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Two new oribatid mites from the Republic of Rwanda. *Plasmobates zarae* sp. n. (Acari, Plasmobatidae) and *Basilobelba spasmenosi* sp. n. (Acari, Basilobelbidae)

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Abstract

Two new species of oribatid mites, *Plasmobates zarae* **sp. n.** and *Basilobelba spasmenosi* **sp. n.** are described from the Republic of Rwanda. They can easily be differentiated from other species by a number of characters.

Plasmobates zarae **sp. n.** is differentiated the following characters. four types of particular cerotegumental layers. Integument slightly foveate to smooth on prodorsum; foveate on notogaster; ventral region rugose to smooth.Large rostral setae inserted on protuberance, whip-shaped, with longitudinal pucker; interlamellar setae rod-shaped with triangular scales; interlamellar setae small. Medial band on prodorsum extending to anterior of central part, but not reaching rostrum. Bothridium horn-shaped; opening basally incised with rectilinear wall, internal bothridial rings dentate. Sensillus whip-like, with minute triangular scales. Variably distributed circumgastric macropores. Opisthosomal gland apophysis flat, triangular in lateral view and cylindrical in posterolateral view. Six pairs of notogastral setae, all situated posterior to opisthosomal gland level. Aggenital setae not detected; three pairs of adanal setae; two pairs of anal setae present. Nymphal scalps simple without anterior tuft or filaments, with dentate peripheral ridge. Larval scalp shaped like Chinese hat.

Basilobelba spasmenosi **sp. n.** is characterized by the combination of the following characters: Cerotegument: thick basal layer with amorphous coat and cavities of different sizes, as well as structures resembling small cauliflowers. Setation: *simple*: notogastral, epimeral, genital, anal; *simple long*, *basally barbate*: *le*, *ro* setae; *simple*, *whip-shaped*: *ex* setae; *medium length*, *sharpened tip with thorns on surface*: *in* setae, leg setae; *Flabellate*: setae situated in ventral neotrichous zone. Thorn-like barbs and more or less parallel longitudinal grooves present on body surface of *le*, *ro*, *in* and leg setae. Prodorsum: rostrum finger-shaped, relative sizes of setae: *le* > *ro* > *in* > *ex*. Prodorsal cuticular surface smooth with shallow transversal furrow and two oblique furrows determining two triangular structures. Large humpbacked *CSO* situated anterior to and in medial line with *in* setal insertion, dorsal bothridial opening. Notogaster swollen, hemispheric; nine pairs of minute setae, only h_1, h_2, *h*3 easily identifiable, cuticular wart and dimple clearly visible. Humeral apophysis with longitudinal furrow dorsally. Elongate chelicera with *cha*, *chb* setae, behind them a series of scales directed dorsoventrally. Epimeral setation 3-1-3-3, adanal-aggenital neotrichy with between 8-10 setae. Nymphal scalps with very particular bean-shaped structure on either side of the decoupage zone, surrounding horn-like structure. Scalps with cuticular polyhedral reticulate to ovoid structure, often forming a cavity, either completely perforated or with a thin cuticular layer resembling an interior membrane.

Keywords
Republic of Rwanda, *Plasmobates zarae* sp. n., *Basilobelba spasmenosi* sp. n., Afrotropic Ecozone

Introduction

This paper is the second on material collected in Rwanda, housed at the Natural History Museum in Geneva, Switzerland. Two new species, *Plasmobates zarae* sp. n. and *Basilobelba spasmenosi* sp. n. are described. At present the family Plasmobatidae consists of four genera: *Orbiculobates* Grandjean, 1961; *Malgachebates* Fernández, Cleva, Theron, 2011; *Plasmobates* Grandjean, 1929 and *Solenozetes* Grandjean, 1931. Over the course of many years the authors have studied members of the family Plasmobatidae, principally those collected from the Afrotropic ecozone (formerly known as Ethiopian zone). This resulted in the description of the genus *Malgachebates* and included a summary of the principal characteristics of each genus of the family (Fernandez et al. 2011). Fernandez et al. (2013) analyzed some problematic aspects of the genus *Solenozetes* and presented a redefinition of the genus, as well as the description of *S. makokouensis* Fernandez et al., 2013.

The taxonomy of the family Plasmobatidae is problematic due to succinct original descriptions lacking in detail, or in which important characteristics were neglected.

Plasmobates (*sensu* Subías 2015) consists of the following species: *P. pagoda* Grandjean, 1929, *P. carboneli* Pérez-Íñigo & Sarasola, 1998, *P. hyalinus* Hammer, 1971, *P. asiaticus* Aoki, 1973, *P. africanus* Balogh, 1958 "sp. inq.", *P. foveolatus* Ermilov, Sidorchuk & Rybalov, 2010, *P. machadoi* Balogh, 1958 "sp. inq.", *P. minor* Balogh, 1958 "sp. inq.". The last four species are from the Afrotropic ecozone, and three of the four species are "species inquerendae" (*sensu* Subías 2015) (see Discussion). We continue the study of this group by providing a description of *Plasmobates zarae* sp. n. Despite more than forty years in alcohol, material was in an excellent state of preservation, conserved to the point that adequate SEM studies could be conducted.

The family Basilobelbidae contains two genera: *Basilobelba* Balogh, 1958 and *Xiphobelba* Csiszár, 1961. The taxonomy of Basilobelbidae is not clear, principally relating to the problematic original description of *Xiphobelba* (Csiszar 1961 page: 353) which indicates: “Rostrum pointed, chelicerae attenuated to a point, chela very small reduced” and “The new genus is an ally of *Basilobelba* Bal.1958, differing from it by the peculiar oral organs, resembling those of *Eupelops*”. The oral organs were not illustrated, and other characteristics, such as the cerotegumental layer were neither described nor figured. Only two figures were given, one dorsal with scalps and the other dorsal without scalps. In the description of a second species of the genus, *X. setosa* Aoki, 1968, the chelicera are partly illustrated with the rest of the infracapitulum (Aoki 1968 p: 271, figure 14). Due to several subsequent papers, the taxonomy of *Basilobelba* is becoming clearer, permitting understanding of several aspects of this group.

More recently Fernandez et al. (2015) described a new species of *Basilobelba* (*B. maidililae*) from Vietnam, analysed problematic aspects of the group, and provided a comparison of species of both genera of the family.

Materials and methods

Specimens studied by means of light microscopy were macerated in lactic acid, and observed in the same medium using the open-mount technique (cavity slide and cover slip) as described by Grandjean (1949) and Krantz and Walter (2009). Drawings were made using a Zeiss GFL (Germany) compound microscope equipped with a drawing tube.

Specimens were also studied under a Scanning Electron Microscope (SEM). Specimens preserved in ethanol were carefully rinsed by sucking them several times into a Pasteur pipette, after which they were transferred to buffered glutaraldehyde (2.5%) in Sörensen phosphate buffer: pH 7.4; 0.1 m for two hours. After postfixation for 2hr. in buffered 2% OsO_4 solution and being rinsed in buffer solution; all specimens were dehydrated in a series of graded ethanol and dried in a critical point apparatus. After mounting on Al-stubs with double sided sticky tape, specimens were gold coated in a sputter apparatus (Alberti and Fernandez 1988, 1990a, 1990b; Alberti et al. 1991, 1997, 2007; Fernandez et al. 1991). SEM micrographs were taken using a SEM FEI-Quanta Feg 250; with 10 Kv and working distance (WD) variable.

Measurements: total length (tip of rostrum to posterior edge of notogaster); width (widest part of notogaster) in micrometers (μm). Leg setation was studied using standard, polarized and phase contrast microscopes are provisional, due to the fact that only adult specimens were available for study. Setal formulae of the legs include the number of solenidia (in parentheses); tarsal setal formulae include the famulus (ε). For *Plasmobates zarae* we added SEM images of leg setae as detail in order to clarify a number of particularities.

Morphological terminology

Morphological terms and abbreviations used herein are those developed by F. Grandjean (1928–1974) (cf. Travé and Vachon 1975). For the setae types Evans (1992);

ornamentation of cuticular surfaces Murley (1951, *ex*: Evans *op.cit*) were used. Some specific morphological characters have never been described before in detail, and hence no terminology or abbreviations exist. For the sake of clarity we include the following in the text and on the figures: bean-shaped structure (*h.sc*); macropores (*mp*); medial band extension (*m.b*); polyhedral reticulate to ovoid structure (*s.r.s*); promontories of podocephalic canal (*a.o.g*); thin cuticular layer (*t.c.l*).

New taxa description

Plasmobates zarae sp. n.

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Figures 1–42, Table 1

Etymology. The specific epithet "*zarae*" is derived from (ζάϱα, Grec=pucker, English) due to longitudinal pucker present on *ro* setae.

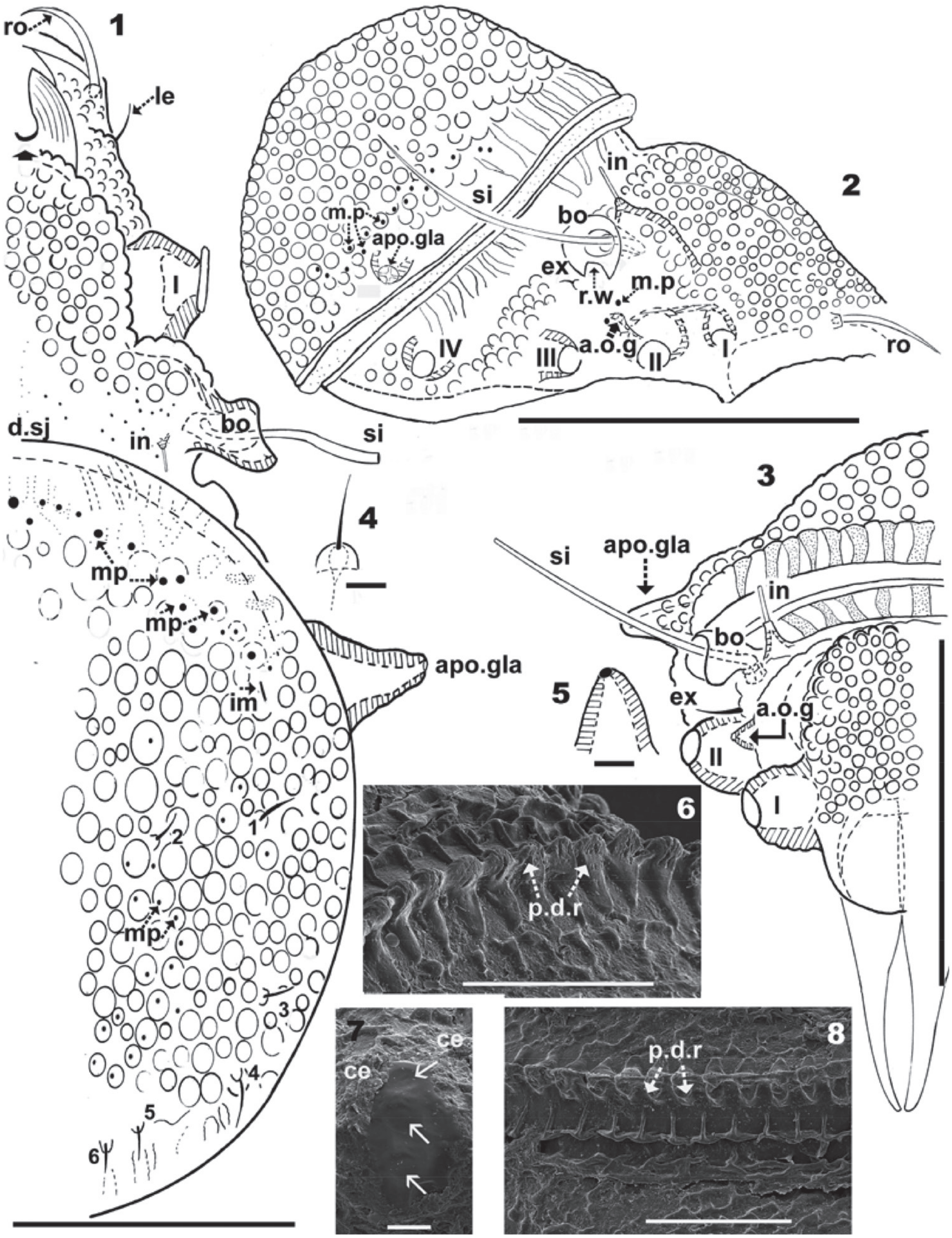
Material examined. Holotype: Female and two paratypes (adult females): "73/2. Kayove-Rwanda; 2100 mts. 15/V/1973" Leg. P.Werner; deposited in the Collection of the Natural History Museum of Geneva (M.H.N.G), Switzerland; preserved in 70% ethanol. Material studied for SEM: three specimens, not deposited.

