

Convergent evolution of sexually dimorphic glands in an amphi-Pacific harvestman family

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Abstract. Sexually dimorphic traits are widespread in animals, and include sex-specific weapons, ornamentation and, although less noticed, glands and associated structures. In arachnids, certain lineages of the order Opiliones exhibit diverse forms of dimorphism in the armature and length of appendages (common in Laniatores), as well as in the presence of sexually dimorphic glands (mostly investigated in Cyphophthalmi), positing harvestmen as promising models to study sexual dimorphism. Whereas the evolution and ecological significance of armature have been the focus of recent attention, sexually dimorphic glands remain understudied in groups other than Cyphophthalmi, despite being widespread in Opiliones. We therefore selected the amphi-Pacific family Zalmoxidae as an ideal taxon to investigate the evolutionary dynamics of this trait. We first describe four new species of Palaeotropical *Zalmoxis*, including a species with sexually dimorphic glands, and describe the morphology of zalmoxid species with sexually dimorphic glands using scanning electron microscopy. Using a previously assembled six-locus dataset supplemented with new terminals, and applying stochastic character mapping, we infer that sexually dimorphic glands evolved once in the Neotropics and at least four times in the Palaeotropical zalmoxids, revealing the evolutionary lability of this trait.

Additional keywords: Arachnida, Laniatores, Opiliones, sexual dimorphism, stochastic character mapping, Zalmoxidae.

Received 8 March 2020, accepted 16 July 2020, published online 16 November 2020

Introduction

Sexually dimorphic traits encompass an array of charismatic morphological phenomena that have convergently evolved in several animal taxa (Andersson 1994). Among the iconic examples are traits that serve as weapons in male–male competitions, such as beetle horns and deer antlers (Andersson 1994; Emlen *et al.* 2005), as well as ornamentation, as exemplified by peacock tails and lion manes. Other common sexually dimorphic traits are glands and their associated cuticular modifications, which may produce pheromones or secrete substances offered as nuptial gifts (Vahed 1998; Wyatt 2012; Kunz *et al.* 2012, 2013).

In arachnids, certain lineages within the order Opiliones (‘harvestmen’ or ‘daddy-long-legs’) typify pronounced sexual dimorphism, which often affects the armature and elongation of appendages, and the presence of glands (Martens 1973; Willemart *et al.* 2010; Buzatto and Machado 2014; Painting

et al. 2015). The most speciose suborder, Laniatores, also known as armoured harvestmen, encompasses great part of this diversity; at least one-third of the ~30 families display sexual dimorphism in robustness, size or armature of chelicerae, pedipalps or legs (Buzatto and Machado 2014), and more than half of the families of Laniatores exhibit sexually dimorphic glands, particularly in the legs (reviewed in Willemart *et al.* 2007, 2010; Proud and Felgenhauer 2011, 2013; Pérez-González *et al.* 2016; Alegre *et al.* 2019). The dimorphic armature has been the focus of recent attention, specifically in the Neotropical laniatorean family Gonyleptidae, in terms of morphology, ecology and evolutionary history. Apophyses on leg IV, elongated femur IV, or elongated second pairs of legs of some gonyleptid species are used in ritualised male–male combats to defend territories and obtain access to females (Willemart *et al.* 2009; Buzatto *et al.* 2011; Zatz *et al.* 2011). Both sexual dimorphism

and male polyphenism (with occurrence of ‘minors’ and ‘majors’) appear to have evolved several times in the group and are associated with alternative reproductive tactics (Buzatto *et al.* 2014; Buzatto and Machado 2014).

By contrast to armature, much less is known about sexually dimorphic leg glands with respect to ecological significance and evolutionary dynamics, although such glands are widespread in Opiliones. These glands can usually be identified by correlation with swollen appendage segments in males, and they differ from the incrassate sexually dimorphic armature by the absence of spines and the presence of associated pores and sometimes setae (Willemart *et al.* 2010). Descriptions of species with gland-like swollen leg parts abound in the taxonomic literature of Laniatores (Willemart *et al.* 2010; Alegre *et al.* 2019) and similar glands are present in the tarsus of leg IV in males of all Cyphophthalmi. Ultrastructural and histological investigations also confirm the presence of glandular tissue that secrete chemicals through tegumental pore openings (Willemart *et al.* 2010; Proud and Felgenhauer 2011, 2013; Alegre *et al.* 2019). Accordingly, the only published behavioural evidence of their function are studies on two gonyleptid species, in which the males have been observed touching the substrate with these glands, probably to spread chemicals (Fernandes and Willemart 2014; Murayama and Willemart 2015). Male sexually dimorphic leg glands usually occur on the distalmost podomeres (metatarsus and tarsus), but they have been reported in all leg segments of all leg pairs (Willemart *et al.* 2010). Although their distribution across harvestman diversity is suggestive of independent acquisitions of these structures, it remains untested whether sexually dimorphic glands are homologous at smaller phylogenetic scales (i.e. intrafamilial or intrageneric levels). One ideal taxon for examining the evolutionary dynamics of gland evolution is the leaf-litter-dwelling circum-Pacific family Zalmoxidae. Males of many species exhibit a sexually dimorphic leg IV that is typically incrassate and armed with spines, but some species with putative sexually dimorphic glands are also known (Willemart *et al.* 2010; Sharma *et al.* 2011, 2012; Sharma 2012). A six-locus molecular phylogeny of the family reconstructed the Palaeotropical genus *Zalmoxis* as a monophyletic group nested within a Neotropical radiation that dispersed across the Pacific in the Cretaceous (Sharma and Giribet 2012). Intriguingly, species with remarkably similar sexually dimorphic gland morphology occur on both sides of the Pacific, but it is unclear if these glands are homologous. Furthermore, although more recently the taxonomy of the group has been revisited (Sharma *et al.* 2011, 2012; Sharma 2012), numerous species remain to be described.

Here, we expand upon the known dimensions of sexual dimorphism in Zalmoxidae through the description of four new species, one of which presents sexually dimorphic glands. Included among the four is the first species endemic to Vanuatu, expanding the known range of Zalmoxidae. We add this species to the molecular phylogeny of Zalmoxidae using standard workhorse markers and reconstruct the evolution of sexually dimorphic glands, towards assessing the homology of the glands across the family.

Materials and methods

Taxonomy

Specimens of the four species here described have been deposited in MCZ (Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA). Brightfield images of holotypes and paratypes were taken with a Leica M60 stereomicroscope with a Leica MC120 digital camera, driven by LAS X software (Leica Microsystems, Wetzlar, Germany). Focus stacking was performed using a series of images at different focal planes using the software Helicon Focus (ver. 6.7.2, Helicon Soft, Kharkiv, Ukraine). Terminology used in species descriptions followed guidelines in Acosta *et al.* (2007).

Scanning electron microscopy

Appendages were dissected out from specimens preserved in 95% ethanol using fine forceps and a razor blade. Whole appendages for taxonomy were cleansed using a Branson 200 sonicator (Branson Ultrasonics Corporation, CT, USA) for 1 min in 95% ethanol and air-dried. For examination of the tarsus and metatarsus, appendages were sonicated in distilled water (1 min), followed by a round in a 10% detergent solution (Alconox) (1 min), and a final wash in distilled water (1 min). These samples were then immersed in 100% acetone and air-dried. Samples for taxonomy and for tarsal morphology were mounted on stubs with carbon adhesive tabs (Electron Microscopy Science, Hatfield, PA, US), coated with Pt–Pd targets (EMS300T D Dual Head Sputter Coater, Electron Microscopy Science) and imaged on a Zeiss Ultra-Plus FESEM or a Zeiss Supra FESEM (field emission scanning electron microscope) (Zeiss, Jena, Germany) at the Center for Nanoscale Systems (Harvard University). Scanning electron micrographs were adjusted for contrast and brightness in Photoshop C6 (Adobe, San Jose, CA, USA), and their backgrounds were edited in black for enhanced contrast and clarity. Figures were assembled using Adobe Illustrator C6.

Morphological and phylogenetic analysis

We sampled tarsi I–IV of 101 specimens previously sequenced in a biogeographic study of the family Zalmoxidae (Sharma and Giribet 2012). Two terminals, both corresponding to the first known *Zalmoxis* from Vanuatu, were added to this phylogeny; one of these species, for which sufficient material was available for taxonomic efforts, is formally described below.

Surveyed terminals comprise the species *Z. bendis* Sharma, Buenavente, Clouse, Diesmos & Giribet, 2012, *Z. cardwellensis* Forster, 1955, *Z. cuspanalis* Roewer, 1927, *Z. darwinensis* Goodnight & Goodnight, 1948, *Z. falcifer* Sharma, 2012, *Z. furcifer* Sharma, 2012, *Z. gebeleizis* Sharma, Buenavente, Clouse, Diesmos & Giribet, 2012, *Z. kaktinsae* Sharma, 2012, *Zalmoxis kotys* Sharma, Buenavente, Clouse, Diesmos & Giribet, 2012, *Z. mendax* Sharma, 2012, *Z. mitobatipes* Roewer, 1926, *Z. cf. neocaledonicus*, *Z. perditus* Sharma, 2012, *Z. princeps* Sharma, 2012, *Z. pygmaeus* Sorensen, 1886, *Z. sabazios* Sharma, Buenavente, Clouse, Diesmos & Giribet, 2012, *Z. zilbelthiurdos* Sharma, Buenavente, Clouse, Diesmos & Giribet, 2012, *Ethobunus cf. foliatus*, *E. tarsalis*

Banks, 1909, *E. zalmoxiformis* Roewer, 1949, *Pachylicus spinatus* Goodnight & Goodnight, 1983, as well as undescribed species of *Zalmoxis*, *Ethobunus* and *Pachylicus*. Four of the *Zalmoxis* sp. are formally described: *Zalmoxis curupira*, sp. nov., *Zalmoxis therianthropes*, sp. nov., *Zalmoxis adze*, sp. nov., and *Zalmoxis bilbo*, sp. nov. Of the terminals examined, 65 had a male available, and only these were used to score the character state of the sexually dimorphic basitarsal gland, in addition to analysis of the original species descriptions when possible. Coding for *Z. falcifer* and *Z. fuscifer* was based only on the original species descriptions.

Sequencing was performed using the approaches previously detailed by Sharma and Giribet (2012). Nucleotide sequences for six markers were individually aligned *de novo* with MUSCLE (ver. 3.8.31, see <http://www.drive5.com/muscle>; Edgar 2004) and concatenated into a final matrix. The resulting matrix was analysed under maximum likelihood using the software RAXML (ver. 8, see <https://github.com/stamatak/standard-RAXML>; Stamatakis 2014) with the same substitution models and heuristics implemented in a previous work (Sharma and Giribet 2012).

Ancestral state reconstruction and analysis of phylogenetic signal

Ancestral state reconstruction was conducted using a stochastic character mapping method (Huelsenbeck *et al.* 2003), as implemented in the R package *phytools* (ver. 0.7.5, see <https://github.com/liamrevell/phytools>; Revell 2012). The pruned phylogeny, including all terminals for which character states could be scored (males present), was used as the basis for comparative analyses. To measure phylogenetic signal of the character ‘tarsus condition’, we calculated its retention index (ri) with the R package *phangorn* (ver. 2.5.5, see <https://github.com/KlausVigo/phangorn>; Schliep *et al.* 2017) and computed the Fritz and Purvis *D* statistic (Fritz and Purvis 2010) with the *phylo.d* function of the R package *caper* (ver. 1.0.1, D. Orme, R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac and W. Pearse, see <https://CRAN.R-project.org/package=caper>). A low retention index indicates high level of homoplasy. *D* compares the observed character distribution with the expected distribution generated by random character state shuffling (*D* = 1) or by character state transitions following Brownian motion (*D* = 0). Conversely, small *D* values (*D* < 0) indicate high phylogenetic signal.

Taxonomy

Order **OPILIONES** Sundevall, 1833
 Suborder **LANIATORES** Thorell, 1876
 Infraorder **GRASSATORES** Kury, 2002
 Family **ZALMOXIDAE** Sørensen, 1886

Type genus. *Zalmoxis* Sørensen, 1886 (type species *Zalmoxis robustus* Sørensen, 1886, by subsequent designation; Roewer 1949).

Zalmoxis curupira, sp. nov.

(Fig. 1–3)

Types. Male holotype (MCZ IZ-131112 [DNA102487]) from Bukit Linggua, near Doloduo, north Sulawesi, Indonesia (GG Locality 498: 0.57553°N, 123.89506°E), 297-m elev., leg. R.M. Clouse, G. Giribet, C. Rahmadi, 17 June 2006; on SEM stubs (MCZ IZ-131112). *Paratypes:* one female (MCZ IZ-131112), same collecting data as holotype; extracted for DNA and in ethanol and on SEM stubs (MCZ IZ-131112).

Diagnosis

Distinguished from congeners by the broad, incrassate, and sexually dimorphic femur IV of males, with two long spines ectally, and incrassate patella and tibia. Additionally, distinguishable by three rows of tubercles along the dorsal midline of the free tergites, whereas other species of the genus have either a belt of tubercles or inconspicuous tubercles.

