cnp-e

Dataset description

Object name: Ministerio de Ciencia y Tecnología de Argentina (Sistema Nacional de Datos Biológicos - SNDB)

Distribution: http://datos.sndb.mincyt.gob.ar/portal/datasets/resource/162
Metadata language: English
Date of metadata creation: 2013-01-09
Hierarchy level: Dataset

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