Diagnosis (adult female). Cerotegumental layer. *Amorphous*: bothridial zone, tubercle of seta *in*, *ro* setae insertion, lateral gland, epimeral zone, genital plate and surrounding zone, anal plate and surrounding zone. *Layer with small tubercles*: internal bothridial zone. *Mixed-layer* (mushroom-like microtubercles associated with irregular cauliflower-like microtubercles): infracapitulum, epimeral zone, lateral body zone, basal zone lateral gland. Integument: prodorsum, small foveate to smooth; notogaster, foveate; ventral region rugose to smooth.

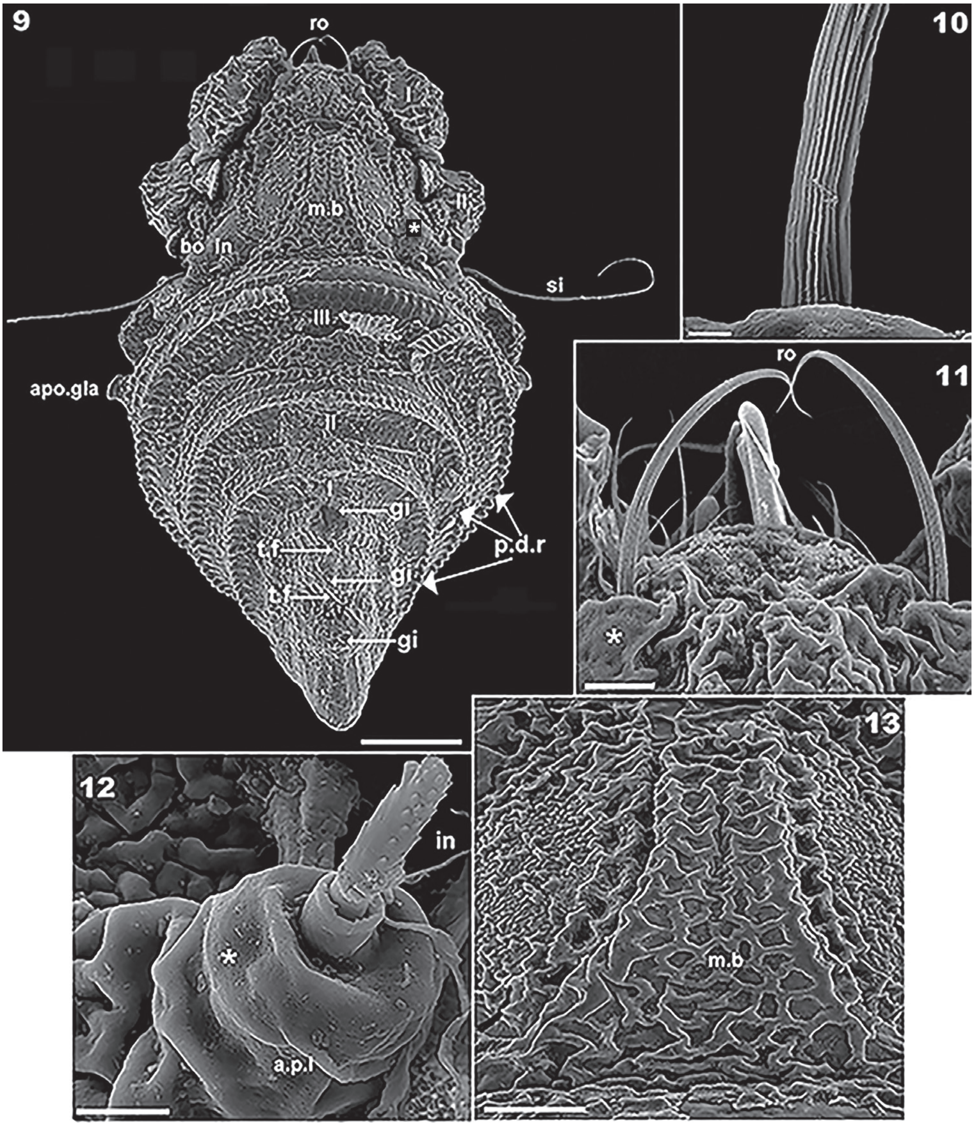
Setation: *simple*: lamellar, notogastral, exostigmatal, epimeral, genital, aggenital anal; *whip-shaped, with longitudinal pucker*: rostral setae; *rod-shaped with triangular scales*: interlamellar setae; *simple, basally inflated*: subcapitular *a*; *simple spur*: *m*.

Prodorsum: medial band extension on central part towards anterior, not extending to rostrum. Interlamellar setae inserted on large protuberances, lamellar setae small, rostral setae large, with longitudinal cuticular puckers, inserted on protuberances. Large horn-shaped bothridium, directing laterally, rectilinear wall with basally incised opening. Internal bothridial rings dentate. Whip-shaped sensillus with minute, triangular scales; exostigmatal seta small. Rostrum medially incised, posterior of incision rounded. Notogaster: fovea situated in smooth zone with circumgastrically distributed macropores on fovea margins or inside fovea. Opisthosomal gland apophysis flat, triangular in lateral view, cylindrical in posterolateral view. Six pairs of notogastral setae, all situated posterior to level of opistosomal gland. Lateral region: opening of podocephalic canal on large promontories.

Ventral region: epimeral setal formula (3-1-2-2). Seven pairs of genital setae; aggenital setae not detected. Three pairs of adanal setae, two pairs of anal setae. Scalps



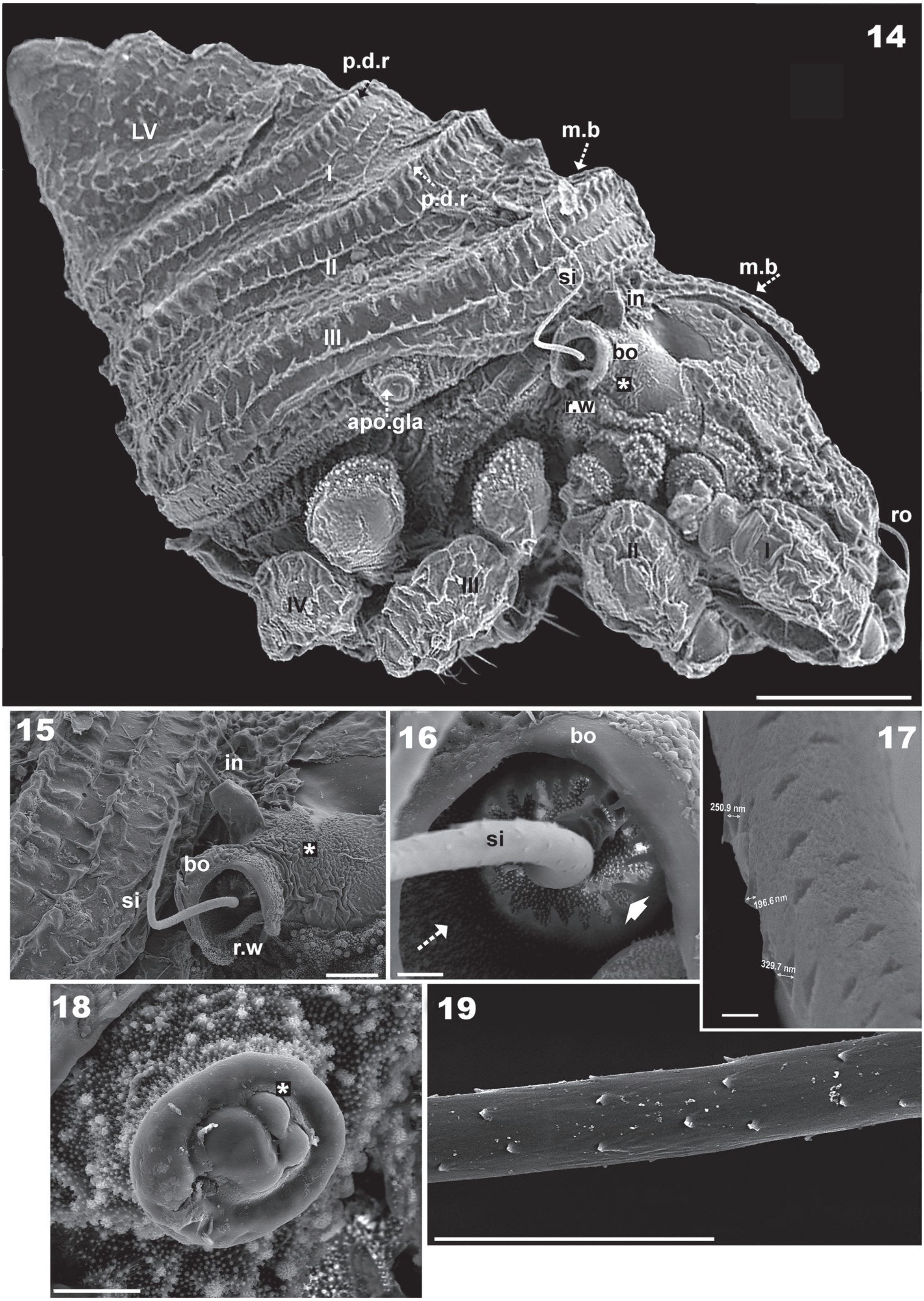
Figures 1–8. *Plasmobates zarae* sp. n. Adult; **1–5** optical microscopy image **6–8** SEM **1** dorsal view **2** lateral view **3** frontal view **4** lamellar setae **5** promontories podocephalic canal **6** dentate peripheral ridge (*p.d.r*), lateral inclined view **7** cerotegumental layer and cuticular microsculpture **8** dentate peripheral ridge (*p.d.r*), frontal view. Abbreviations: see Materials and methods. Scale: **1** = 100 μm; **2** = 200 μm; **3** = 70 μm; **8** = 50 μm; **6** = 30 μm; **7** =10 μm; **4, 5** = 5 μm.