Description

Total length of male holotype (female paratype in parentheses) 4.28 mm (3.15 mm), greatest width of prosoma 1.38 mm (1.10 mm), greatest width of opisthosoma 2.79 mm (2.10 mm); length-to-width ratio 1.53 (1.50). Body dark brown to reddish brown in ethanol, depending upon incidence of light. Eyes present on sides of ocularium, with a triad of minute, blunt tubercles arranged in a triangle. Anterior margin of carapace with one median peg and three small pairs of pegs above coxae of leg I. First scutal groove of mesotergum V-shaped, subsequent grooves more U-shaped; grooves lighter in colour than remaining area of tergites. Last tergite of fused scutal area with a pair of small tubercles flanking the midline. Three rows of tubercles along the dorsal midline of the free tergites, beginning with the posterior margin of the fused scutal area; midline bears the most prominently sized tubercular row. Coxa IV with prominent tubercles, visible dorsally (Fig. 1).

Ventral prosomal complex of male with coxae II and III meeting in midline, coxae I and IV not so. Anterior and posterior margins of coxae III with tubercular bridges to adjacent coxae, and coxae I–III with setose tubercles. Coxa II with two small ventrally oriented small tubercles adjacent to the endites. Coxa III with a single posterodistal tubercle. Coxae IV of male incrassate, in apposition to spiracles of second opisthosomal segment. Genital operculum subtriangular, typical of Zalmoxidae. Opisthosomal sternites with regular rows of denticle belts. Anal plate with three rows of spines: four medium spines in anterior row, two spines in median row (possibly broken), and two prominent spines in posterior row (Fig. 1).

Chelicera (Fig. 2A, 3A) sexually monomorphic, with prominent bulla. Ventroectal surface of proximal segment granulated. Palp (Fig. 2B, 3B) robust, with megaspines, typical of zalmoxids. Legs I–IV (Fig. 2, 3) of both sexes with tubercles on femora, patellae, and tibiae. Leg IV of both sexes with prominent tubercles on ventroectal surface of femora, greatly exaggerated in male. Male femur IV with five prominent tubercles on ventral surface, with an additional pair

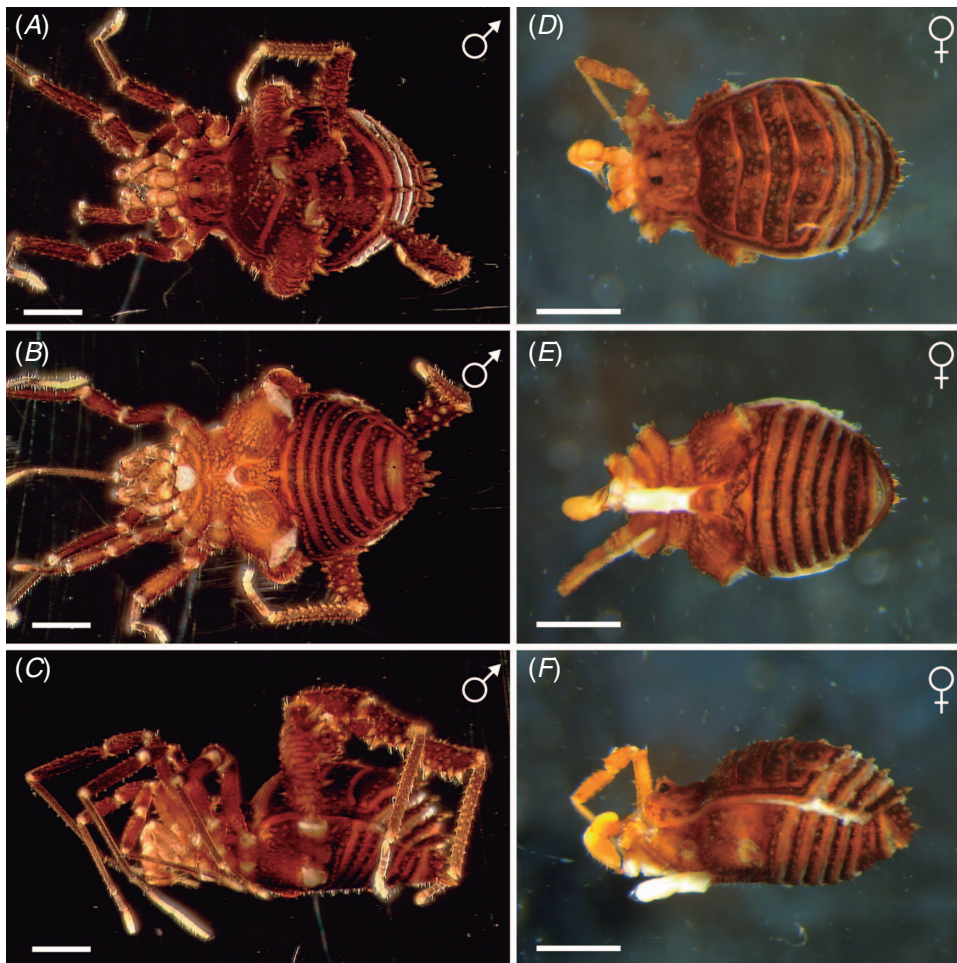


Fig. 1. *Zalmoxis curupira*, sp. nov. (A) Holotype, dorsal view. (B) Holotype, ventral view. (C) Holotype, lateral view. (D) Female paratype, dorsal view. (E) Female paratype, ventral view. (F) Female paratype, lateral view. Scale bars: 1 mm.

on ventrodistal margin; dorsally with setose tubercles approximately alternating in size. Male patella IV with one prominent ventral tubercle. Male tibia IV with distally incrassate region marked by pronounced ventral tubercles. Male metatarsus IV with a regular row of ventral setose tubercles (not so in female). Tarsal claws simple; tarsal formula 3 : 6 : 5 : 6 (Fig. 2, 3, Tables 1, 2).

Penis with clear rutrum and pergula, typical of zalmoxids. Rutrum with two pairs of distal setae forming two rows. Pergula with a triad of setae at the apposition of the rutrum and pergula juncture. Two pairs of lateral setae on pergula margin; additional latero-dorsal pair of setae visible in ventrolateral view. One pair of small setae on lateral margin, immediately proximal of pergula (Fig. 3*H, I*).

Distribution

Known only from its type locality in north Sulawesi.

Etymology

Noun in apposition. In Brazilian folklore, Curupira is a red-skinned boy, or dwarf, with backward feet. Curupira confuses

hunters and forest wanderers by leaving reversed footprints and emitting deceiving whistles. The epithet alludes to the fact that leg IV of harvestmen, in particular zalmoxids, are oriented posteriorly (backwards), and also alludes to the Neotropical origins of the Palaeotropical genus *Zalmoxis*.

Zalmoxis therianthropes, sp. nov.

(Fig. 4–6)

Types. Male holotype (MCZ IZ-131124 [DNA102502]) from Bukit Kanrapih, Sulawesi Selatan, Indonesia (GG Locality 509: 5.04222°S, 119.73527°E), 1888-m elev., leg. R.M. Clouse, G. Giribet, C. Rahmadi, 27 June 2006; extracted for DNA and on SEM stubs (MCZ IZ-131124). *Paratypes:* one female (MCZ IZ-131124), same collecting data as holotype; in ethanol and extracted for DNA and on SEM stubs.

Diagnosis

Distinguished from congeners by the absence of a conspicuous sexually dimorphic leg IV and penis with a lanceolate rutrum and a medially subdivided pergula, whereas

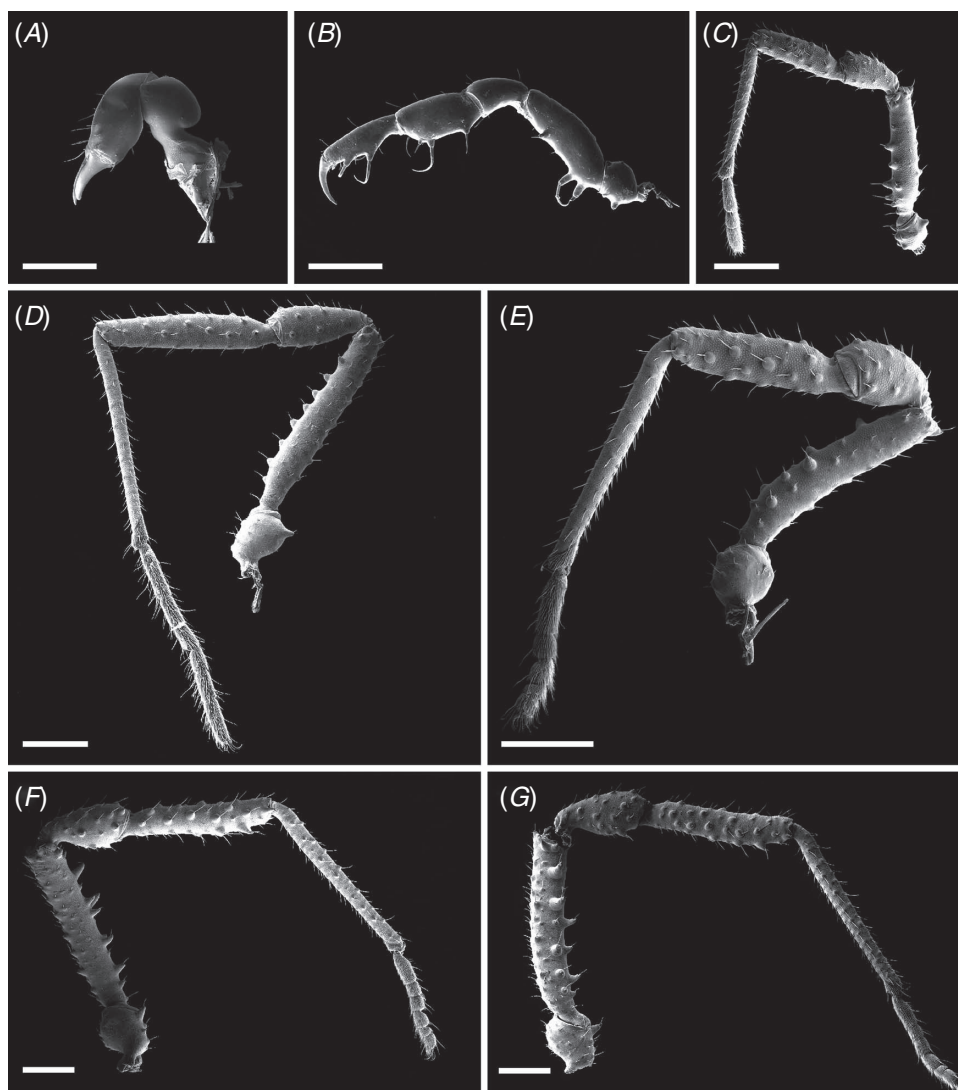


Fig. 2. *Zalmoxis curupira*, sp. nov., female paratype. (A) Left chelicera. (B) Left pedipalp. (C) Left leg I. (D) Left leg II. (E) Left leg III. (F) Left leg IV (mesal view). (G) Right leg IV (ectal view). Scale bars: 500 μ m.

pergula in other species of the genus is usually discreetly curved up. Additionally, body atypically incrassate for Zalmoxidae in both sexes, and palp of male with swollen tibia.

Description

Total length of male holotype (female paratype in parentheses) 3.64 mm (3.36 mm), greatest width of prosoma 1.25 mm (1.18 mm), greatest width of opisthosoma 2.24 mm (2.20 mm); length-to-width ratio 1.62 (1.52). Body dark brown, trochanters and tarsi light brown. Eyes on the sides of the ocularium. Ocularium wider than long, removed from anterior margin of the carapace; with three pointed tubercles arranged in a triangle. First scutal groove of mesotergum U-shaped, subsequent ones less so, almost parallel to each other. Margins of the free tergites without conspicuous belt of tubercles (Fig. 4).

Ventral prosomal complex of male with coxae II and III meeting in midline, coxae I and IV not so. Anterior and posterior

margins of coxae III with tubercular bridges to adjacent coxae, and coxae I–III with setose tubercles. Coxae IV of male of similar size to female. Genital operculum subtriangular, typical of Zalmoxidae. Spiracles visible. Opisthosomal sternites and anal plate without prominent tubercular ornamentation (Fig. 4).

Chelicera (Fig. 5A, 6A) smooth and monomorphic, with bulla. Palp of male (Fig. 5B, 6B) with enlarged tibia, with four macrosetae mesally and three ectally.

Legs I–IV (Fig. 5, 6) of both sexes with small setiferous tubercles on femur, patella, tibia and metatarsus. Leg IV of both sexes with similar degree of granulation, femur with four distal tubercles ectally. Distalmost tubercle curved towards patella. Patella, tibia and metatarsus IV with regularly spaced setiferous tubercles larger than in the other legs, slightly more pronounced in male. Tarsal claw simple; tarsal formula: 3 : 6 : 5 : 6 (Fig. 5, 6, Tables 3, 4).