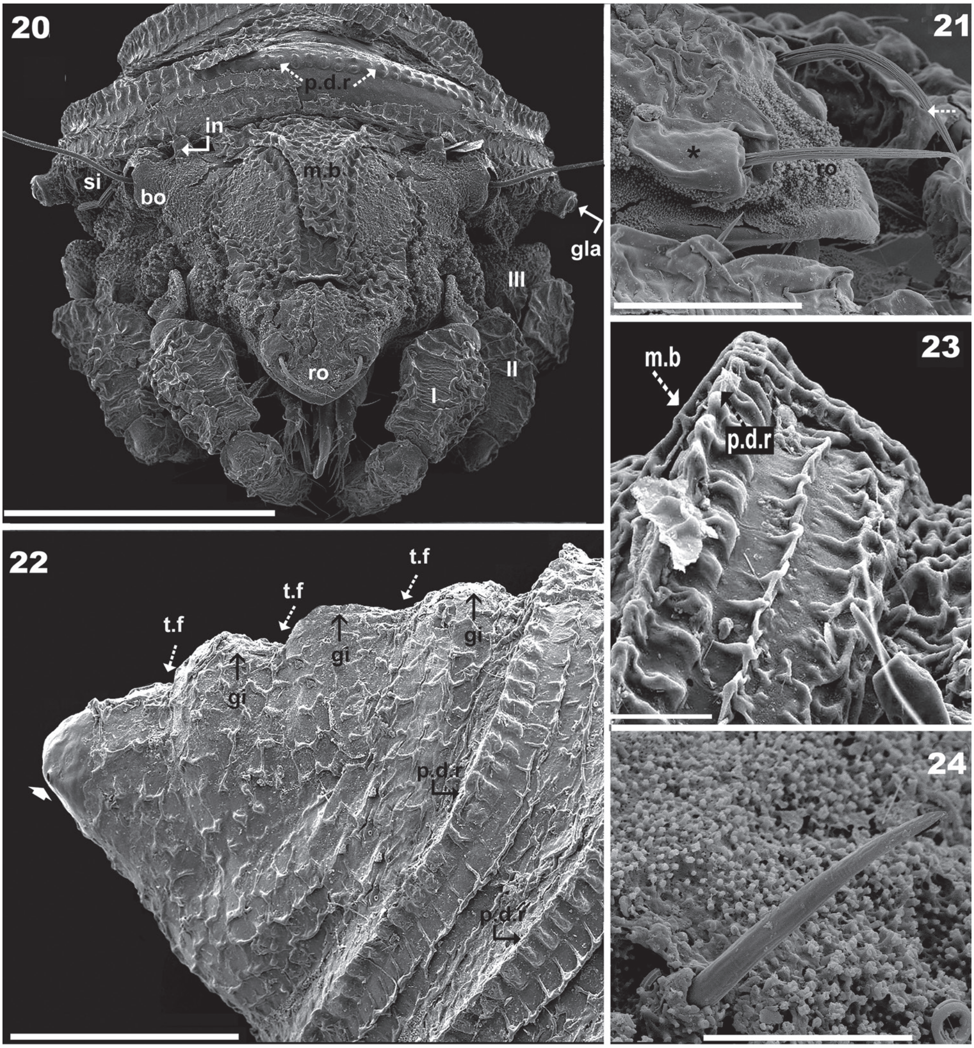
Figures 9–13. *Plasmobates zarae* sp. n. Adult; SEM micrographs. **9** dorsal view with scalp and cerotegument layer **10** rostral setae, detail **11** rostral setae, general view **12** interlamellar seta **13** medial band detail, dorsal view. Abbreviations: see Materials and methods. Scale: **9** = 100 µm; **11, 13** = 20 µm; **12** = 5 µm; **10** = 2 µm.

multilayered, medial band extending anteriorly from each scalp. Medial band covers central zone, firmly adhered to prodorsal surface. Nymphal scalps with dentate peripheral ridge. Setae hardly discernible, scalps simple without anterior tuft of filaments. Chinese hat-shaped larval scalp differing greatly from nymphal scalps

Description. *Measurements.* SEM: total length with scalps 580–615 µm × 600 µm (measurements on three specimens). Total length without scalps 433–438 µm × 435µm (measurements on three specimens). Notogastral width without scalps 248–



Figures 14–19. *Plasmobates zarae* sp. n. Adult; SEM. **14** lateral view with scalp and cerotegumental layer **15** bothridium and interlamellar seta, lateral view **16** bothridium, internal structures **17** sensillus, superficial scales (high magnification) **18** lateral gland **19** sensillus detail. Abbreviations: See Materials and methods. Scale: **14** = 100 µm; **15** = 20 µm; **18** = 10 µm; **16, 19** = 5 µm; **17** = 1 µm.



Figures 20–24. *Plasmobates zarae* sp. n. Adult; SEM. **20** frontal view **21** *ro* seta **22** tritonymphal scalp with medial bands, lateral view **23** larval scalp detail **24** *ag* seta detail. Abbreviations: see Materials and methods. Scale: **20** = 200 µm; **22** = 20 µm; **23** = 50 µm; **21** = 10 µm; **24** = 5 µm.

253 µm × 250 µm. Light microscopy: 612–656 µm × 639 µm (measurements on three specimens). Specimens with scalps ovoid, elongate in dorsal view. (Figures 1, 9, 22). In lateral view specimens with scalps appear pyramidal (Figure 14); without scalps anterior triangular and posterior rounded (Figure 2).

Colour. Specimens without cerotegument and scalps dark yellowish to medium brown.

Cerotegument (scalps not considered). Thick complex layer with elaborate pattern, composed of wax layer and amorphous cement layer covering entire body and legs. *Amorphous layer* (Figure 33, indicated in all Figures with *): external bothridial zone of prodorsum (Figures 14, 15), tubercles of *in* setae (Figure 12), *ro* setae insertion zone

(Figure 21), *gla* (Figure 18), epimeral zone (Figure 25) subcapitular setae *h* (Figure 26), genital plate and surrounding zone, anal plate and surrounding zone (Figure 32). *Small tubercules*: internal bothridial zone (Figure 16 indicated by5). *Mixed-layer* (Figures 28, 29, 30): mushroom-like microtubercles (*mus*) diameter 0.02–0.6 µm, height 0.2–1.9 µm associated with irregular cauliflower-like microtubercles (*cau*) diameter 1.2–1.9 µm, height 1.4–3.1 µm. Distribution: infracapitulum, epimeral zone, lateral body zone, basal zone of *gla* (Figures 18, 19, 25, 31). Legs: Trochanters covered by *mixed-layer*, femur, genu, tibia, covered by *amorphous layer* with prominent folds. Tarsus: *amorphous layer* with subtle folding and several smooth areas (Figures 14, 20, 35, 36, 38, 39, 42).

Integument. lateral microsculpture of prodorsum faintly foveate to smooth (Figures 1, 2, 3), dorsal microsculpture of prodorsum flat, foveate (Figure 7 indicated by h). Notogaster: foveate in vicinity of notogastral border (Figures 1, 2, 3), posterior notogastral zone smooth, posterolateral notogastral zone presenting ridges anterior to macropore zone (Figures 1, 2, 3). Distribution of macropores circumgastric (Figures 1, 2). Ventral region rugose to smooth on subcapitulum (Figure 25), epimeral zone, surrounding genital and anal openings and genital and anal plates.

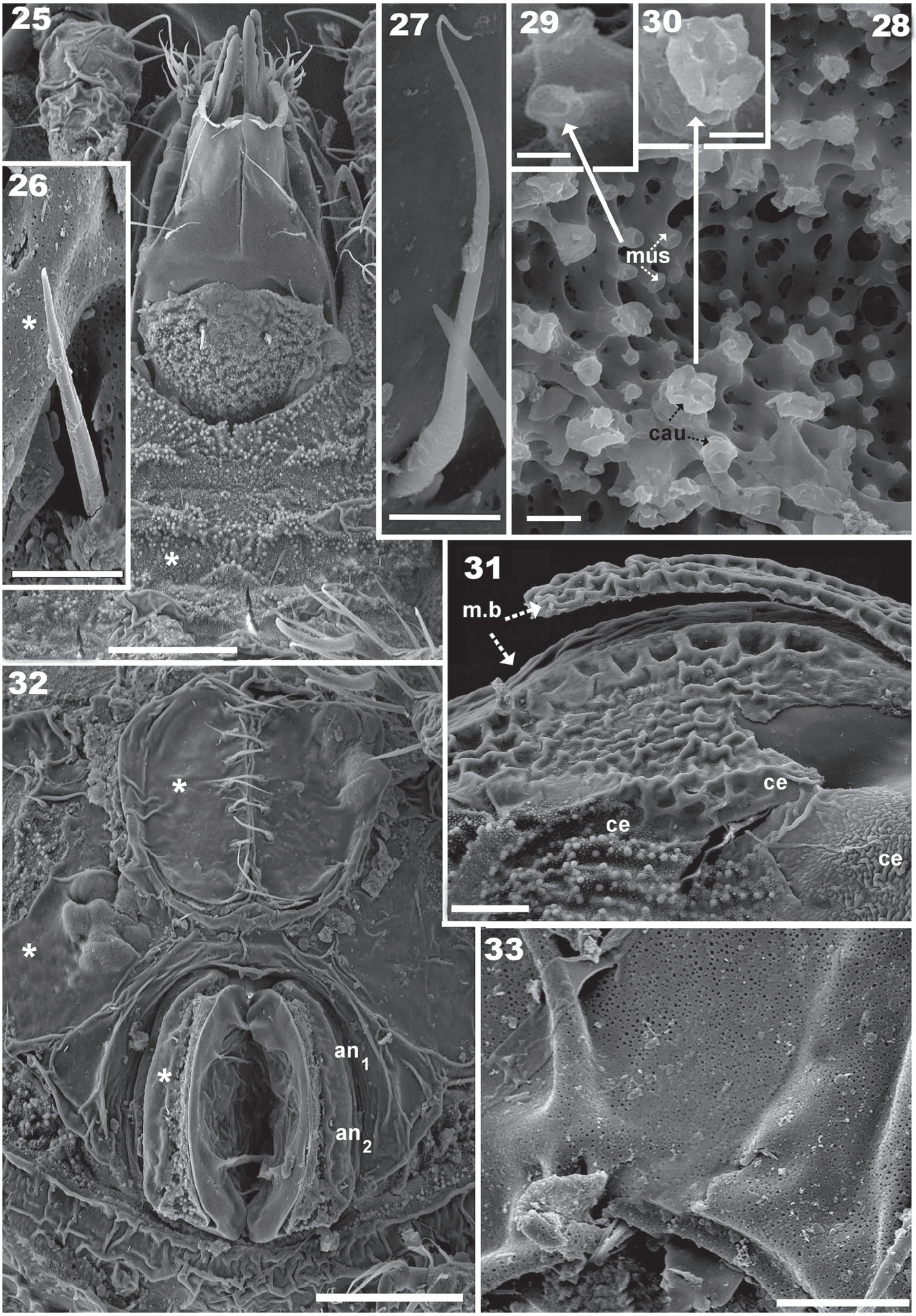
Setation. Lamellar (Figure 4), notogastral, exostigmatal, epimeric, genital, aggenital (Figure 24) and anal setae simple; *ro* setae whip-shaped, with longitudinal pucker (Figures 9, 10, 11); *in* setae (10–13 µm) rod-shaped with triangular scales (Figure 12); subcapitular setae simple, slightly basally inflated (Figure 27) 30–36 µm *a*; *h* simple, spur-shaped (Figure 26) 16–21 µm.

Prodorsum. Medial band extension (*m.b*) observed on central part towards anterior, not extending to rostrum, terminating anterior to *le* setal insertion level on specimens with scalps (Figures 9, 13, 14, 20, 31). Elevated zone surrounding medial band extension (Figure 14). Interlamellar setae (*in*) inserted near bothridial base on large protuberances, extending upward and inclined backward (Figures 9, 12, 14, 15). Lamellar setae (*le*) small, inserted on small protuberances (Figure 4), rostral setae (*ro*) (58-61 µm) inserted on protuberances, cuticular folds at base of setae (Figures 10, 11, 14, 20, 21). Large laterally directing horn-shaped bothridium (Figures 14, 20). Semicircular lateral bothridial opening, basally incised with thin rectilinear wall (*r.w*) (Figures 14, 15). Internal bothridial rings dentate with triangular teeth (Figure 16).

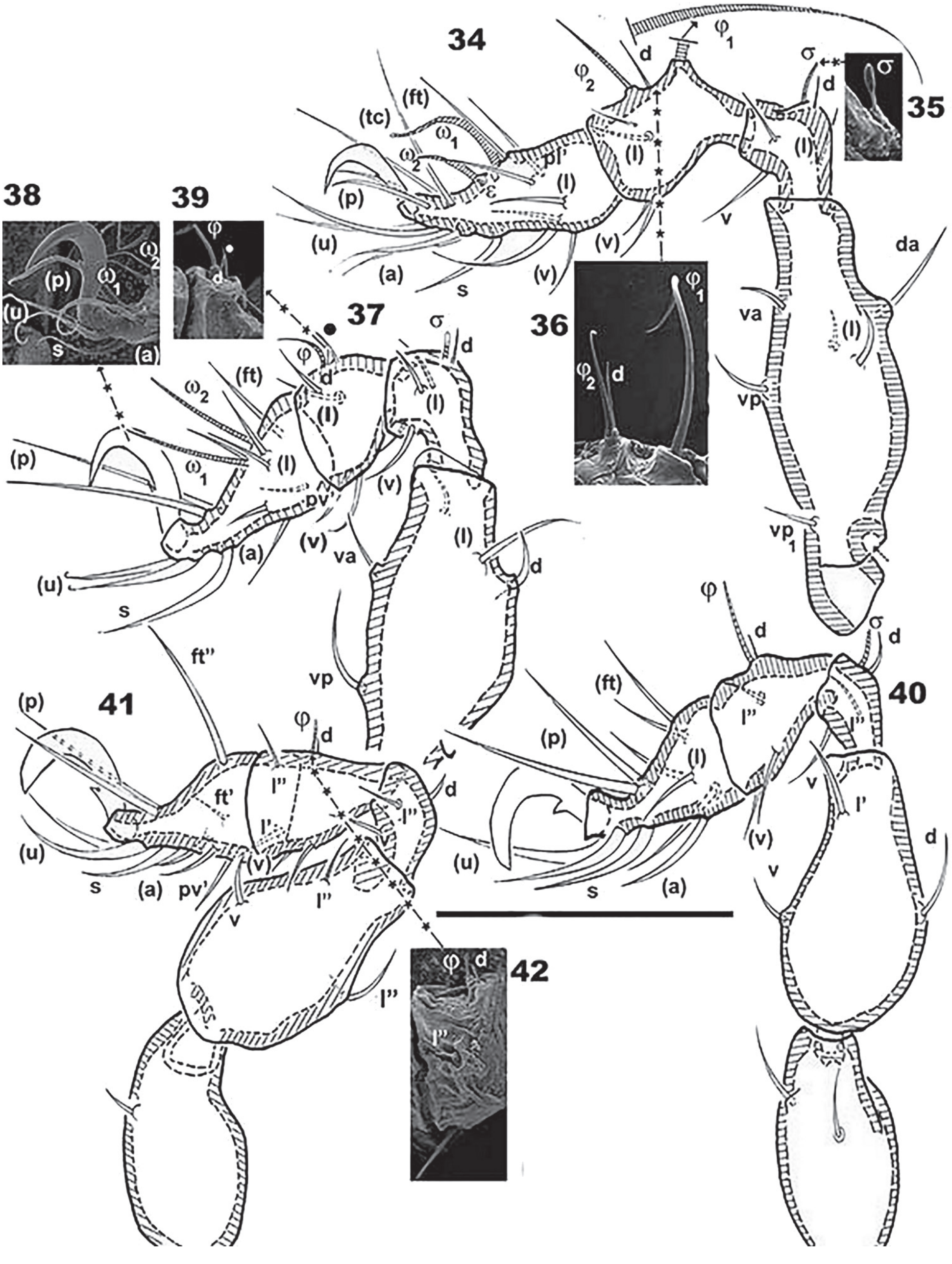
Whip-shaped filiform sensillus (*si*) (80-106 µm) with minute triangular scales, height 196 nm, length 603-987 nm (Figure 17), exostigmatal setae (*ex*) small. Narrow medial incision on rostrum, in dorsal view posterior end of incision rounded (Figure 1).

Notogaster. Circumgastrically distributed macropores (*mp*) of varying diameter (0.3–1 µm) situated in small foveae on smooth zone, either on periphery or internally to foveated notogastral pattern (Figures 1, 2). In dorsal view anterior zone *mp* clearly visible (Figure 1), but those located near *gla* need to be observed in lateral or posterior views (Figure 2) due to notogastral shape, in order to obtain the best impression of their distribution.

Distribution of *mp*: a) single line in anterior notogastral zone; b) linear in anterior lateral zone near *gla*; c) irregularly distributed on posterior notogastral zone (setal zone) (Figure 1).



Figures 25–33. *Plasmobates zarae* sp. n. Adult; SEM. **25** epimeral zone **26** subcapitular seta *h* **27** subcapitular seta *a* **28** cerotegumental layer **29** detail cerotegumental "cauliflower" (*cau*) **30** detail cerotegumental "mushroom" (*mus*) **31** prodorsum with *m.b*. **32** anogenital region **33** detail cerotegumental layer. Abbreviations: see Materials and methods. Scale: **25** = 50 µm; **26** = 5 µm; **27** = 5 µm; **28** = 1 µm; **29** = 0.5 µm; **30** = 0.5 µm; **31** = 20 µm; **32** = 50 µm; **33** = 5 µm.



Figures 34–42. *Plasmobates zarae* sp. n. Adult; Optical and SEM observations. **34** leg I, antiaxial **35** detail with SEM; solenidium s genu I **36** detail with SEM, solenidia j_1, j_2 and setae *d*, femur I **37** leg II, antiaxial **38** apical zone, tarsus II (detail with SEM) **39** solenidium j_1, dorsal setae *d* and complementary seta indicated byl, tibia I (detail SEM observation) **40** leg III, antiaxial **41** leg IV, antiaxial **42** tibia I, solenidium j and setae *d* (detail SEM observation). Abbreviations: see Materials and methods. Scale: **34–42** = 70 µm.

In dorsal view opisthosomal gland (*gla*) apophysis observed as flat triangle, but appears cylindrical in lateral and lateroposterior views, directing slightly obliquely forward (Figures 1, 9, 14, 18); opening with protuberances (Figure 18). Six notogastral setae on small protuberances (Figure 1), all setae situated behind level of *apo.gla*.

Lateral region. Exobothridial seta (*ex*) small but clearly discernible (Figure 3); two macropores situated one above and one below promontories of podocephalic canal (Figure 2 surrounding *a.o.g*). Opening of podocephalic canal on large promontories (Figures 2, 3, 5). Tubercles of interlamellar setae more or less cylindrical (Figures 12, 15); setae *ro* inserted on tubercles (Figures 12, 14); notogastral border clearly discernible even after long preservation in lactic acid; sejugal depression deep, easily discernible.

Ventral region. Specimens with cerotegument: plate-like cerotegumental structures on epimeres resulting in irregular levels on upper surface, epimeral furrows easily discernible with *mus*, *cau* and amorphous cerotegumental layer (Figure 25). Flat lateral cerotegumental zones, deep epimeral furrows 1 and 2. Epimeres III, IV small, epimeral setal formula (3-1-2-2). Seven pairs of genital setae in a single longitudinal line; aggenital setae not detected; three pairs of adanal setae; two pairs of anal setae.

Gnathosoma. Subcapitulum suctorial with short tube. Subcapitular setae large, especially *a*, *m* (Figure 25).

Legs (Table 1). Legs differ from those of congeners studied by the authors (See Table 1), particularities illustrated on SEM micrographs (Figures 34–42). Setal and solenidial formulae (trochanter to tarsus): I(1-6-4-5-19-1) (1-2-2); II(1-4-5-6-12-1) (1-1-2); III(2-3-3-4-11-1) (1-1-0); IV(1-3-4-5-10-1) (0-1-0).

Scalps. Exuviae of immature stases adhering one on top of the other, creating a multilayered structure. Each scalp extending anteriorly into a medial band (*m.b*) (Figures 14, 20, 23, 31) covering central zone, adhering to prodorsal surface (Figure 20) and extending backward towards *ro* setal insertion (Figure 20). Sometimes *m.b* is slightly detached (Figure 14).

Cerotegumental layer: medial band covered by thick amorphous layer with a network of round to polygonal depressions (Figure 13). Nymphal scalps with dentate peripheral ridge (*p.d.r*) (Figures 6, 8, 14, 20, 22, 23). Setae hardly discernible, scalps simple without anterior tuft of filaments. Larval scalp unlike the others, broad and elevated, Chinese hat-shaped, with three gibbose areas (*gi*) separated by transverse furrows (*t.f*) (Figure 22). In lateral view insertion of *dp* setae clearly visible (Indicated by Figure 22).

Remarks. SEM is vital in order to observe aspects such as: 1) dorsal seta *d* associated with solenidium hardly discernible (detailed drawings are included to facilitate understanding) 2) clavate shape of small solenidia is problematic, as doubt regarding the exact shape remains if using only optical microscopy 3) the position of dorsal seta *d* relative to j_1 and j_2 on tibia II differs from *Solenozetes makokouensis* and *Malgachebates peyrierasi*. Changed angles of observation and rotation of specimens in SEM clarified the situation. 4) accessories available in SEM facilitated measurements of minute triangular scales of the sensillus with great precision 5) protuberances situated around the opening of the lateral gland also had to be observed from different angles. See Discussion for comparison with congeners.

Table 1. *Plasmobates zarae* sp. n. adult, legs.

	Femur	Genu	Tibia	Tarsus	Observations
Leg I					
Setae	*da*,(*l*),*va*,*vp*, vp_1	(*l*),*v*,*d*	*d*,(*l*),(*v*)	*pl'*,(*l*),(*v*),(*ft*),(*tc*),(*p*), (*u*),(*a*),*S*,(*v*),e	Crispinate (socket-like) dorsal femur, solenidium s clavate, *d* seta positioned near j_2, usually near j_1
Solenidium	-	s	j_1, j_2	w_1, w_2	
Leg II					
Setae	*l"*,*d*,*va*,*vp*	*d*,(*l*),(*v*)	*d*,l,(*l*)(*v*)	(*ft*),(*l*),(*p*)(*u*),*S*,(*a*),*pv'*	crispinate (socket-like) dorsal femur, solenidium s clavate, genu with one *d* seta near j, also another associated minute seta indicated by l
Solenidium		s	j	w_1, w_2	
Leg III					
Setae	*d*,*l'*,*v*,	*d*,*l"*, *v*	*d*,*l"*,(*v*)	(*ft*),(*p*),(*u*),*S*,(*a*),(*l*)	
Solenidion		s	j	-----------------	
Leg IV					
Setae	(*l*),*v*	*d*,*l"*,*v*	*d*,(*l*),(*l*)	(*ft*),*pv'*,(*a*),*S*,(*u*),(*p*)	
Solenidion		---------	j	---------------------	

Basilobelba spasmenosi sp. n.

http://zoobank.org/F4ABFDAF-99F0-45DB-876C-DD914C7852A7

Figures 43–74; Table 2

Etymology. The specific epithet "*spasmenosi*" is derived from (Σπασμένος, Grec = broken, English), due to characteristics of scalps with cavities or perforations.