Penis with rutrum and pergula, forming a triangular outline; rutrum lanceolate and pergula subdivided medially, with

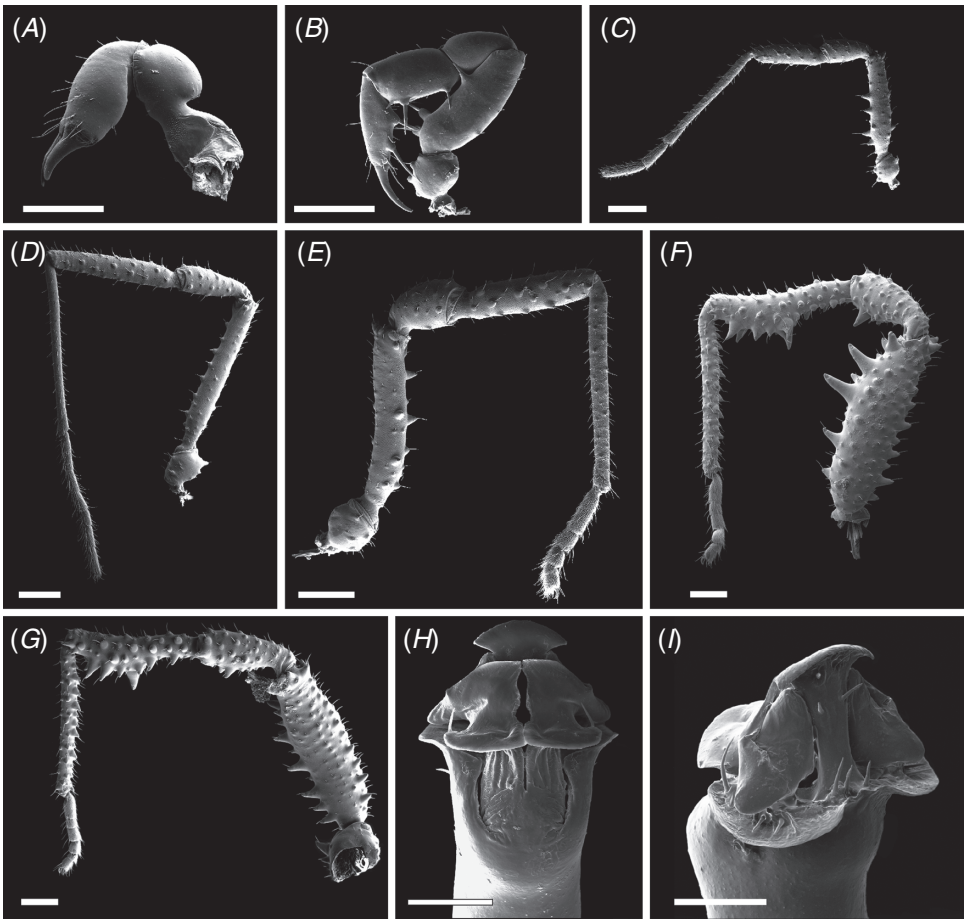


Fig. 3. *Zalmoxis cucupira*, sp. nov., male paratype. (A) Left chelicera. (B) Left pedipalp. (C) Left leg I. (D) Left leg II. (E) Right leg III. (F) Left leg IV (ectal view). (G) Right leg IV (mesal view). (H) Genitalia, dorsal view. (I) Genitalia, ventrolateral view. Scale bars: A–G, 500 µm; H, I, 100 µm.

Table 1. Appendage measurements (length/width) of *Zalmoxis cucupira*, sp. nov. holotype
Tr, trochanter; Fe, femur; Pa, patella; Ti, tibia; Mt, metatarsus; Ta, tarsus

	Tr	Fe	Pa	Ti	Mt	Ta	Total
Leg I	0.38/0.32	1.34/0.26	0.67/0.30	0.88/0.27	1.68/0.12	0.90/0.14	5.85
Leg II	0.53/0.37	1.92/0.25	0.93/0.31	1.56/0.27	2.19/0.12	1.77/0.10	8.90
Leg III	0.49/0.42	1.53/0.28	0.69/0.42	1.17/0.31	1.91/0.15	0.96/0.14	6.75
Leg IV	0.69/0.69	2.26/0.73	1.37/0.50	1.73/0.48	2.11/0.21	0.97/0.15	9.13
Palp	0.31/0.28	0.83/0.29	0.41/0.23	0.49/0.30	–	0.56/1.97	2.60
	Proximal	Second	Distal				
Chelicera	0.79/0.4	1.08/0.37	0.33/0.14				

Table 2. Appendage measurements (length/width) of *Zalmoxis cucupira*, sp. nov. female paratype (MCZ IZ-131112)
Tr, trochanter; Fe, femur; Pa, patella; Ti, tibia; Mt, metatarsus; Ta, tarsus

	Tr	Fe	Pa	Ti	Mt	Ta	Total
Leg I	0.31/0.23	1.03/0.21	0.49/0.24	0.71/0.21	1.14/0.10	0.57/0.11	4.25
Leg II	0.53/0.33	1.55/0.23	0.78/0.31	1.33/0.27	1.75/0.11	1.67/0.11	7.61
Leg III	0.44/0.31	1.55/0.21	0.54/0.33	0.89/0.25	1.44/0.12	0.8/0.12	5.66
Leg IV	0.56/0.45	1.75/0.33	0.91/0.41	1.41/0.28	1.99/1.16	1.00/1.13	7.62
Palp	0.31/0.27	0.70/0.27	0.43/0.23	0.49/0.28	–	0.57/0.18	2.50
	Proximal	Second	Distal				
Chelicera	0.67/0.36	1.03/0.34	0.30/0.13				

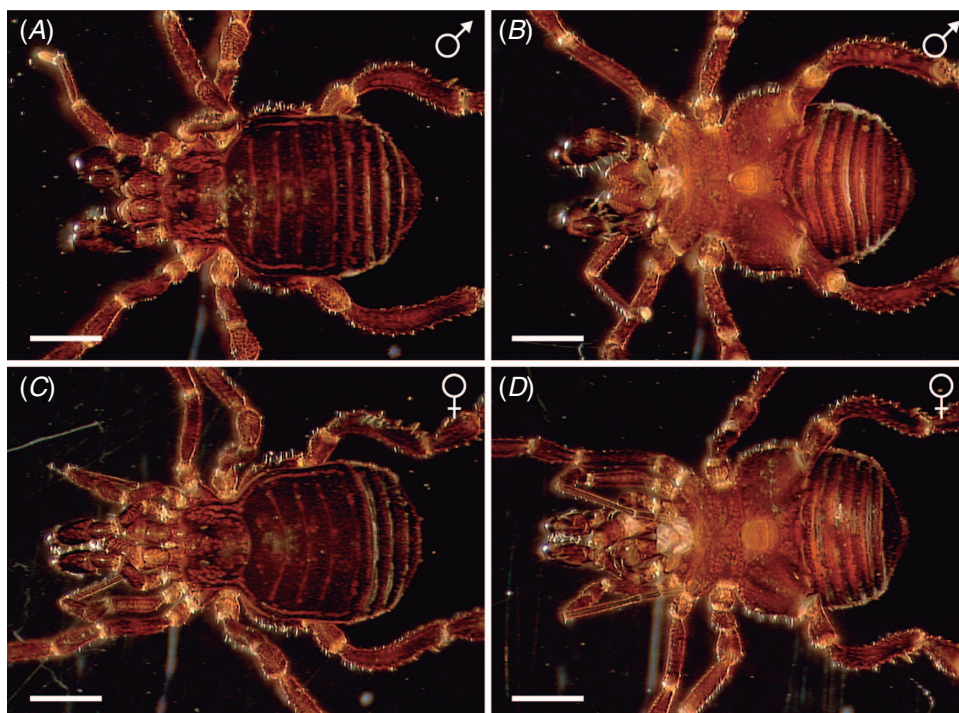


Fig. 4. *Zalmoxis therianthropes*, sp. nov. (A) Holotype, dorsal view. (B) Holotype, ventral view. (C) Female paratype, dorsal view. (D) Female paratype, ventral view. Scale bars: 1 mm.

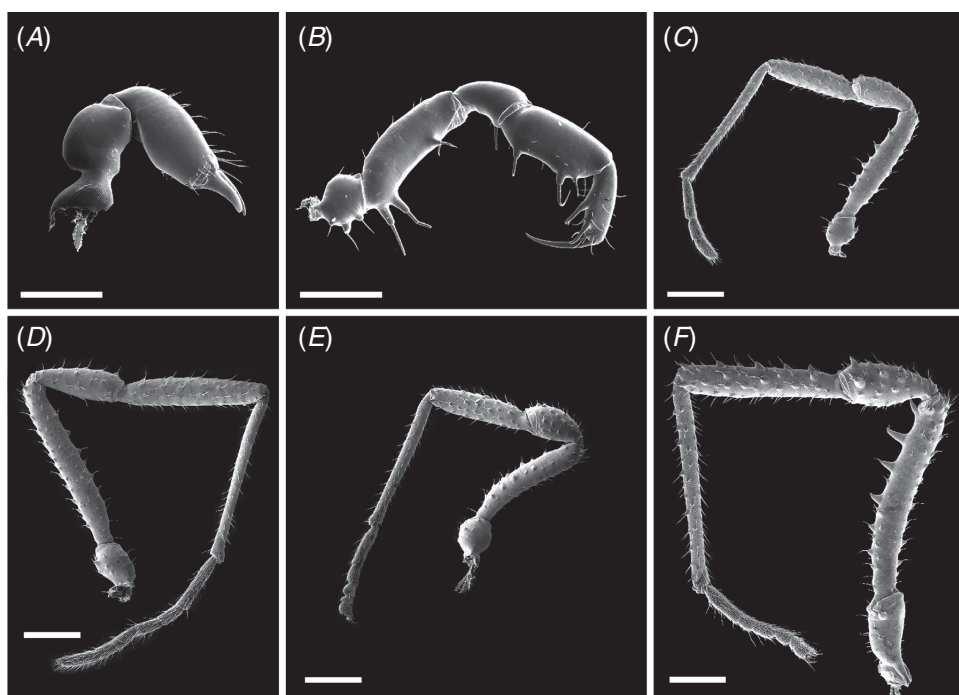


Fig. 5. *Zalmoxis therianthropes*, sp. nov., female paratype. (A) Right chelicera. (B) Right pedipalp. (C) Left leg I. (D) Right leg II. (E) Left leg III. (F) Left leg IV (ectal view). Scale bars: 500 μ m.

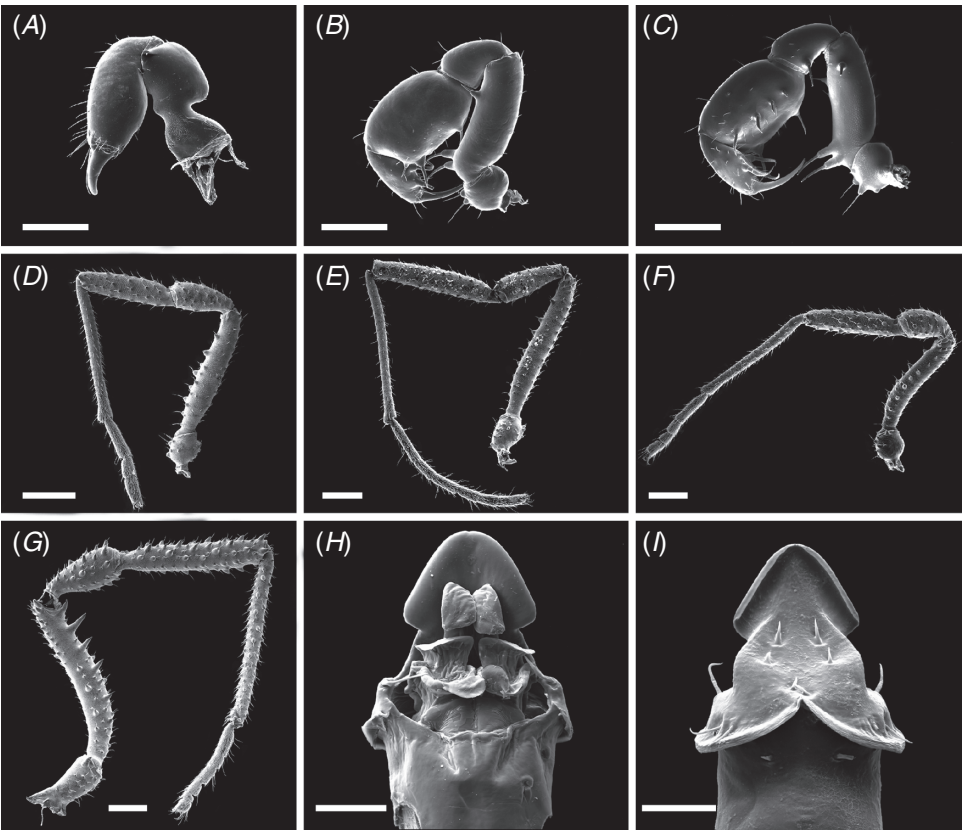


Fig. 6. *Zalmoxis therianthropes*, sp. nov., male paratype. (A) Left chelicera. (B) Left pedipalp (ectal view). (C) Right pedipalp (mesal view). (D) Left leg I. (E) Right leg II. (F) Left leg III. (G) Right leg IV (ectal view). (H) Genitalia, dorsal view. (I) Genitalia, ventral view. Scale bars: A–G, 500 µm; H, I, 100 µm.