Material examined. Holotype: Female and two paratypes (adult females):. "73/2. Kayove-Rwanda; 2100 mts.15/V/1973" Leg. P.Werner; material deposited in the Collection of the Natural History Museum of Geneva (M.H.N.G), Switzerland; preserved in 70% ethanol. Material studied for SEM: three specimens, not deposited.

Diagnosis (adult female). Cerotegument. Thick basal layer with amorphous coat, perforations of various sizes, and structures resembling small cauliflowers. Setation. *Simple*: notogastral, epimeral, genital, anal; *simple*, *long*, *basal barbs*: *le*, *ro* setae; *simple*, *whip-shaped*: *ex* setae; *medium length*, *sharpened tip with thorn-like barbs on surface*: *in* setae, leg setae; *flabellate*: setae situated in ventral neotrichy zone. Thorn-like barbs and more or less parallel longitudinal grooves on body of *le*, *ro*, *in* and leg setae. Prodorsum. Rostrum finger-shaped, relative sizes of setae: *le* > *ro* > *in* > *ex*. Prodorsal cuticular surface smooth, with shallow transversal furrow and two oblique furrows delineating two triangular structures. Large humpbacked *CSO* situated anterior to and in medial line with *in* setal insertion, dorsal bothridial opening. Notogaster. Swollen, hemispheric, with nine pairs of minute setae. Only h_1, h_2, h_3 easily identifiable. Cuticular wart and dimple clearly visible. Humeral apophysis with longitudinal furrow dorsally. Chelicera elongate, series of dorsoventrally directing scales behind setae *cha*, *chb*. Epimeral setation 3-1-3-3, adanal-aggenital neotrichy with 8-10 setae. Nymphal Scalps. Particular bean-shaped structure on either side of the decoupage zone around horn-like structure. Scalps with polyhedral reticulate to ovoid cuticular structure. Polyhedral reticulate cuticular structure often appears either completely perforated or with a thin cuticular layer resembling an interior membrane.

Description. *Measurements.* SEM: total length without scalps 618–598 × 605 µm; width without scalps 310–290 × 303 µm (measurements on three specimens). Light microscopy: 660–632µm × 643 µm; width 325–315 × 320 µm (measurements on three specimens).

Shape. Elongated oval (Figures 43, 49, 53).

Colour. Specimens without cerotegument brown, slightly shiny when observed in reflected light.

Cerotegument. Present only on prodorsum, notogastral anterior zone, ventral region and legs. Thick basal layer with amorphous coat and perforations of various sizes (indicated by¿ Figure 47). Small structures on surface resembling cauliflowers (*cau*) of different sizes (Figures 47, 56, 59, 60).

Setation. Simple: notogastral, epimeral, genital, anal (Figures 50, 52, 54); *simple*, *long*, *basal barbs*: *le*, *ro* setae (Figures 48, 55); *simple*, *whip-shaped*: *ex* setae (Figures 43, 46); *medium length*, *sharpened tip with thorns on surface*: *in* setae (Figures 43, 44, 46, 58, 59, 61), leg setae. These setae are very particular, with large thorn-like barbs basally and small thorn-like barbs distally. *Flabellate* (Figure 56): setae situated in ventral neotrichous zone. Particular to *le*, *ro*, *in* and leg setae (Figure 60) is the presence of thorn-like barbs and more or less parallel longitudinal grooves on setal body (Figures 58, 59, 60).

Integument. Smooth

Prodorsum. Rostrum finger-shaped (Figures 43, 46). Rostral setae *ro* laterally inserted on large promontories 107 µm (96–109 µm); *le* setae 155 µm (151–159 µm); *in* setae on small promontories 46 µm (48–50 µm); *ex* 40 µm (38–42 µm). Relative sizes of setae: *le* > *ro* > *in* > *ex*.

Prodorsal cuticular surface smooth with a shallow transversal furrow situated anterior to *in* setal insertion (Figure 46 indicated by 5). Two oblique furrows (Figure 46 indicated by a) delimiting two triangular structures (Figure 46 indicated by *). Large humpbacked *CSO* situated in front of and in medial line with *in* setal insertion (Figures 43, 46, 53). More or less parallel *le* setae with criss-crossing tips (Figure 46). Two oblique furrows and two triangular structures conspicuous in dorsal view of prodorsum.

Ovoid bothridial opening dorsally (Figures 43, 48, 53); in medial zone ovoid loop directing anteriorly and slightly obliquely (Figure 61 indicated by¿). Sensillus long, setiform, generally directing backward, both sides barbate (Figure 61).

Frontal view. Beak-shaped rostral margin (Figure 57). Large humpbacked *CSO* clearly visible, slightly anterior to interlamelar setal insertion.

Notogaster. Swollen, hemispheric (Figure 53), bearing four-layered exuviae (larval, protonymphal, deutonymphal and tritonymphal) stacked to resemble a low tower (Figure 43), fixed anteriorly and posteriorly by particular structures (See Scalps). Dorsosejugal furrow large, rectilinear, well delimited (Figure 44). After removal of exuviae, glabrous notogastral surface becomes visible (Figure 53), bearing nine pairs of minute setae

(Figure 52). Only h_1, h_2, h_3 setae easily identifiable (h_2 and h_3 always in very close proximity to each other), h_1 setae identified by relative position to lyrifissures (See Discussion). Unidentified setae indicated by * (Figure 52). Lyrifissures *ih* and *ips* identified as pores, other lyrifissures probably present but difficult to identify due to ornamentation. Anterior notogastral zone (Figures 43, 44) bearing cuticular wart (*ve*) hooking arched tritonymphal buckle (*co.bu*) by coaptation. Depression in posterior zone (Figure 53, dimple (*f*) indicated by rounded dotted zone) housing the *us* zone of tritonymphal horn by coaptation. (Figure 69 indicated by *us* and ¿). SEM observations of this small depression is necessary from different angles, hence it is indicated by a rounded zone (See Scalps). Humeral apophysis easily discernible, with dorsal longitudinal furrow (Figure 43).

Lateral region. Only pedotectum I present (Figure 49); *Pd I* large lamina; the border can be followed a short distance to *ex* setae; *h.ap* clearly discernible as a structure with longitudinal furrow (Figure 43).

Ventral region. Subcapitulum diarthric, cerotegumental layer observed only behind *h* setal insertions (Figure 55). Subcapitular setae faintly barbate on either side, sharply tipped. Setae differing greatly in shape (see Figure 55): *a* (42 µm ± 3 µm) simple, sharply tipped; *m* (55 µm ± 3 µm); *h* (48µm ± 3 µm). Chelicera (Figure 51) elongate, with *cha*, *chb* setae. Series of dorsoventrally directing scales posterior to *cha*, *chb* setae; the largest is found dorsally, appears darker, followed by small transparent scales. Small movable digit (Figure 51) (see Discussion).

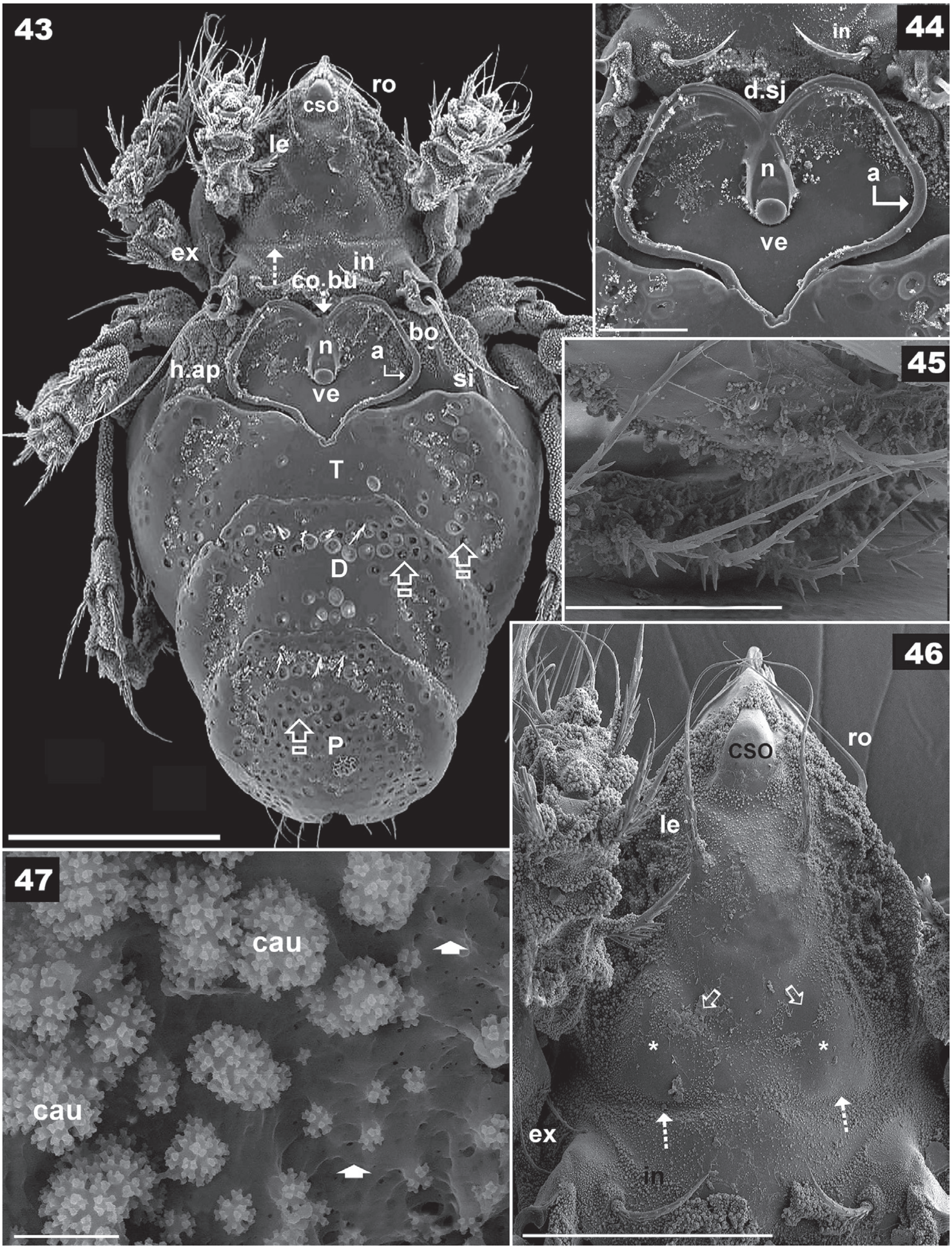
Epimeres I, II typical morphology, ventrosejugal furrow easily discernible, other epimeres not visible. Epimeral setation 3-1-3-3 (Figures 49, 54). Aggenital and adanal setae difficult to identify due to adanal-aggenital neotrichy. Neotrichy originates laterally to anal opening, is very prominent on posterior zone, number of setae varies between 8-10 (See Discussion).

Legs (Figures 70–74, Table 2). Leg shape similar to *Basilobelba retiarius* (Grandjean, 1959), moniliform with bulbous segments and large peduncles (Figures 70–74), femoral peduncles being largest. Tarsi particularly shaped, narrower between bulb and claw on legs I-IV. Cerotegumental layer covering segments but only basal zone of setae (Figure 60).

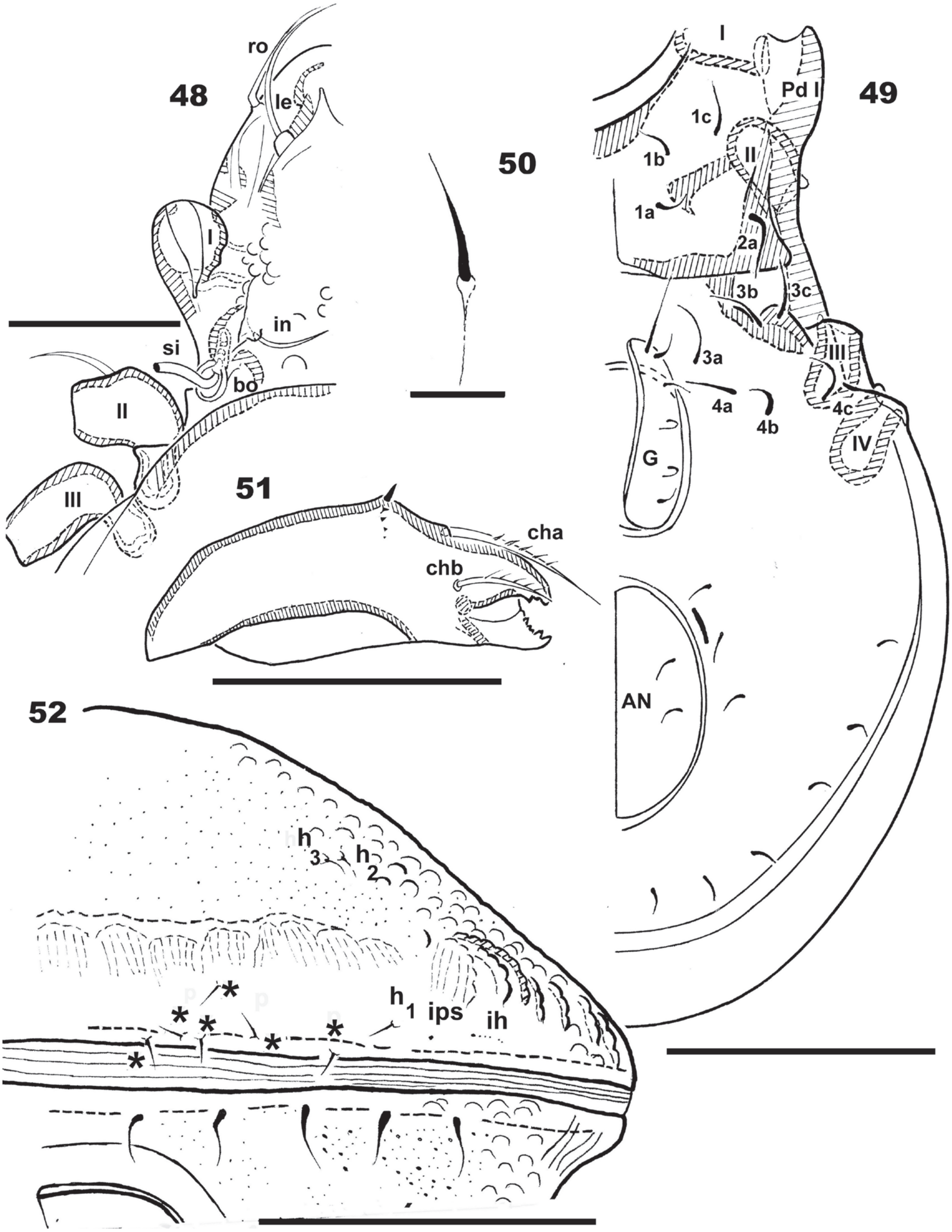
Setal formulae: I(1-6-4-5-20-1) (1-2-2); II(1-2-4-4-14-1) (1-1-2); III(2- 3-3-4-12-1) (1-1-0); IV(1-3-2-4-10-1) (0-1-0). Setae *d* present on all femurs, genua and tibiae. On tibia I (Figure 70) seta *d* is small and hardly discernible (Figure 73), situated on the same promontory as solenidion φ_2. On all other tibiae (II, III, IV) (Figures 71, 72, 74),genua and femurs setae *d* large and barbate (Figures 70, 71, 72, 74).

Nymphal Scalps. Limited number of specimens and the necessity of dissection impeded comprehensive study of scalps, for this reason our study was limited to deutonymphal and tritonymphal scalps. Observed particularities: Very particular bean-shaped structures are found on either side of the decoupage zone around the horn-like structure (Figures 63, 64, 65, 66).

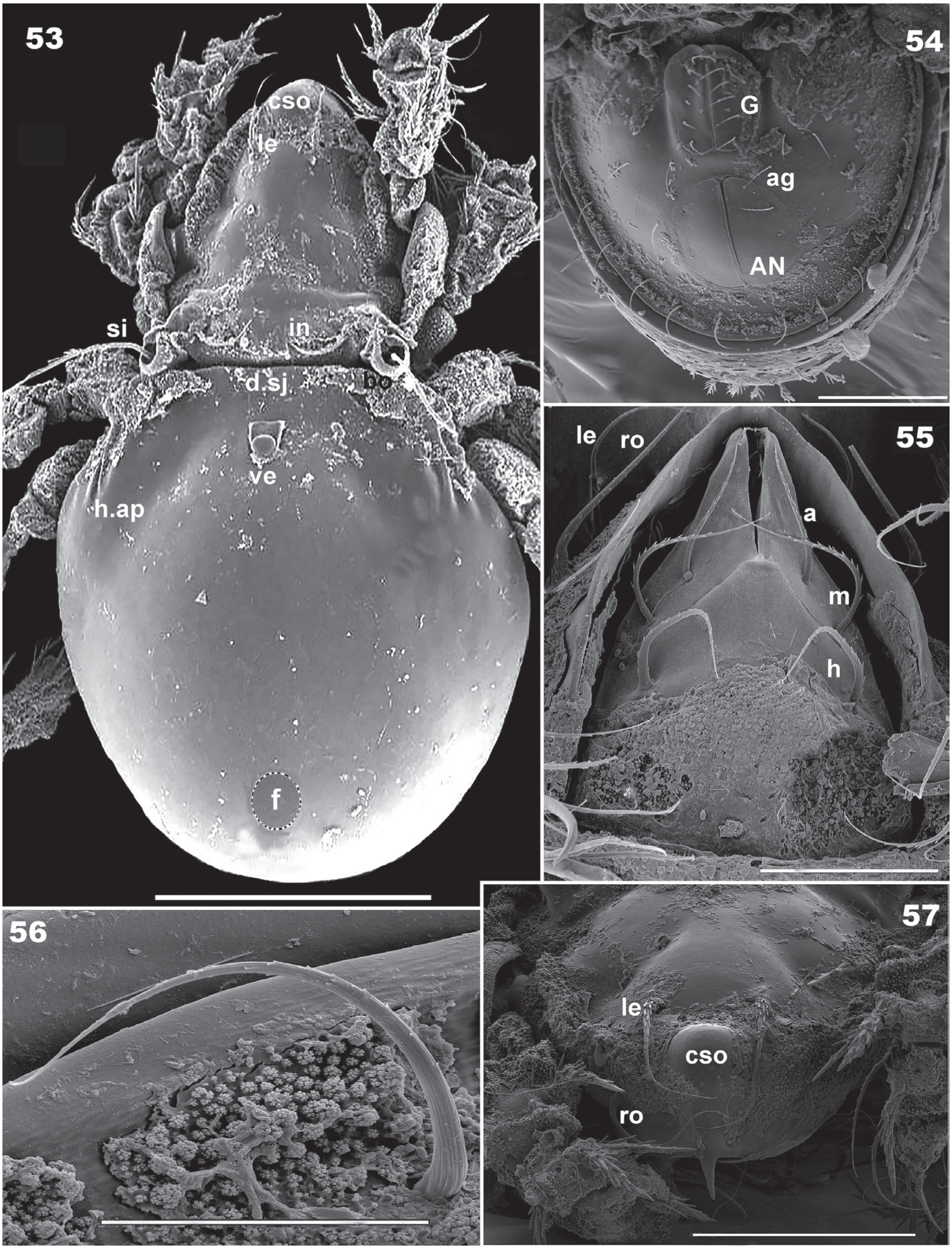
Scalps present polyhedral reticulate to ovoid cuticular structure (*s.r.s*), most visible on internal side (Figure 69) but also on entire scalp (Figures 63–69). Internally scalps present a very thin cuticular layer (*t.c.l*) (Figure 69) covering polygonal-ovoid structures. The *s.r.s* often appearing either completely perforated or with a thin cuticular



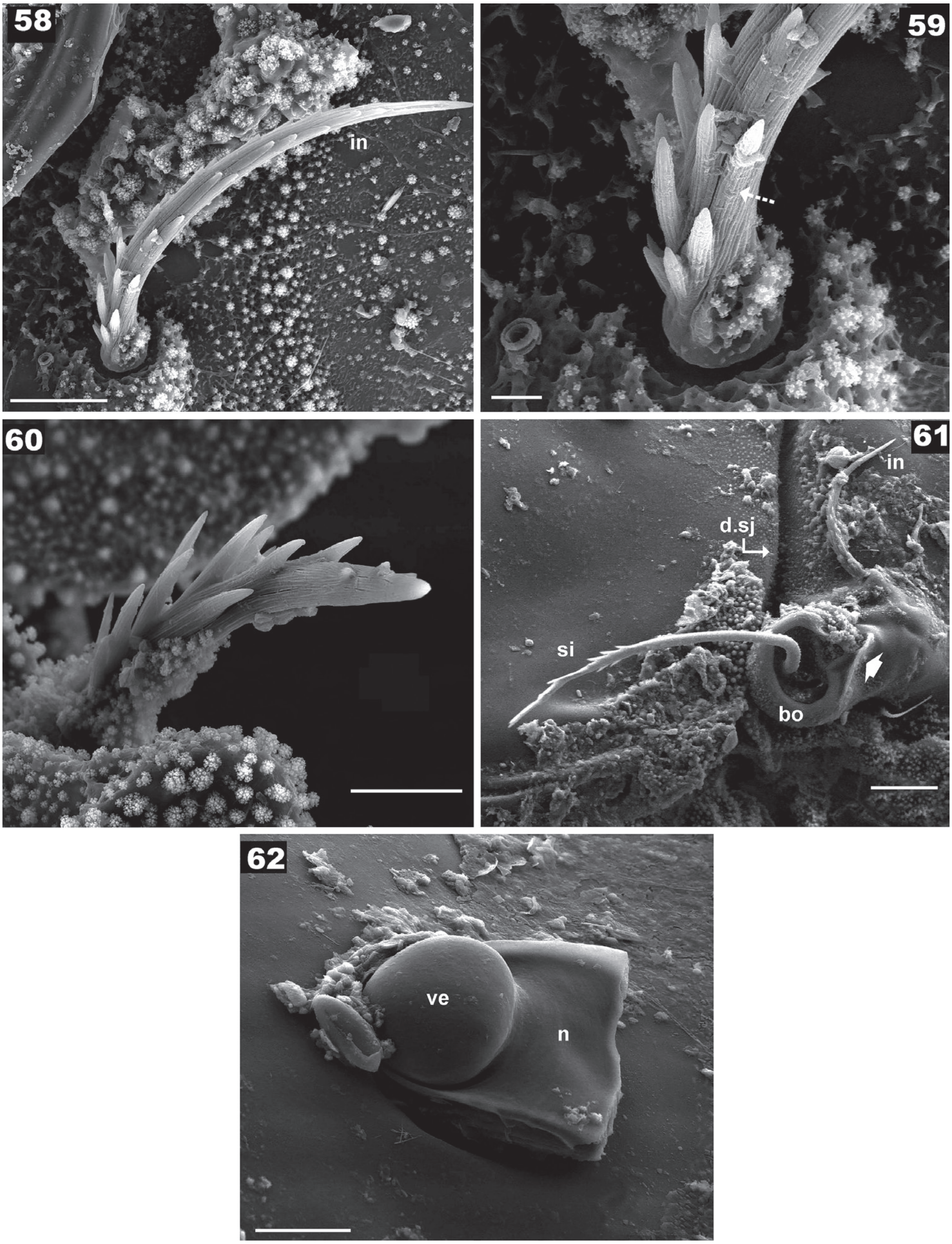
Figures 43–47. *Basilobelba spasmenosi* sp. n. Adult; SEM. **43** dorsal view with scalps **44** dorsal view with scalp **45** posterior zone of scalps **46** prodorsum anterior zone **47** arched tritonymphal buckle. Abbreviations: see Materials and Methods. Scale bars: **43** = 200 µm; **44** = 50 µm; **45** = 20 µm; **46** = 50 µm; **47** = 2 µm.