Table 3. Appendage measurements (length/width) of *Zalmoxis therianthropes*, sp. nov. male paratype
Tr, trochanter; Fe, femur; Pa, patella; Ti, tibia; Mt, metatarsus; Ta, tarsus

	Tr	Fe	Pa	Ti	Mt	Ta	Total
Leg I	0.38/0.25	1.18/0.22	0.63/0.26	0.86/0.23	1.42/0.11	0.85/0.11	5.32
Leg II	0.47/0.33	1.86/0.27	0.89/0.31	1.53/0.29	1.94/0.13	2.2/0.12	8.89
Leg III	0.50/0.39	1.36/0.26	0.69/0.36	1.14/0.30	1.71/0.14	1.03/0.13	6.43
Leg IV	0.95/0.39	2.16/0.4	1.06/0.46	1.94/0.36	2.43/0.22	1.34/0.18	9.88
Palp	0.38/0.35	0.86/0.35	0.57/0.30	0.77/0.56		0.61/0.22	3.19
Chelicera	Proximal 0.84/0.43	Second 1.29/0.47	Distal 0.35/0.14				

Table 4. Appendage measurements (length/width) of *Zalmoxis therianthropes*, sp. nov. female paratype
Tr, trochanter; Fe, femur; Pa, patella; Ti, tibia; Mt, metatarsus; Ta, tarsus

	Tr	Fe	Pa	Ti	Mt	Ta	Total
Leg I	0.36/0.24	1.04/0.20	0.55/0.23	0.76/0.20	1.28/0.10	0.78/0.10	4.77
Leg II	0.50/0.29	1.57/0.22	0.78/0.26	1.25/0.23	1.56/0.10	1.82/0.10	7.48
Leg III	0.40/0.28	1.02/0.18	0.56/0.28	0.85/0.21	1.29/0.10	0.81/0.10	4.93
Leg IV	0.81/0.31	1.77/0.26	0.88/0.36	1.42/0.25	1.94/0.15	1.1/0.11	7.92
Palp	0.36/0.29	0.74/0.28	0.48/0.23	0.59/0.29		0.52/0.16	2.69
Chelicera	Proximal 0.81/0.35	Second 1.09/0.34	Distal 0.29/0.13				

lateral edges facing down. Rutrum with two pairs of setae, and one pair of setae at the apposition of the rutrum and pergula juncture. Pergula with two pairs of setae on lateral margin and one dorsolateral pair; additionally, one pair of setae on the basis, immediately proximal to pergula (Fig. 6*H, I*).

Distribution

Known only from the type locality in south-western Sulawesi.

Etymology

Noun in apposition. Therianthropes are mythological creatures that have mixed human and animal identities. The epithet refers to the recently discovered cave paintings in South Sulawesi, which are among the oldest symbolic drawings of early human history.

Zalmoxis adze, sp. nov.

(Fig. 7, 8)

Types. Male holotype (MCZ IZ-131138 [DNA102525]) from Virarata National Park, Central Province, Papua New Guinea (RMC Locality 461: S9.44402, E147.34083), 815-m elev., leg. R.M. Clouse, Awei, 31 March 2006; extracted for DNA and on SEM stubs. Three female paratypes (MCZ IZ-131137; DNA102524), same collecting data as holotype; three in 96% ethanol and one extracted for DNA.

Diagnosis

Distinguished from congeners by males with the broad incrassate sexually dimorphic femur IV with two long spines ectally, and incrassate patella and tibia with sharp apophysis. Flea-like habitus with ventrally inserted leg IV in male, whereas other species in the genus have leg IV laterally inserted.

Description

Total length of male holotype (female paratype in parentheses) 2.26 mm (1.77 mm), greatest width of prosoma 0.88 mm (0.78 mm), greatest width of opisthosoma 1.48 mm (1.34

mm); length-to-width ratio 1.52 (1.32). Body brown to orange. Eyes present on the sides of an ocularium. Anterior margin of carapace with pointed peg medially; two pairs of discrete pegs in the lateral margin and one between those two and the midline peg. First scutal groove of mesotergum thicker than others and orthogonal to antero-posterior axis, subsequent grooves progressively more U-shaped; grooves darker in colour than remaining area of tergites. Free tergites with belt of tubercles (Fig. 7).

Ventral prosomal complex of male with coxae II and III meeting in midline, coxae I and IV not so. Coxae IV large and visible in dorsal view; one dorsal projection pointing ectally. Genital operculum subtriangular, typical of *Zalmoxidae*. Spiracles visible. Opisthosomal sternites with single belt of tubercles and two antero-posterior bands of depigmented cuticle flanking the ventral midline. Anal plate with an anterior row of four tubercles and posterior row of three tubercles (Fig. 7).

Chelicera (Fig. 8*A*) mostly smooth, but with granulation on the ectal-ventral surface of proximal segment; bulla present. Palp (Fig. 8*B*) with slightly swollen tibia. Legs I–III with granules on femur, patella, tibia and metatarsus. Leg II with proximal metatarsus slightly thicker than distally. Leg IV with femur ectal surface with three large tubercles distally and three smaller proximally; patella swollen, with large tubercles; tibia greatly enlarged, with a row of seven tubercles increasing in length from most proximal to distal; first six tubercles with blunt tip; distalmost tubercle bifurcated, curved and pointed. Tarsal claws simple; tarsal formula: 3 : 6 : 5 : 6 (Fig. 8, Table 5).

Penis with rutrum, and pergula with discrete margin. Rutrum with two pairs of setae; one pair at the apposition of rutrum and pergula. Pergula with two pairs of setae on the lateral margin; longest of these visible in dorsal view. Basis with one pair of setae on the lateral margin (Fig. 8*H, I*).

Distribution

Known only from type locality in the Central Province of Papua New Guinea.

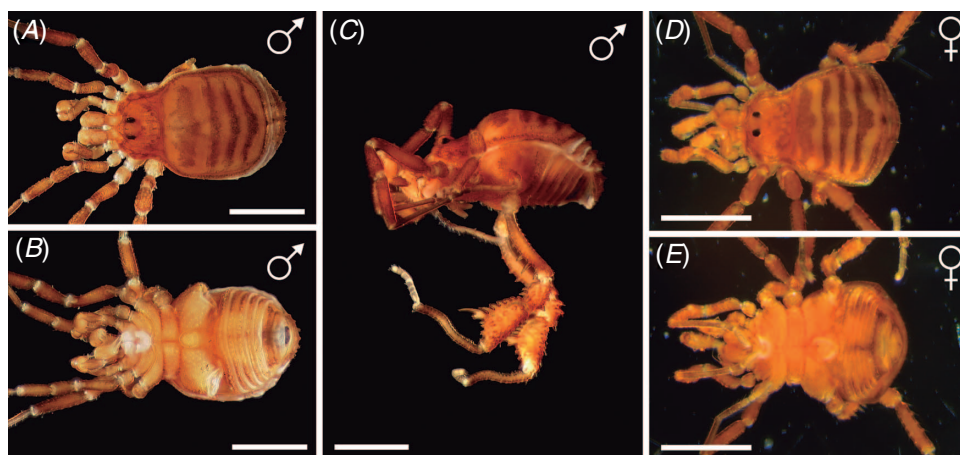


Fig. 7. *Zalmoxis adze*, sp. nov. (A) Holotype, dorsal view. (B) Holotype, ventral view. (C) Holotype, lateral view. (D) Female paratype, dorsal view. (E) Female paratype, ventral view. Scale bars: 1 mm.

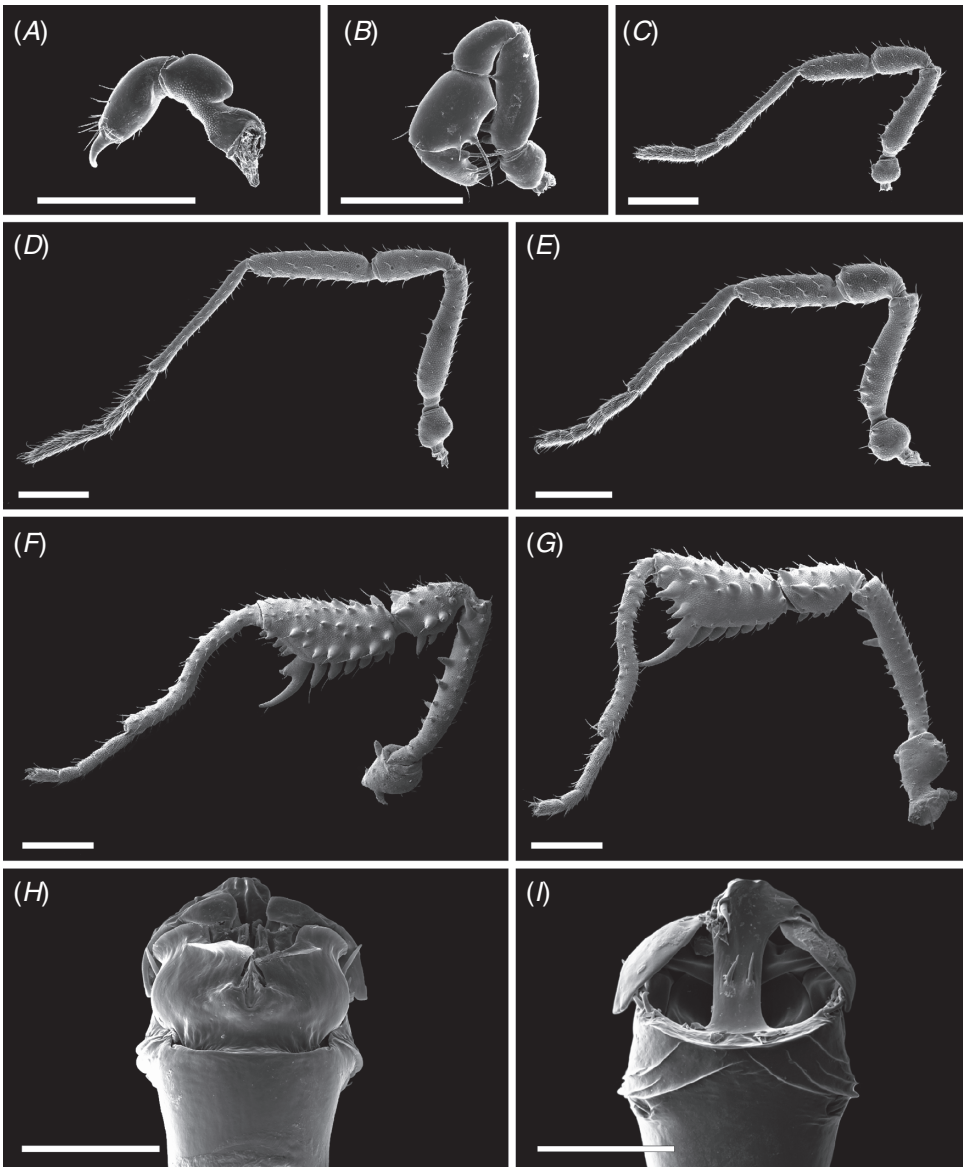


Fig. 8. *Zalmoxis adze*, sp. nov., male paratype. (A) Left chelicera. (B) Left pedipalp. (C) Left leg I. (D) Left leg II. (E) Left leg III. (F) Left leg IV (ectal view). (G) Right leg IV (mesal view). (H) Genitalia, dorsal view. (I) Genitalia, ventral view. Scale bars: A–G, 500 µm; H, I, 100 µm.

Table 5. **Appendage measurements (length/width) of *Zalmoxis adze*, sp. nov. holotype**
Tr, trochanter; Fe, femur; Pa, patella; Ti, tibia; Mt, metatarsus; Ta, tarsus

	Tr	Fe	Pa	Ti	Mt	Ta	Total
Leg I	0.21/0.19	0.69/0.17	0.41/0.20	0.50/0.17	0.79/0.08	0.56/0.09	3.16
Leg II	0.34/0.25	0.98/0.19	0.58/0.21	0.84/0.19	1.07/0.08	1.09/0.08	4.90
Leg III	0.28/0.27	0.81/0.19	0.45/0.24	0.64/0.20	0.99/0.13	0.65/0.10	3.82
Leg IV	0.44/0.34	1.15/0.19	0.61/0.31	0.90/0.44	1.28/0.12	0.70/0.08	5.08
Palp	0.20/0.18	0.49/0.17	0.30/0.15	0.34/0.25		0.28/0.13	1.61
Chelicera	Proximal 0.33/0.15	Second 0.42/0.14	Distal 0.10/0.04				

Etymology

Noun in apposition. Adze is a curved tool used for wood carving and that was commonly crafted by early Oceanian societies. The shape of the large tibial apophysis of males of this species is reminiscent of this tool.

Zalmoxis bilbo, sp. nov.

(Fig. 9–11)

Types. Male holotype (MCZ IZ-131200 [DNA106883]) from Tanakar, near Butmas, Mt Espiritu Santo, Vanuatu (15.36824°S, 166.97804°E), 705-m elevation, leg. C. Rabeling, E. O. Wilson, 16 November 2011, Winkler extraction from leaf litter. Genitalia in SEM stub; body in 96% ethanol. Three female paratypes MCZ IZ-131200 (DNA106883), three male paratypes MCZ IZ-131200 (DNA106883), appendages in SEM stubs; specimens dried out and reimmersed in ethanol. MCZ IZ-131202 (DNA106885): two male paratypes and one female paratype, used for gland SEM, in 96% ethanol, good preservation state.

Diagnosis

Distinguished from congeners by the swollen second basitarsal article of leg IV of males, whereas other species are either monomorphic or with a different combination of tarsomeres swollen. Additionally, distinguished by pigmentation of the sternites, with a black stripe medially.