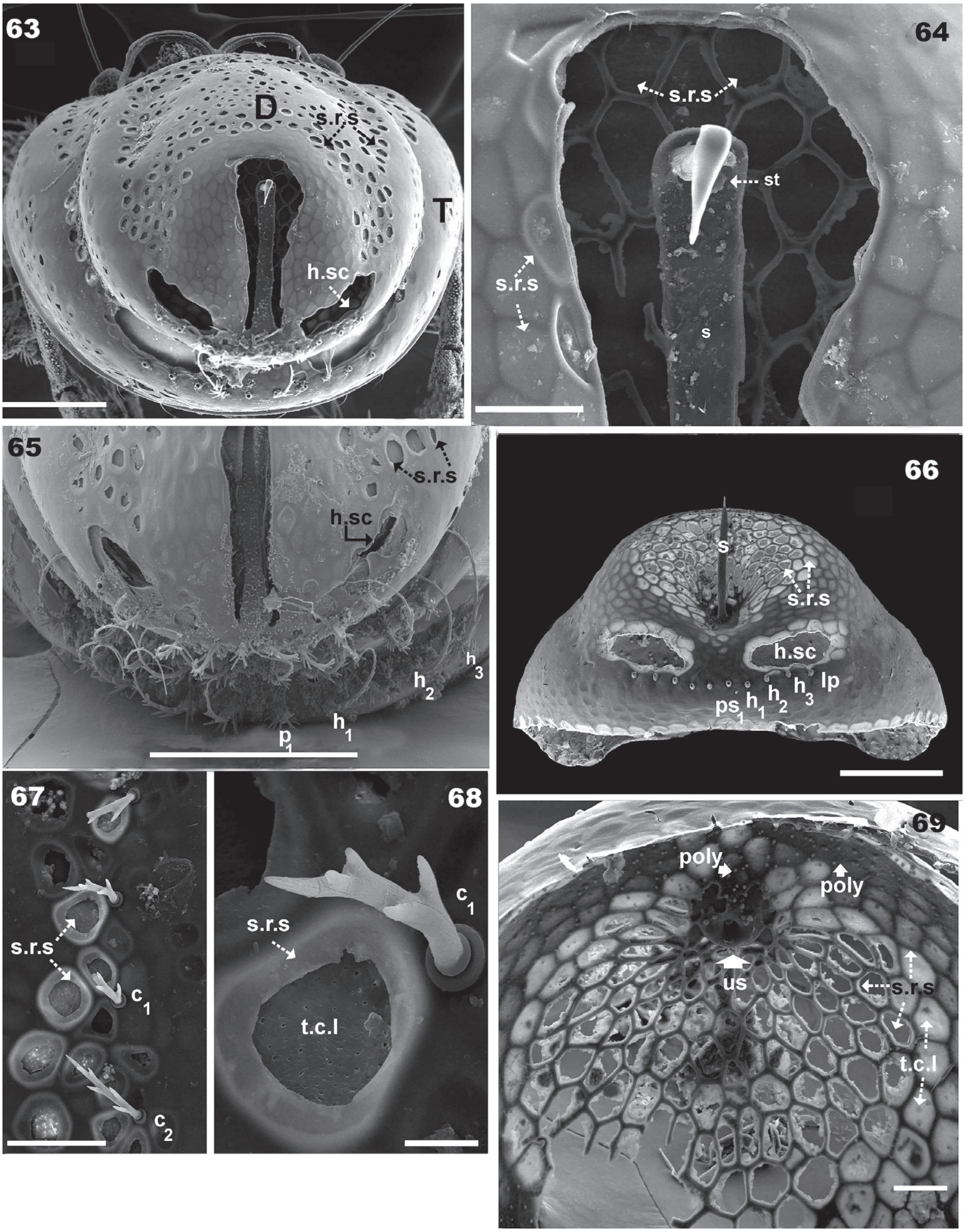
Figures 48–52. *Basilobelba spasmenosi* sp. n. Adult; Optical microscopy. **48** anterior zone prodorsum **49** ventral zone without subcapitulum **50** ventral setae **51** Chelicera, lateral view **52** notogaster posterior view. Abbreviations: see Materials and methods. Scale bars: **52** = 100 µm; **49** = 70 µm; **50** = 25 µm; **48, 51** = 20 µm.



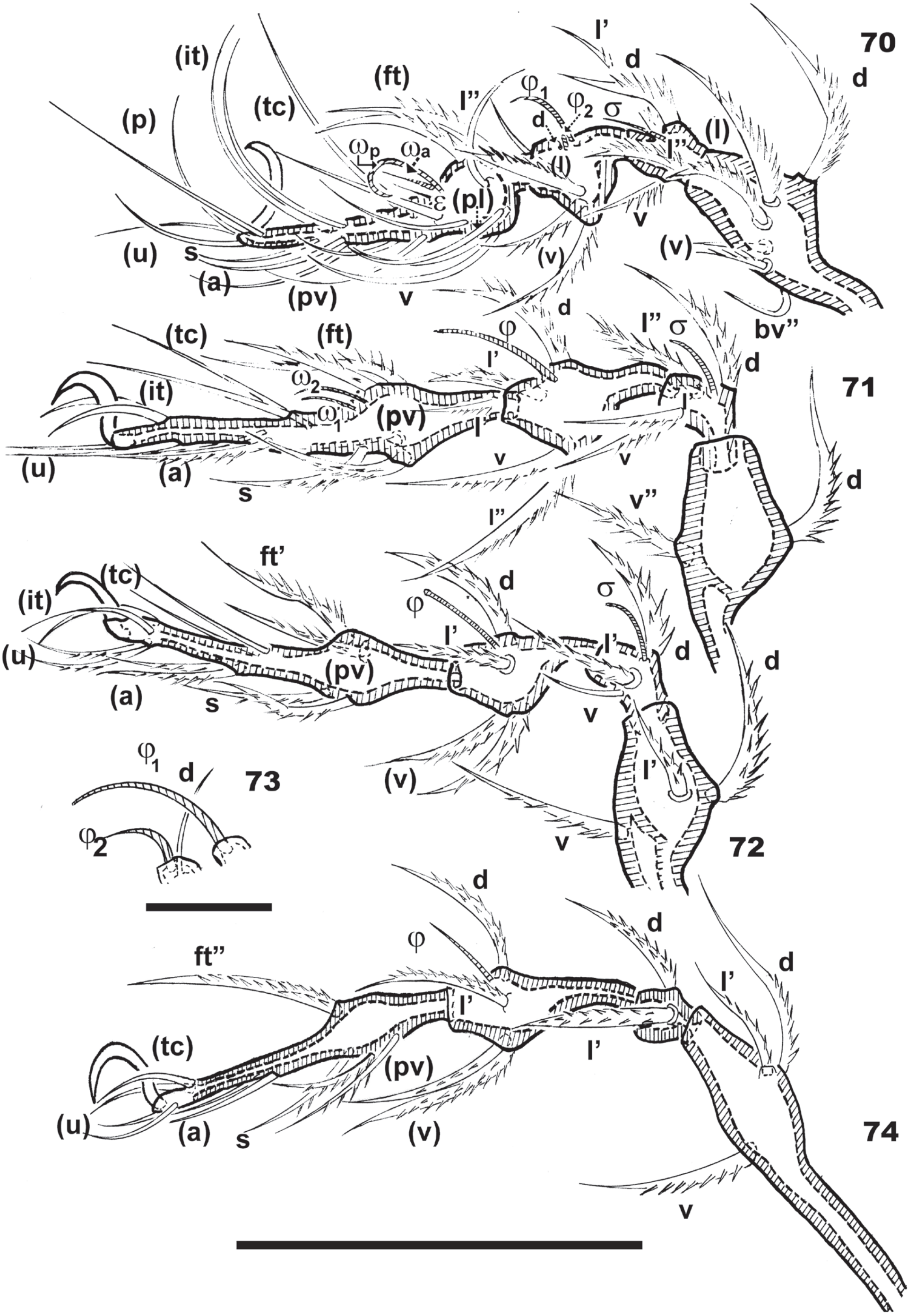
Figures 53–57. *Basilobelba spasmenosi* sp. n. Adult; SEM. **53** general view without scalps **54** genito-anal zone **55** subcapitulum, ventral view **56** ventral setae **57** frontal view. Abbreviations: see Materials and methods. Scale bars: **53** = 200 µm; **54** = 100 µm; **55** = 20 µm; **56** = 20 µm; **57** = 100 µm.



Figures 58–62. *Basilobelba spasmenosi* sp. n. Adult; SEM. **58** *in* seta **59** detail *in* seta **60** legs, dorsal setae **61** bothridium **62** wart, with part of tritonymphal buckle. Abbreviations: see Materials and methods. Scale bars: **58** = 10 µm; **59** = 2 µm; **60** = 10 µm; **61** = 20 µm; **62** = 10 µm.



Figures 63–69. *Basilobelba spasmenosi* sp. n. Adult; SEM. **63** posterior view deutonymphal and tritonymphal scalps **64** posterior zone deutonymphal scalps, detail **65** dorso-posterior view deuto and tritonymphal scalps **66** tritonymphal scalps, posterior view **67** deutonymphal scalp, detail, anterior zone **68** detail *s.r.s* and *c1* seta, deutonymphal scalp **69** interior view, tritonymphal scalp. Abbreviations: see Materials and methods. Scale bars: **63** = 100 µm; **64** = 20 µm; **65** = 50 µm; **66** = 100 µm; **67** = 20 µm; **68** =5 µm; **69** = 20 µm.



Figures 70–74. *Basilobelba spasmenosi* sp. n. Adult; Optical observations. **70** leg I antiaxial **71** leg II antiaxial **72** leg III antiaxial **73** details tibia I solenidium j1, j2 and dorsal seta **74** leg IV, antiaxial. Abbreviations: See Materials and methods. Scale bars: **70, 71, 72, 74** = 100 µm; **73** = 25 µm.

Table 2. *Basilobelba spasmenosi* sp. n. adult, legs.