Description

Total length of male holotype (female paratype MCZ IZ-131200 in parentheses) 2.98 mm (2.92 mm), greatest width of prosoma 1.05 mm (1.05 mm), greatest width of opisthosoma 2.03 mm (2.03 mm); length-to-width ratio 1.46 (1.43). Body dark brown to dark orange. Eyes present on the sides of an ocularium; integument around eyes black. Anterior margin of carapace with a median peg, and three small pairs of pegs on the lateral margin. First three scutal grooves of mesotergum nearly orthogonal to body midline; last groove slightly curved posteriorly. Free tergites without conspicuous belts of tubercles (Fig. 9).

Ventral prosomal complex of male with coxae II and III meeting in midline, coxae I and IV not so. Coxae IV of male not enlarged with respect to female, and visible dorsally. Genital operculum subtriangular, typical of Zalmoxidae. Spiracles visible. Opisthosomal sternites with fine granules and two bands of depigmented cuticle flanking the ventral midline, which leaves the medial dark stripe that characterises the species. Anal plate without conspicuous tubercles (Fig. 9).

Chelicera (Fig. 10A, 11A) monomorphic, mostly smooth, with bulla; ventroectal surface of proximal segment granulated. Palp (Fig. 10B, 11B) with megaspines, typical of zalmoxids. Legs (I–IV) with tubercles on femur, patella, tibia and metatarsus, more conspicuous on leg IV; smaller pointed tubercles proximally on the mesal surface. Male femur IV with three distal pointed tubercles ectally; distalmost tubercle curved towards patella; tibia with row of pointed tubercles, two distal ones longer and curved distally; metatarsus distally

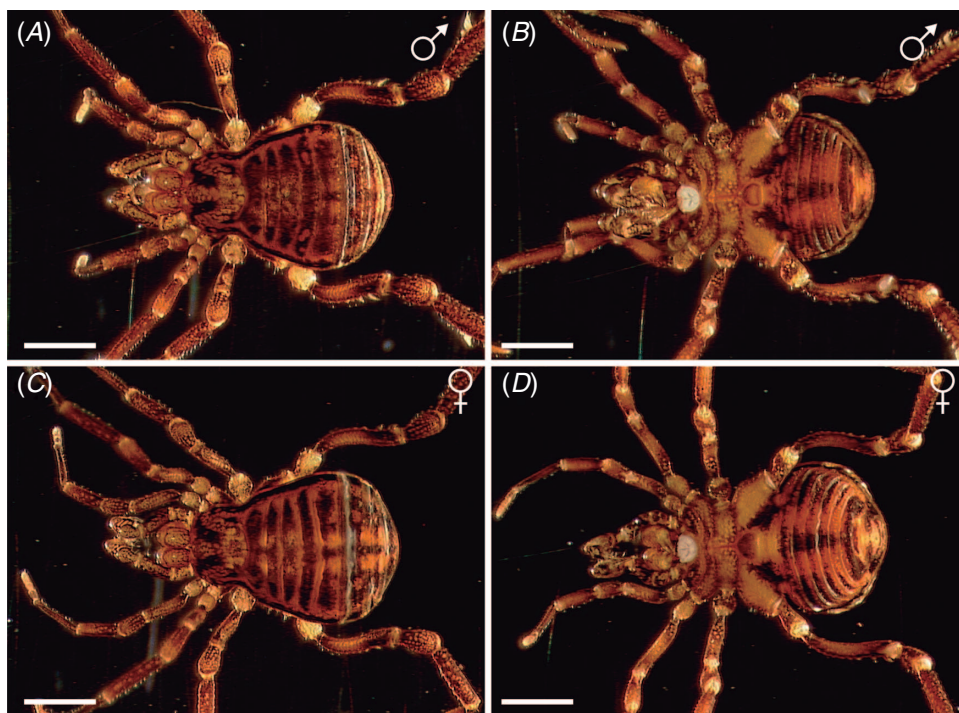


Fig. 9. *Zalmoxis bilbo*, sp. nov. (A) Holotype, dorsal view. (B) Holotype, ventral view. (C) Female paratype, dorsal view. (D) Female paratype, ventral view. Scale bars: 1 mm.

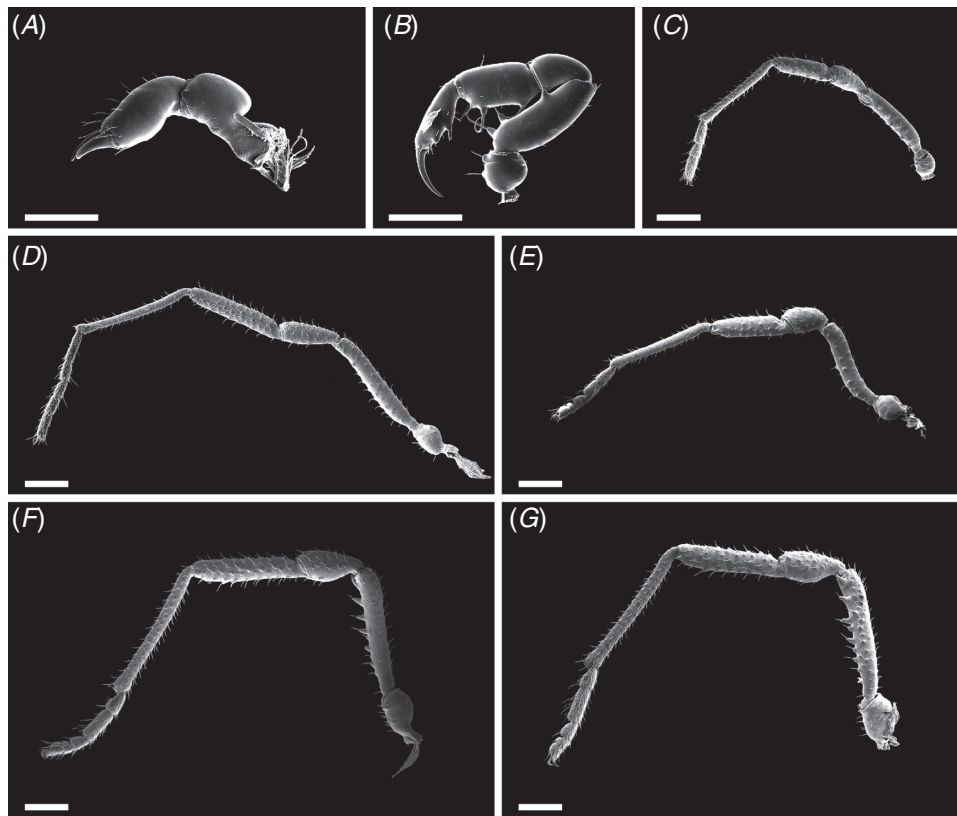


Fig. 10. *Zalmoxis bilbo*, sp. nov., female paratype. (A) Left chelicera. (B) Left pedipalp. (C) Left leg I. (D) Left leg II. (E) Left leg III. (F) Right leg IV (mesal view). (G) Left leg IV (ectal). Scale bars: 500 µm.

enlarged; first tarsomere (proximal to distal) slightly enlarged, second tarsomere greatly enlarged. Tarsal claws simple; tarsal formula: 3 : 5 : 5 : 5 (Fig. 10, 11, Tables 6, 7).

Penis with rutrum and pergula with lateral margins exceeding the width of the basis, cup-shaped. Rutrum triangular with wide base; two pairs of setae medially. One pair of setae on the apposition of rutrum and pergula, aligned with the rutrum setae along the penis axis. Pergula with three pairs of setae on lateral margin. One pair of setae on the basis, proximal to pergula (Fig. 11*H, I*).

Distribution

Known only from type locality.

Etymology

Noun in apposition. The name refers to J. R. R. Tolkien's character Bilbo Baggins, a big and hairy-footed hobbit that appears in a number of his novels, in reference to the swollen sexually dimorphic tarsus of this species.

Morphology of the sexually dimorphic basitarsus

The basitarsus of males of the following species is swollen in comparison with the remaining tarsomeres of the leg and with the corresponding region of the females: *Zalmoxis bendis* (Borneo), *Z. bilbo*, sp. nov. (Vanuatu), *Zalmoxis* sp. (MCZ IZ-131140 [DNA102527]) (Papua New Guinea), *Zalmoxis* sp.

(DNA 102358-5) (New Caledonia), *Ethobunus* sp. (DNA 103875) (Costa Rica), *E. zalmoxiformis* (Costa Rica), and *E. tarsalis* (Costa Rica). Variation occurs with respect to which tarsomere is swollen, and whether the swollen tarsomere occurs on leg III or IV, or both (Fig. 12). A different tarsal count between sexes occurs in three species: in *Z. bendis*, males have four and five tarsomeres on legs III and IV respectively; whereas females have five and five. In *Ethobunus* sp. and in *E. tarsalis* (see Goodnight and Goodnight 1983), males have four and six tarsomeres on legs III and IV respectively; whereas females have five and six.

Males of *Zalmoxis zilbelthiurdos* (Borneo) exhibit a swollen metatarsus IV (Sharma *et al.* 2012), but the ultrastructure of this species was not investigated here. A slightly swollen metatarsus III also occurs in *Z. derzelas* Sharma, Buenavente, Clouse, Diesmos & Giribet, 2012 (Mindoro, Philippines), *Z. gebeleizis* (Panay, Philippines) and *Z. roeweri* Pérez-González, Sharma & Proud, 2016 (Fiji), but there is no evidence of glands (Pérez-González *et al.* 2016). The tarsus of these species is sexually monomorphic.

Zalmoxis bendis

Tarsomere I (proximal to distal) of leg III is greatly swollen (Fig. 13*A, B*). The ventral surface of tarsomere I has ~65 sulcated setae (*sensu* Willemart *et al.* 2010) that occupy the distal two-

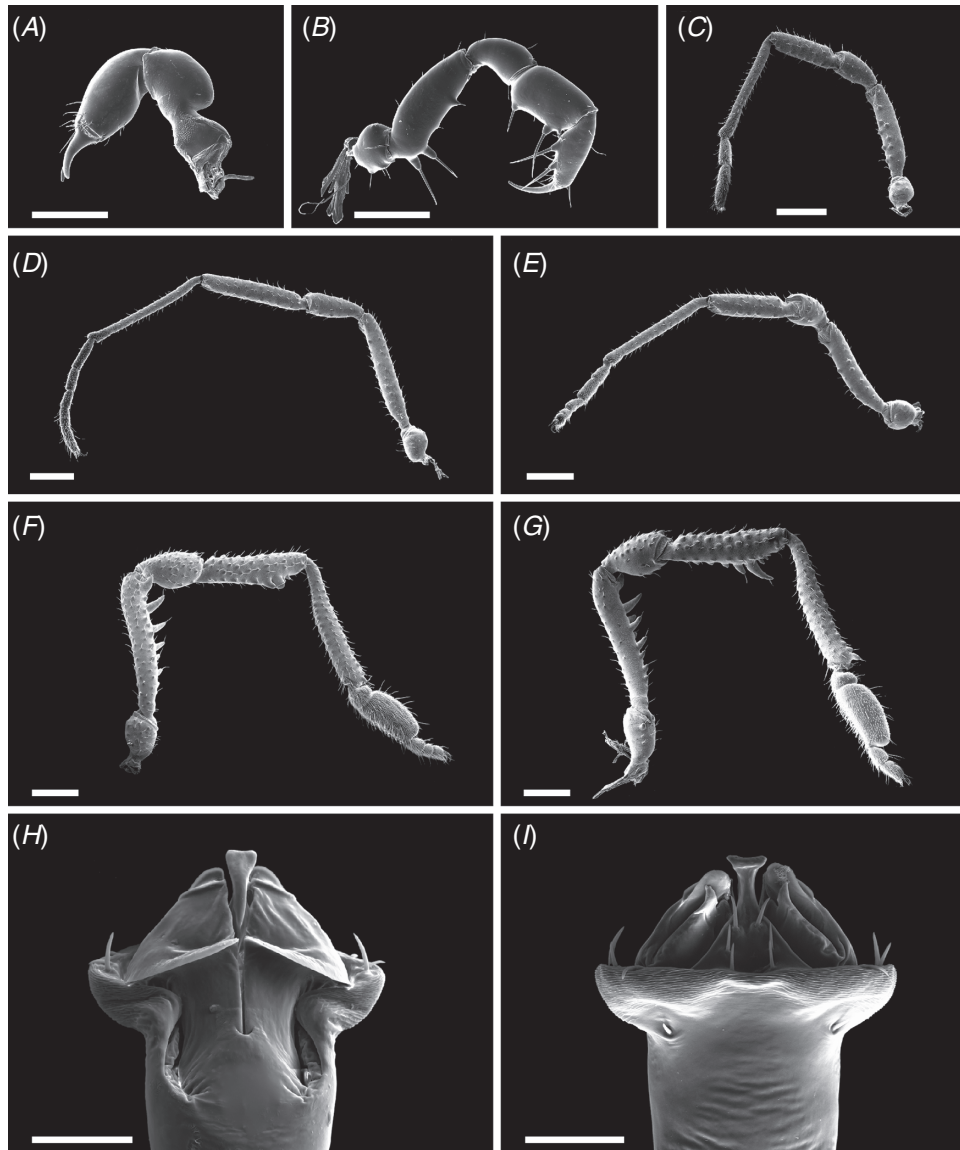


Fig. 11. *Zalmoxis bilbo*, sp. nov., male paratype. (A) Left chelicera. (B) Right pedipalp. (C) Left leg I. (D) Left leg II. (E) Left leg III. (F) Right leg IV (ectal view). (G) Left leg IV (mesal view). (H) Genitalia, dorsal view. (I) Genitalia, ventral view. Scale bars: A–G, 500 µm; H, I, 100 µm.

Table 6. Appendage measurements (length/width) of *Zalmoxis bilbo*, sp. nov. male paratype MCZ IZ-131200

Tr, trochanter; Fe, femur; Pa, patella; Ti, tibia; Mt, metatarsus; Ta, tarsus

	Tr	Fe	Pa	Ti	Mt	Ta	Total
Leg I	0.36/0.25	0.96/0.20	0.50/0.23	0.67/0.21	1.15/0.10	0.68/0.12	4.32
Leg II	0.43/0.28	1.38/0.21	0.71/0.26	1.17/0.21	1.5/1.11	1.42/0.10	6.61
Leg III	0.35/0.29	1.08/0.20	0.49/0.32	0.83/0.23	1.32/0.12	0.83/0.12	4.90
Leg IV	0.62/0.36	1.53/0.26	0.73/0.38	1.10/0.32	1.48/0.25	1.16/0.37	6.62
Palp	0.27/0.30	0.74/0.28	0.46/0.24	0.50/0.32		0.54/0.19	2.51
	Proximal	Second	Distal				
Chelicera	0.78/0.38	1.06/0.35	0.32/0.12				

Table 7. Appendage measurements (length/width) of *Zalmoxis bilbo*, sp. nov. female paratype (MCZ IZ-131200)
Tr, trochanter; Fe, femur; Pa, patella; Ti, tibia; Mt, metatarsus; Ta, tarsus

	Tr	Fe	Pa	Ti	Mt	Ta	Total
Leg I	0.27/0.23	0.93/0.20	0.47/0.23	0.65/0.19	1.1/0.10	0.67/0.10	4.09
Leg II	0.40/0.27	1.32/0.20	0.71/0.24	1.08/0.20	1.42/0.10	1.31/0.11	6.24
Leg III	0.36/0.28	1.02/0.19	0.55/0.30	0.79/0.21	1.25/0.12	0.82/0.12	4.79
Leg IV	0.65/0.32	1.43/0.21	0.77/0.35	1.18/0.24	1.7/0.15	1.03/0.15	6.76
Palp	0.31/0.29	0.73/0.26	0.44/0.20	0.50/0.28		0.53/0.18	2.51
Chelicera	Proximal 0.72/0.33	Second 0.96/0.32	Distal 0.27/0.13				

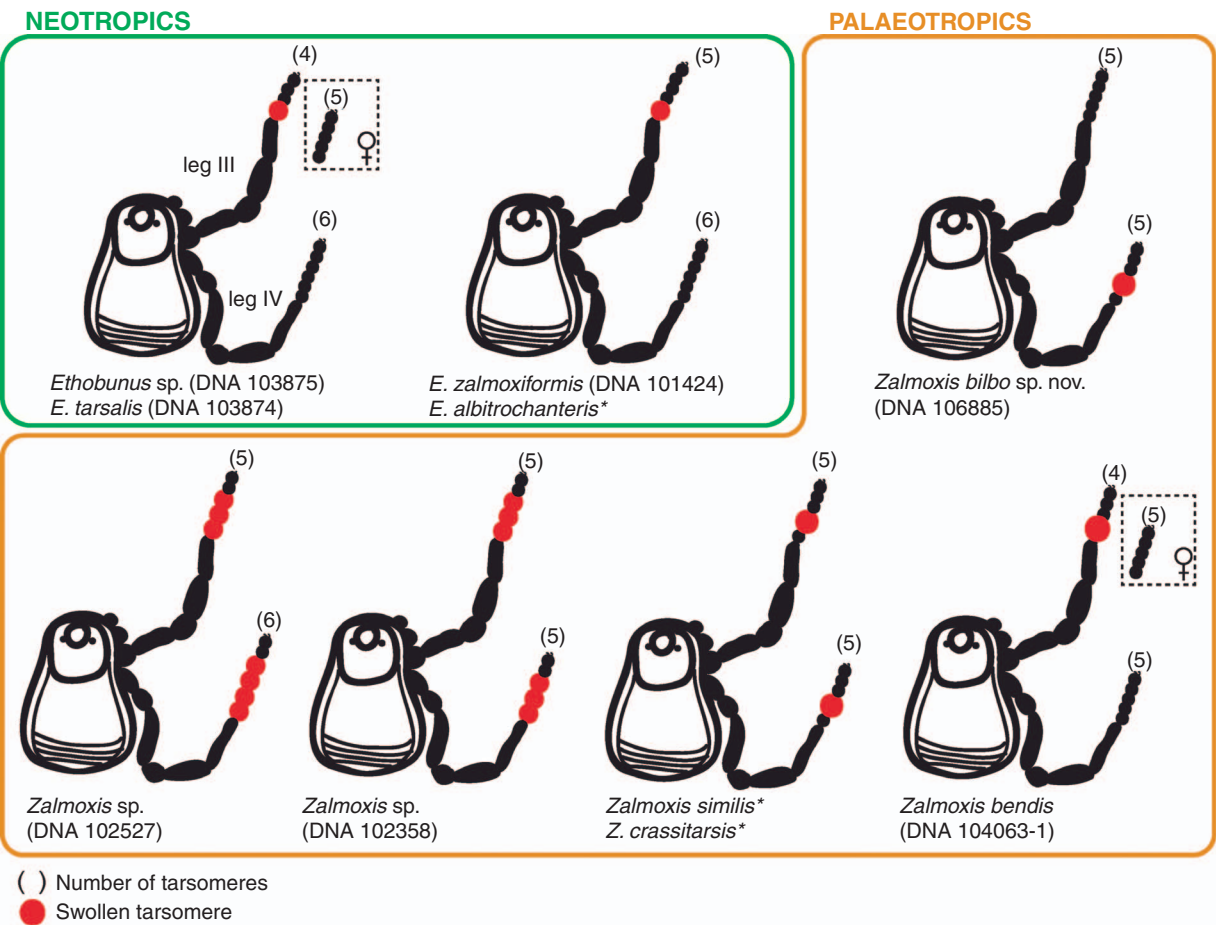


Fig. 12. Summary of the species of Zalmoxidae that have swollen tarsomeres. Schematics depict legs III and IV of males, indicating sexually dimorphic tarsomeres in red; the number of tarsomeres is shown in parenthesis. Cases where females have a different number of tarsomeres appear in dashed boxes. Asterisks denote information inferred only from the literature.

thirds of the tarsomere (Fig. 13B). This field of sulcated setae is devoid of sensilla chaetica and trichomes, which cover the adjacent surfaces of the tarsomere. Each sulcated seta (approximate length: 50 µm) is inserted in a pit with a small socket and a semicircular single row of pores. The shaft is curved, tapers to a fine tip, and has longitudinal ridges that form grooves (Fig. 13D, E). In leg IV, all the five tarsomeres of the males have the same width and height, and are of a comparable size to the tarsomeres in legs III and IV of females. Females have five tarsomeres in both legs III and

IV, and do not present sulcate setae (Fig. 13C). The corresponding region is covered with trichomes and sensilla chaetica (Fig. 13C).

Zalmoxis bilbo, sp. nov.

Tarsomere II (proximal to distal) of leg IV of males is greatly swollen (Fig. 14A, B). The ventral surface of tarsomere II has ~40 equally spaced sulcated setae. This region is devoid of sensilla chaetica and has additionally a few interspersed

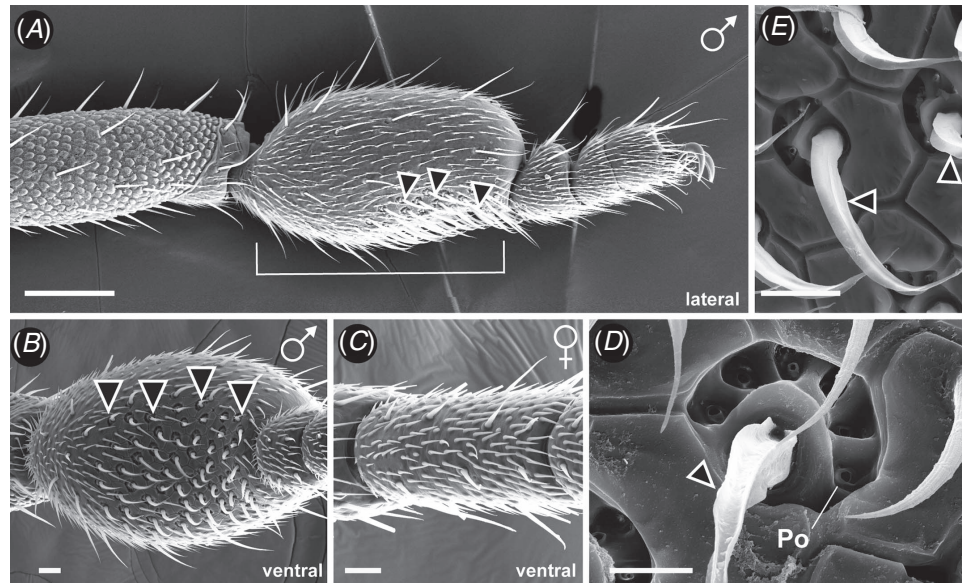


Fig. 13. *Zalmoxis bendis*, morphology of basitarsus III. (A) Male distal leg III, lateral view. (B) Male second tarsomere III (proximal–distal), ventral view. (C) Female second tarsomere III (proximal–distal), ventral view. (D) Sulcated seta, basal shaft and insertion. (E) Sulcate seta. Black arrowhead, sulcated seta. Bracket, first tarsomere. Po, pore. Scale bars: A, 100 μ m; B, C, 20 μ m; D, 5 μ m; E, 10 μ m.

trichomes among sulcated setae. Each sulcated seta is inserted in a socket and is not in a pit as is the case of *Z. bendis*. Two discrete groups of pores (5–10) occur adjacent to the socket (Fig. 13E, G). Proximally, the shaft has two longitudinal flaps that are folded into a groove that gradually widens towards the apex (Fig. 14E, F, H, I). The two longitudinal flaps project from the axis of the shaft, which has a lumen (Fig. 14E, G, I). The distal portion of the shaft is flat and abruptly tapers to a fine tip, conferring on it an acuminate leaf aspect (Fig. 14E). In females, the corresponding region of tarsomere II lacks sulcated setae and presents only sensilla chaetica and trichomes (compare Fig. 14C, D). Tarsomere I of leg IV of males is also slightly shorter and swollen, but sulcated setae and pores were not detected on its ventral surface.

Zalmoxis sp. MCZ IZ-131140

Tarsomeres I–III of legs III and IV of males of *Zalmoxis* sp. are moderately swollen (compare Fig. 15A, B). The ventral surface of these tarsomeres bears trichomes and sulcated setae (4–6) that occur in two parallel rows (Fig. 15D). Sulcated setae have more robust shafts and their longitudinal grooves do not form a single canal as in *Z. bilbo*, sp. nov. (see above) (Fig. 15E). Pores occur adjacent to the socket (6–9) (Fig. 15E). The female does not present sulcated setae (Fig. 15C).

Zalmoxis sp. MCZ DNA102358-5

Tarsomeres I–III of legs III and IV of males of *Zalmoxis* sp. are moderately swollen (compare Fig. 16A–D). No sulcated setae occur on the ventral surface of the swollen tarsomeres of the males. We observed sensilla chaetica and trichomes uniformly distributed on the ventral surface of tarsomeres I–III of both sexes. A higher concentration of tegumental

pores was observed on the ventral surface of swollen tarsomeres of leg IV of the male (Fig. 16E, F).

Ethobunus sp. MCZ DNA 1043875

Tarsomere I of leg III of males of *Ethobunus* sp. is moderately swollen (compare Fig. 17A, B). This tarsomere has sulcated setae ventrally, interspersed with trichomes (Fig. 17D, E). The shaft of the sulcated setae is inserted in a socket, in the vicinity of which a few pores occur. The shaft has longitudinal grooves that do not form a clear groove as in *Z. bilbo*, sp. nov. (Fig. 16E). The corresponding region of the female has sensilla chaetica inserted in a similar disposition as sulcated setae on the male as well as trichomes (Fig. 17C).

Ethobunus zalmoxiformis

Males of *Ethobunus zalmoxiformis* have a discreetly swollen tarsomere I of leg III (Fig. 18A, B). Similar to *Ethobunus* sp., the ventral surface of this tarsomere in the male bears sulcated setae and associated pores, which are not present in the female (Fig. 18C–E). The condition of tarsus IV could not be assessed because the available male specimen lacked legs IV.

Phylogenetic analysis and character evolution

The maximum-likelihood tree inferred from the dataset of Sharma and Giribet (2012) (augmented with the addition of *Zalmoxis bilbo*, sp. nov., and a second undescribed Vanuatuan species MCZ IZ-131205, DNA106901) recovered an identical topology with respect to the original terminals (Fig. 19). Each Vanuatuan species was recovered in a clade with species from Fiji with high nodal support (BS = 94–100%) (Fig. S1).

The following terminals were scored as present for sexually dimorphic basitarsal glands: *Zalmoxis bendis*

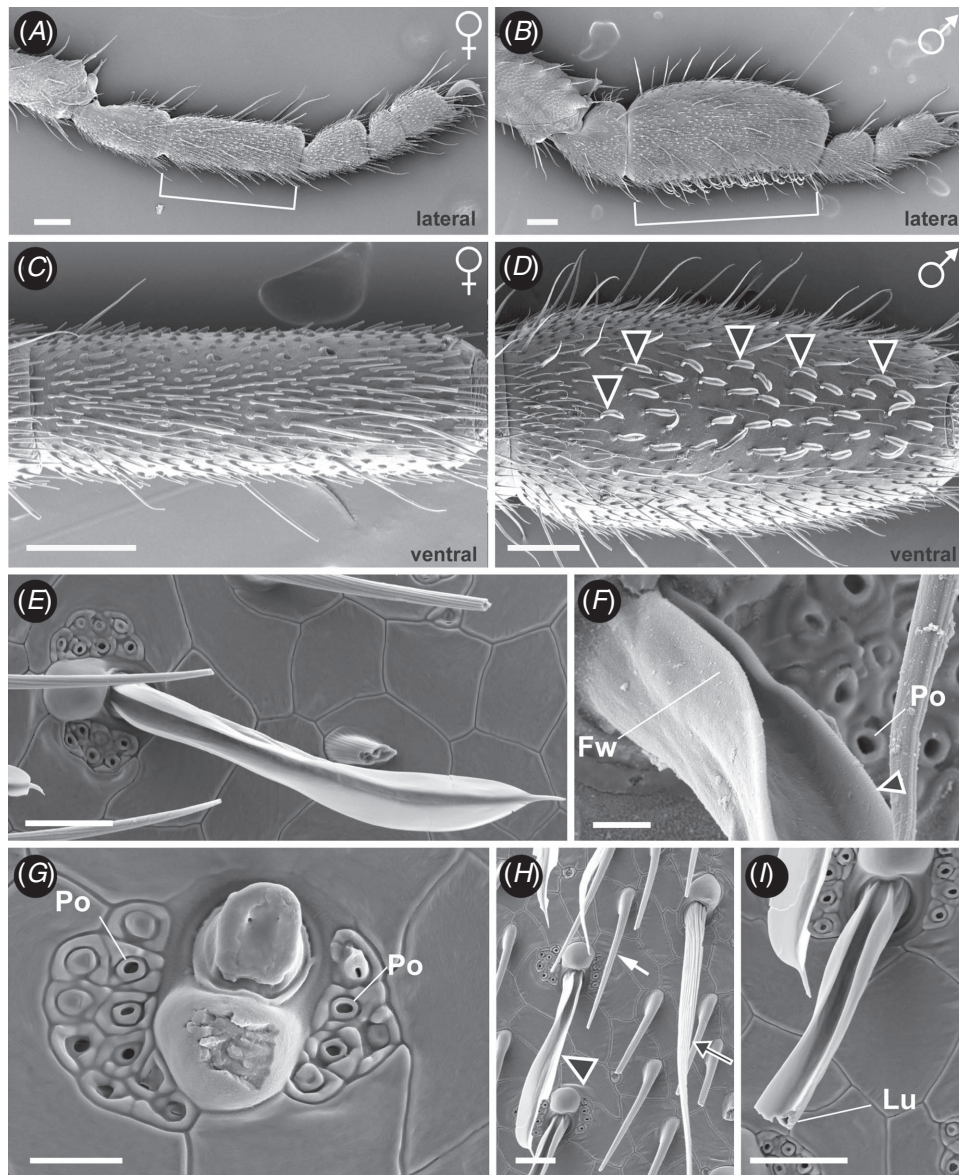


Fig. 14. *Zalmoxis bilbo*, sp. nov., morphology of basitarsus IV. (A) Female distal leg IV, lateral view. (B) Male distal leg IV, lateral view. (C) Female second tarsomere IV (proximal–distal), ventral view. (D) Male second tarsomere IV (proximal–distal), ventral view. (E) Sulcated seta, ventral view. (F) Basal shaft of sulcated seta. (G) Basally broken shaft of sulcated seta and associated pores, frontal view. (H) Ventral surface of the second tarsomere of a male. (I) Medially broken shaft of sulcated seta. Black arrow, sensillum chaeticum. Black arrowhead, sulcated seta. Bracket, second tarsomere. Fw, furrow. Lu, lumen. Po, pore. White arrow, trichome. Scale bars: A–D, 100 μ m; E, 10 μ m; F, 2 μ m; G, 5 μ m; H, I, 10 μ m.

(Borneo), *Z. bilbo*, sp. nov. (Vanuatu), *Zalmoxis* sp. MCZ IZ-131140 (New Guinea), *Zalmoxis* sp. MCZ DNA102358-5 (New Caledonia), *Ethobunus* sp. MCZ IZ-79937, *E. zalmoxiformis*, and *E. tarsalis* (Neotropics, Costa Rica) (Table S1). Ancestral state reconstruction supported the hypothesis that sexually monomorphic tarsi is the plesiomorphic condition for *Zalmoxis* and Zalmoxidae, and that sexually dimorphic tarsi evolved independently at least five times (Fig. 19). All *Zalmoxis* species with sexually dimorphic glands occur in separate clades that are clearly

plesiomorphically monomorphic. The three *Ethobunus* species with dimorphic tarsus form a clade, making the dimorphic condition the most likely reconstruction in their most recent common ancestor (Fig. 19). Accordingly, the retention index of this character is $ri = 0.33$, also indicating a high degree of homoplasy. D was measured as -0.1448621 . The probability that the $E(D)$ results from random phylogenetic structure is 0 and of $E(D)$ resulting from a Brownian phylogenetic structure is 0.635. We interpret these values as indicating evidence of phylogenetic

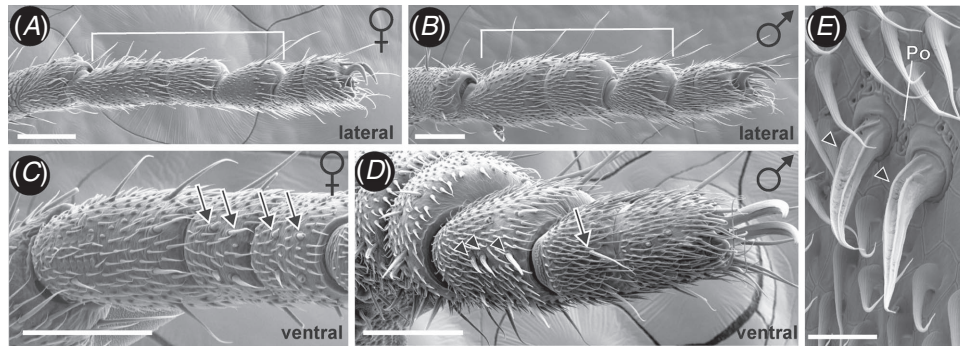


Fig. 15. *Zalmoxis* sp. MCZ IZ-131140, morphology of basitarsus III and IV. (A) Female distal leg III, lateral view. (B) Male distal leg III, lateral view. (C) Female second tarsomere IV (proximal–distal), ventral view. (D) Male second tarsomere IV (proximal–distal), ventrolateral view. (E) Sulcated seta. Black arrowhead, sulcated seta. Bracket, basitarsus (three tarsomeres). Black arrow, sensillum chaeticum. Po, pore. Scale bars: A–D, 100 µm; E, 10 µm.

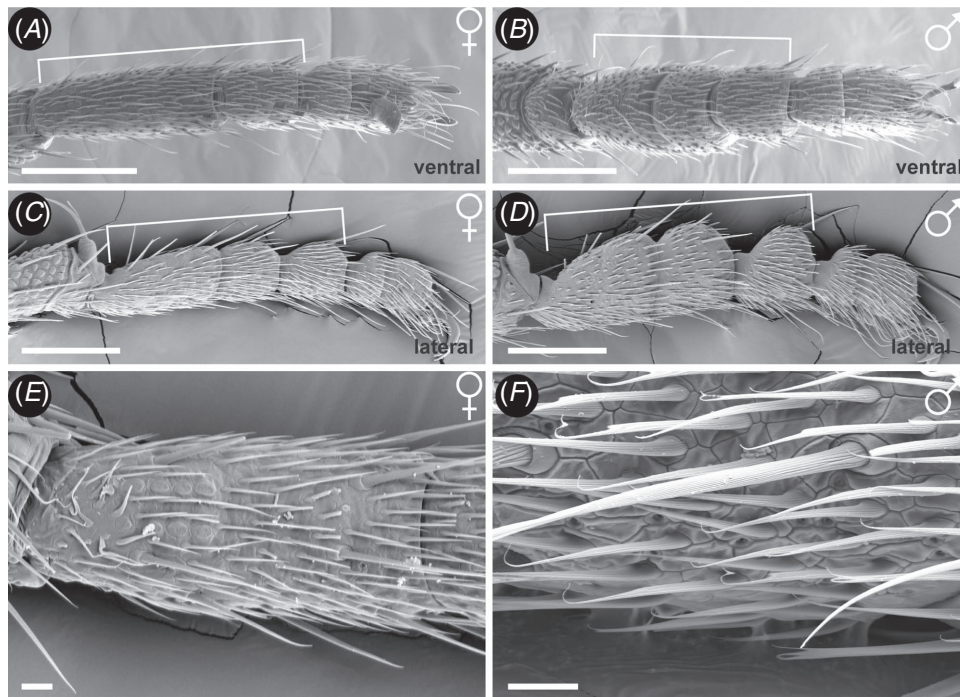


Fig. 16. *Zalmoxis* sp. MCZ DNA102358–5, morphology of basitarsus III and IV. (A) Female distal leg III, ventral view. (B) Male distal leg III, ventral view. (C) Female distal leg IV, lateral view. (D) Male distal leg IV, lateral view. (E) Female first tarsomere IV (proximal–distal), ventral view. (F) Male first tarsomere IV (proximal–distal), ventrolateral view. Bracket, basitarsus (three tarsomeres). Po, pore. Scale bars: A–D, 100 µm; E, F, 10 µm.

structure consistent with constant rates of character change along the phylogeny and nonrandom evolution.

Discussion

In an effort to improve the taxonomy of the zalmoxid fauna of the Palaeotropics and increase taxon sampling in species with sexual dimorphism, we described four new species, including the first record of *Zalmoxis* from the Vanuatu archipelago. These newly described species contribute to

the repertoire of diverse leg morphologies observed in Zalmoxidae, with respect to dimorphism in armature, and to the presence of sexually dimorphic glands. *Zalmoxis adze*, sp. nov. and *Zalmoxis curupira*, sp. nov. have an incrassate leg IV, whereas *Zalmoxis bilbo*, sp. nov. presents a sexually dimorphic swollen basitarsus IV. *Zalmoxis thieranthropes*, sp. nov., however, shows little or no sexual dimorphism with respect to both these aspects.

Sexual dimorphism in leg armature is widespread in Zalmoxidae, and usually involves an elongated leg IV with

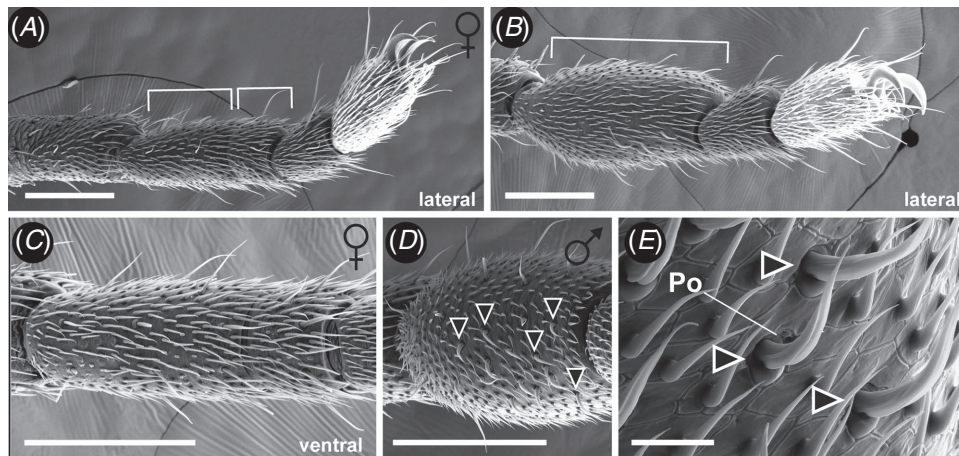


Fig. 17. *Ethobunus* sp., morphology of basitarsus III. (A) Female distal leg III, lateral view. (B) Male distal leg III, lateral view. (C) Female first and second tarsomere III (proximal–distal), ventral view. (D) Male first tarsomere III (proximal–distal), ventral view. (E) Sulcated setae on basitarsus of male. Black arrowhead, sulcated seta. Bracket, first tarsomere. Po, pore. Scale bars: A–D, 100 µm; E, 10 µm.

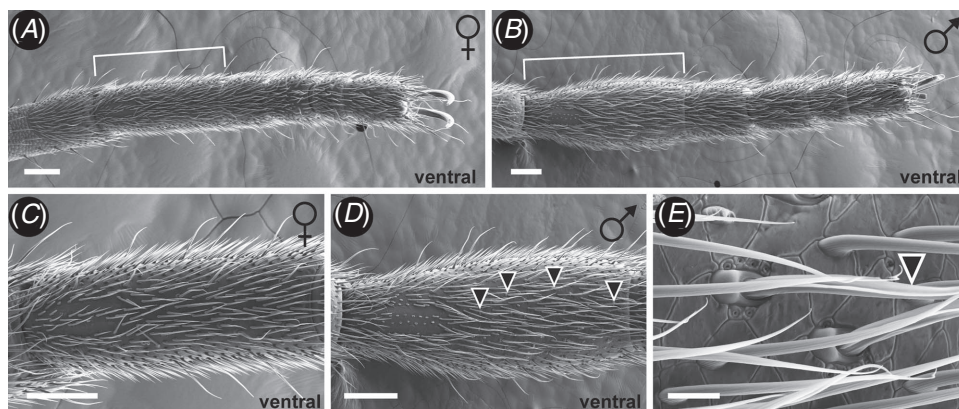


Fig. 18. *Ethobunus zalmoxiformis*, morphology of basitarsus III. (A) Female distal leg III, ventral view. (B) Male distal leg III, ventral view. (C) Female first tarsomere III (proximal–distal), ventral view. (D) Male first tarsomere III (proximal–distal), ventral view. (E) Sulcated setae. Black arrowhead, sulcated seta. Bracket, first tarsomere. Po, pore. Scale bars: A–D, 100 µm; E, 10 µm.

apophyses on the femur, patella or tibia (Sharma *et al.* 2011, 2012; Sharma 2012). At least in one species, *Zalmoxis furcifer*, males are polymorphic and display remarkable elongation of the fourth pair of legs (Sharma 2012). In addition to the basitarsal glands studied here, typical glandular morphology has also been reported for the metatarsus IV of *Guagonia multispina* González-Sponga, 1987 and tibia and patella II of *Stygnoleptes analis* Banks, 1914 (Willemart *et al.* 2010). There is also evidence for sexually dimorphic glands in other zalmoxids, inferred from the presence of swollen leg segments in *Ethobunus* sp. (Willemart *et al.* 2010 and references therein), and in the genus *Minuides* (Kury and Pérez-González 2007). Drawings of *Zalmoxis crassitarsis* Suzuki, 1982 and *Z. similis* Suzuki, 1982 (Bismarck Archipelago, New Guinea) clearly show a swollen tarsomere II in male leg IV, similar to that of *Zalmoxis bilbo*, sp. nov. (Vanuatu), but also in leg III (Suzuki 1982). Males of the

Neotropical species *Ethobunus albitrochanteris* (Roewer, 1933) also present swollen basitarsus III, possibly associated with glands (Goodnight and Goodnight 1983). A putative sexually dimorphic gland has also been reported on the body of a zalmoxid: males of *Minuides milleri* Šilhavy, 1978 have a porous area in the ocularium, which initially was thought to be part of a stridulatory apparatus (Šilhavy 1978; Kury and Pérez-González 2007).

The basitarsi of the species investigated herein are remarkably similar with respect to (1) the swollen condition of the tarsomeres, (2) the presence of ventral pores, and (3) the location of the pores, which are almost always clustered at the base of sulcated setae. These parallel features of the dimorphism are suggestive of homology. Nevertheless, our ancestral state reconstruction results suggest that sexually dimorphic basitarsal glands are homoplastic, having evolved

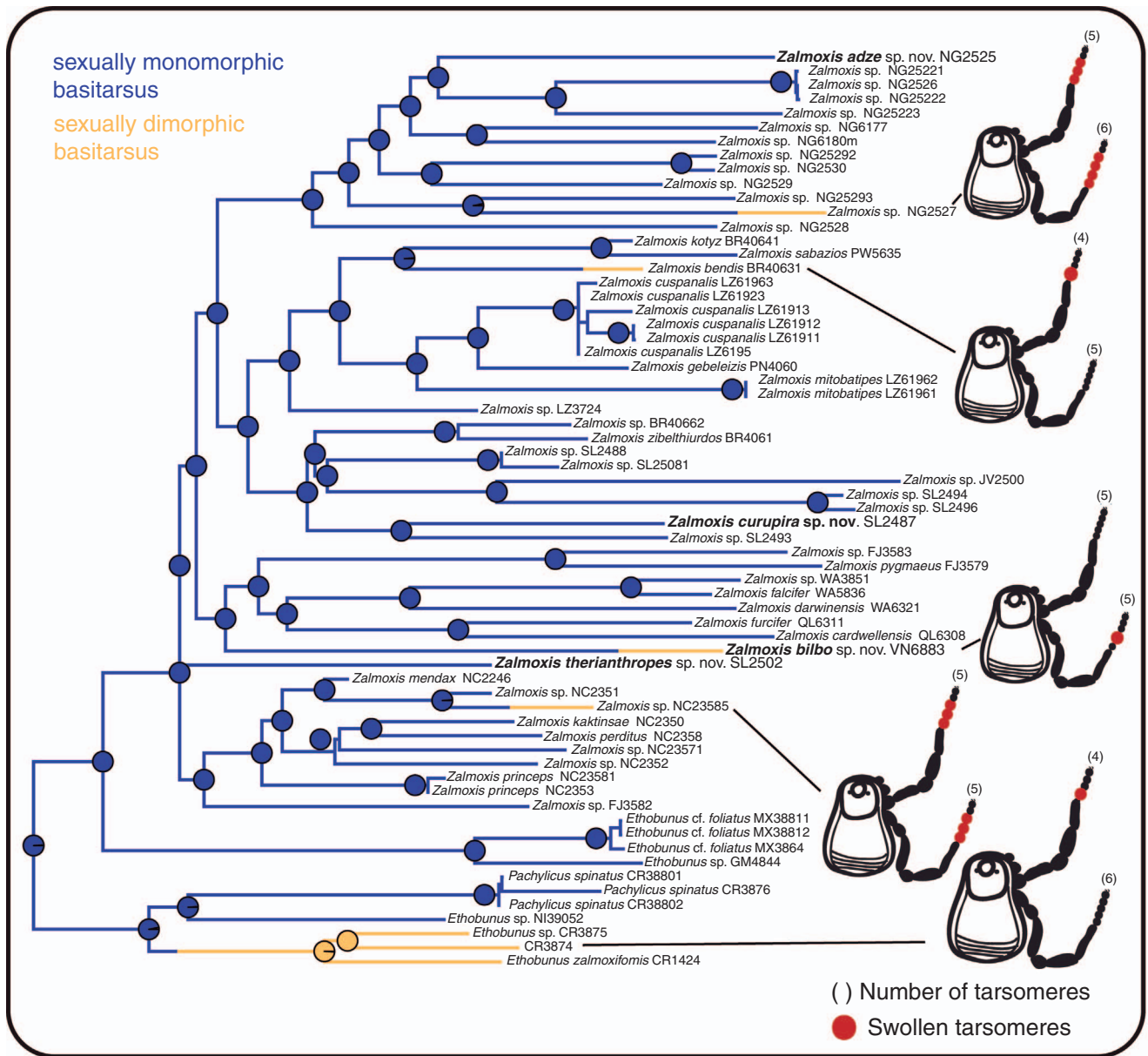


Fig. 19. Stochastic character mapping of the basitarsus condition on the maximum-likelihood tree of 65 terminals of Zalmoxidae for which the basitarsus was coded. Charts on nodes indicate posterior probability of the ancestral states overlaid in a randomly selected stochastic character state mapping. Blue (dark grey online), sexually monomorphic basitarsus. Orange (light grey online), sexually dimorphic basitarsus. Schematics depict legs III and IV of selected terminals, indicating sexually dimorphic tarsomeres in red, and number of tarsomeres in parentheses.

independently (at least five times) in Zalmoxidae. It is worth mentioning that more *Ethobunus* species (Neotropics) are known from the taxonomic literature to have a sexually dimorphic swollen basitarsus III (see above); a better sampling of Neotropical fauna could potentially change this ancestral state reconstruction. Nonetheless, given the available evidence and that *Zalmoxis* terminals showing glands are nested in separate clades that plesiomorphically lack the glands, the inclusion of derived *Ethobunus* species that possibly bear the glands would likely not affect the

reconstruction for *Zalmoxis*. Therefore, our data provide evidence that basitarsal glands evolved at least once in zalmoxids of the Neotropics and multiple times in the zalmoxids of the Palaeotropics.

In the better studied Neotropical harvestman family Gonyleptidae, a sexually dimorphic leg IV (apophyses and femur length) is plesiomorphic for the family, and has been lost and regained several times, which demonstrates the evolutionary lability of this character (Buzatto *et al.* 2014; Buzatto and Machado 2014). The comparability of these

observations with ours raises the possibility that the repeated convergent evolution of a very similar trait may involve shared developmental mechanisms. As an example, in *Onthophagus* Latreille, 1802 beetles, the expression of sexually dimorphic horns of males involves a developmental switch, which allows for a modularity in the emergence of this trait and facilitates the convergent evolution of horns in several species of the genus (Emlen *et al.* 2005). By contrast, the physiology and developmental genetics of sexually dimorphic traits in arachnids remain mostly unexplored, save for studies in the spider *Oedothorax gibbosus* that suggest a strong genetic component behind the occurrence of a male morph with a sexually dimorphic prosomal gland (Maelfait *et al.* 1990; Vanacker *et al.* 2001). A further interesting aspect of basitarsal glands of some zalmoxid species is the reduced number of tarsomeres in males (Fig. 12). This condition could result from either the deletion of one tarsomere or fusion of two adjacent tarsomeres. In the model fruit fly *Drosophila melanogaster*, the subdivision of the tarsal segment into five tarsomeres is under the control of Epidermal Growth Factor Receptor signalling (Campbell 2002; Galindo *et al.* 2002; Kojima 2004), but the role of this signalling pathway in tarsal patterning has not been investigated in any chelicerate to date. Unfortunately, there are also no studies on the postembryonic development of zalmoxids, which could prove useful in determining at which moment in ontogeny the tarsal modification in males occur.

Convergent evolution of specialised structures with deep homology is often difficult to explain using mathematical models, and there is always the alternative, non-parsimonious solution, of multiple losses of such a character. In the case of sexually dimorphic glands, repeated losses of such characters are plausible, as the glands and associated cuticular modifications are often absent in females and juveniles, and thus could result from the early truncation of a developmental process (neoteny). This avenue of research has received little attention (see Rambla 1980), and zalmoxids could serve as a model taxon to study this phenomenon.

An interesting hypothesis to be explored is whether different types of sexual dimorphism (armature versus glands) are related to alternative sexual strategies or mating systems. *Zalmoxis bendis* and *Zalmoxis bilbo*, sp. nov. bear sexually dimorphic glands and display little or no armature dimorphism in leg IV (the same in *Z. similis* Suzuki, 1982), whereas several species display dimorphic armature on leg IV and no sexually dimorphic glands. Nonetheless, at least one species, *Zalmoxis crassitarsis*, exhibits both putative basitarsal glands on legs III and IV, and sexually dimorphic armature on leg IV, demonstrating that both conditions are not mutually exclusive. Furthermore, preliminary data in other family (Gonyleptidae) also shows species with glands and armature, suggesting no correlation between presence of sexually dimorphic glands and male armature (T. M. Costa and R. H. Willemart, unpubl. data). Attempts have been made to understand the function of these glands in laboratory behavioural tests (Dias and Willemart 2016) but we still need more behavioural studies.

On the swollen parts of the legs, zalmoxids also bear atypical setae, which have a channel that seem to allow the

flow of secretions. Analogous setae on the metatarsus III of harvestmen in the family Biantidae have been hypothesised to serve as ‘paint-brushes’ (Alegre *et al.* 2019), and a similar mechanism could occur in Zalmoxidae. The presence of associated setae, the variation in the number of swollen tarsomeres and in which legs they are present pose interesting mechanistic and ultimate questions on the release mechanisms of chemicals, as well as how or why glands are used. Our survey underscores a yet understudied facet of sexual dimorphism in Opiliones and highlights Zalmoxidae as an interesting group for the study of sexual selection and the evolution of sexually dimorphic traits.

Conflicts of interest

Gonzalo Giribet is the Editor-in-Chief and Prashant P. Sharma is an Associate Editor for *Invertebrate Systematics*. Despite these relationships, they did not at any stage have Editor-level access to this manuscript while in peer review, as is the standard practice when handling manuscripts submitted by an editor to this journal. *Invertebrate Systematics* encourages its editors to publish in the journal and they are kept totally separate from the decision-making process for their manuscripts. The authors have no further conflicts of interest to declare.

Declaration of funding

This research was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) 2013/23189-1 and 2015/01815-9, NSF Grant DEB-1144492: ‘Collaborative Research: ARTS: Taxonomy and systematics of selected Neotropical clades of arachnids’ and from internal funds from the Museum of Comparative Zoology.

Acknowledgements

Caitlin M. Baker assisted with access to MCZ specimens. R. M. Clouse, C. Rahmadi, C. Rabeling, and E. O. Wilson collected specimens of the newly described species. Gustavo Hormiga and one anonymous reviewer provided suggestions that improved an earlier draft of this manuscript.

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Handling editor: Shane Ahyong