	Femur	**Genu**	**Tibia**	**Tarsus**
Leg I				
Setae	*d,(l),(v),bv*	*(l),d,v*	*d,(l),(v)*	*(ft),l”,(pl),v,(pv),(tc),(it),(p),(u),s,(a),e*
Solenidium	--------	s	j_1 , j_2	w_1, w_2
Leg II				
Setae	d,v”	d,(l),v	d,(l),v	*(ft),(pv),S,(a),(u),(it),(tc),l’*
Solenidium		s	j	w_1, w_2
Leg III				
Setae	d,l’,v	d,v,l’	d,l’(v)	*ft’,(pv),(tc),(it,)(u),(a),S*
Solenidium				
Leg IV				
Setae	d,l’,v	d,l’	d,l’(v)	*(tc),ft”,(pv)s,(a)(u)*
Solenidium		-----	j	-------

layer (*t.c.l*) resembling an interior membrane (Figures 63, 65, 66, 67). In Figure 64 the *s.r.s* is clearly visible due to transparency, also internally, on both sides of the decoupage zone around the horn. The complexity of these perforated areas is yet more interesting as the polyhedral reticular structure, when not perforated, is more or less rounded, surrounded by a polyhedral structure (Figure 67, 68).

Tritonymphal scalps (Figures 43, 45, 66, 69). Basque beret-shaped (Figure 66) tritonymphal scalp fixed to the adult by two structures, one situated anteriorly and the other posteriorly. Heart-shaped structure (tritonymphal buckle) in anterior part affixing scalp to adult notogaster. Tritonymphal buckle consisting of two loops (*a*) (Figures 43, 44), curving outwards then inwards forming a heart-shaped structure, continuing to meet in the plane of symmetry forming a thong-like structure (*n*) (Figure 44) in order to receive the wart (*ve*) (Figures 53). The *ve* is a round-ovoid structure (Figure 62) situated on the adult cuticular surface, functioning like a snap button, fixing the anterior part of the scalp to the adult cuticle. Depression (*f*) on the posterior adult cuticular surface (Figure 53) is indicated by a dotted round zone with *f* in centre. Observing *f* is difficult, necessitating changes in angle of observation. Zone *f* functions by coaptation with the interior part (*su*) (Figure 69 indicated by ¿) (i.e. the inner curving part of the horn-like structure). Small polyhedral structures (poly) are present (Figure 69 indicated by poly and¿) with similar characteristics to *Basilobelba maidililae* Fernandez et al., 2015 but obtaining high resolution SEM images was impossible due to a technical problem. Setae h_1, h_2, h_3, p_1 clearly visible; setae *lp*, *lm* only visible in some instances, due to the cerotegumental layer impeding observation (Figure 65). Horn-like structure on posterior scalp border (Figure 64, 65) aiding in hooking the deutonymphal scalp.

Deutonymphal scalps (Figures 43, 63, 64, 65). Tritonymphal and deutonymphal scalps differ greatly (Figures 66 and 63). No buckles for adherence observed in anterior zone of deutonymphal scalps, and posterior zone (Figures 63, 64) with horn-like structure fixing the protonynphal scalp found on a mobile strip (*le*) consisting of a section of deutonymphal scalp (See Grandjean 1959). Horn-like structure composed of a style (*s*) and a stylet (*st*) (Figure 64). In common with the tritonymphal scalp, a bean-shaped structure (*h.sc*) is observed. Only posterior setae h_1, h_2, h_3, p_1 are visible.

Remarks. The remarkable perforated structures are not observed in other congeners. At present we are studying another species from Rwanda with bean-shaped structures on scalps, similar to those in *Basilobelba spasmenosi* sp. n.

Discussion

The taxonomy of the genus *Plasmobates* in the Afrotropic ecozone is very complex. Species of the genus *Plasmobates* Grandjean, 1929 recorded in this region are: *P. africanus* Balogh, 1958; *P. foveolatus* Ermilov et al., 2010; *P. machadoi* Balogh, 1958; *P. minor* Balogh, 1958; and *P. zarae* sp. n. Subías (2015) considers *P. africanus* Balogh, 1958; *P. machadoi* Balogh, 1958 and *P. minor* Balogh, 1958 as "sp. inq.", without providing reasons. We analyzed the work of Balogh (1958), but found it impossible to identify the cited species. A comprehensive search in the available collection from this ecozone failed to provide specimens with the characters in the provided text. One comparable species from the region is *P. foveolatus* Ermilov et al., 2010, but the study lacks SEM micrographs, and is not detailed enough to provide a conclusive comparison.

In our opinion the following characteristics permit easy differentiation of species of *Plasmobates* from other congeners, not only those from the Afrotropic ecozone: the cerotegumental layer, shape and insertion type of *ro* and *in* setae, sensillus with scales, promontories of podocephalic canal, distribution of macropores, type of lateral gland, number and distribution of notogastral setae, positions of setae *d* of tibia I and particular setae found on tibia II.

The taxonomy of *Basilobelba* Balogh, 1958 and *Xiphobelba* Csiszár, 1961 were discussed in preceding work (Fernandez et al. 2015). *B. spasmenosi* sp. n. presents all characteristics of the genus and also displays very interesting particularities, permitting easy differentiation from other congeners, such as: shape and characteristics of cerotegumental layer, lamellar and rostral setae, prodorsum, rostrum and *CSO*, infracapitulum, notogastral setae, nymphal scalps with perforated areas and particular distribution of leg setae. The distribution of notogastral setae is especially particular as all nine setae are situated on the posterior notogastral zone.

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The genus *Paraplonobia* Wainstein and *Neopetrobia* Wainstein (Acari, Trombidiformes, Tetranychidae) from Saudi Arabia: new species, new records and key to the world species of *Paraplonobia*

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Abstract

The two tetranychid genera *Paraplonobia* Wainstein and *Neopetrobia* Wainstein (Trombidiformes: Tetranychidae) are reported for the first time from Saudi Arabia. Three new species *Paraplonobia* (*Anaplonobia*) *arabica* Mirza & Alatawi, **sp. n.**, *P.* (*A.*) *haloxylonia* Alatawi & Mirza, **sp. n.** and *P.* (*A.*) *tabukensis* Kamran & Alatawi, **sp. n.** are described and illustrated based on adult females, collected from *Prosopis juliflora* (SW.) Dc. (Fabaceae) and *Haloxylon salicornicum* Bunge (Amaranthaceae) from two different regions of Saudi Arabia. *Neopetrobia mcgregori* (Pritchard and Baker) is redescribed and illustrated based on female collected from *Cynodon dactylon* L. (Poaceae).The diagnostic morphological features including leg chaetotaxy of all known species of the subgenus *Anaplonobia* is tabulated. A key to the world species of the genus *Paraplonobia* is also provided.

Keywords

Hystrichonychini, *arabica*, *haloxylonia*, *tabukensis*, *Prosopis*

Introduction

The genus *Paraplonobia* Wainstein belongs to the tribe Hystrichonychini Pritchard and Baker of the subfamily Bryobinae (Acari: Prostigmata: Tetranychidae). Wainstein (1960) considered *Anaplonobia* and *Paraplonobia* as subgenera of *Aplonobia* Womersley. Later, Tuttle and Baker (1968) proposed *Anaplonobia* and *Paraplonobia* as two valid genera. After that, Gutierrez (1985) categorized the genus *Paraplonobia* into three subgenera: *Anaplonobia* Wainstein, *Brachynychus* Mitrofanov & Strunkova and *Paraplonobia* Wainstein on the basis of coxal setal count and the aspect of peritremes and considered the genus *Anaplonobia* as subgenus of *Paraplonobia* (Gutierrez 1985).

The genus *Paraplonobia* includes 32 species to date, which are widely distributed throughout the world. The subgenera *Anaplonobia*, *Paraplonobia*, and *Brachynychus* include 22, nine and one species, respectively (Baker and Tuttle 1972, Meyer 1987, Bolland et al. 1998, Migeon and Flechtmann 2004).

The subgenera *Anaplonobia* and *Paraplonobia* have a coxal setal formula of 2–2–1–1 while the subgenus *Brachynychus* has a coxal setal formula of 4–3–2–2. The subgenus *Anaplonobia* differs from *Paraplonobia* by having anastomosed peritremes while the later has simple peritremes (Gutierrez 1985, Bolland et al. 1998).

The genus *Neopetrobia* also belongs to the tribe Hystrichonychini and morphologically closely resembles the genus *Paraplonobia* except for the fourth pair of dorsocentral setae f_1 which are widely spaced as compared to setae $c_{1,}$ while f_1 setae are normally spaced as c_1 in *Paraplonobia* (Meyer 1987, Bolland et al. 1998). The genus *Neopetrobia* has been categorized into three subgenera; *Neopetrobia*, *Reckia* Wainstein and *Langella* Wainstein (Gutierrez 1985, Bolland et al. 1998). The subgenus *Neopetrobia* is different from other two subgenera by having integument without tuberculate or reticulate pattern and rounded or spindle shaped dorsal setae and includes ten species to date (Bolland et al. 1998).

A few tetranychid species have been reported from Saudi Arabia (SA): *Bryobia praetiosa* Koch, *Eotetranychus fallugiae* Tuttle & Baker, *Eutetranychus orientalis* (Klein), *E. palmatus* Attiah, *Oligonychus afrasciaticus* (McGregor), *O. pratensis* (Banks), *Tetranychus cinnabarinus* (Boisduval), *T. turkrestzni* (Ugarov & Nikolskii), and *T. urticae* (Koch) (Martin 1972, Alatawi 2011). The genus *Paraplonobia* is poorly known from Arabian peninsula. Previously, two species *P.* (*A.*) *harteni* Meyer and *P.* (*P.*) *dactyloni* Smiley & Baker were reported from Yemen (Meyer 1996; Smiley and Baker 1995).

Two genera, *Paraplonobia* and *Neopetrobia*, are reported upon for the first time from Saudi Arabia with three new species: *Paraplonobia* (*Anaplonobia*) *arabica* sp. n., *P.* (*A.*) *haloxylonia* sp. n. and *P.* (*A.*) *tabukensis* sp. n. which are described and illustrated based on adult females. The male of *P.* (*A.*) *haloxylonia* sp. n. is also described and illustrated. *Neopetrobia mcgregori* (Pritchard & Baker) is redescribed and illustrated based on female.

Diagnostic features of all known species of the subgenus *Anaplonobia* are provided including body morphological features, leg I length in comparison to body length, and leg chaetotaxy (Tables 1 and 2) as well as a key to the world species of the genus *Paraplonobia*.

Table 1. Some morphological diagnostic features of the world species of the subgenus *Anaplonobia*, genus *Paraplonobia*.

Species	Distribution	Dorsal setae		Dorsal Striations		Stylophore anteriorly	Peritremes
		Shape	Distance of dorso-central hysterosomal setae especially (c1, d1, e1) to the setae next in line	Hysterosoma medialy	Propodosomal shield		
[3] *P.* (*A.*) *acharis* (Pritchard & Baker, 1955)	USA	slightly lanceolate	distinctly shorter	widely spaced transverse	dashed, weak, longitudinal	rounded	weak with two irregular branches
[7] *P.* (*A.*) *algarrobicola* (Gonzalez, 1977)	Chile	subspatulate, on tubercles	distinctly shorter	widely spaced transverse	longitudinal	rounded	anastomose
[3] *P.* (*A.*) *ambrosiae* (Tuttle, Baker & Abbatiello, 1976)	Mexico, USA	slender/ setiform	distinctly shorter	widely spaced transverse	tuberculate longitudinal	-	ball like anastomose
[7] *P.* (*A.*) *arabica* Mirza & Alatawi, sp. n.	Jazan, Riyadh, Tabuk	subspatulate, e-f on small tubercules	distinctly shorter	widely spaced transverse	weak, irregular, longitudinal	slightly incised	elongate anastomose
[9] *P.* (*A.*) *boutelouae* Tuttle & Baker, 1968	USA	subspatulate	distinctly shorter	closely spaced transverse	-	-	anastomose
[3] *P.* (*A.*) *brickellia* Baker & Tuttle, 1972	USA	subspatulate	distinctly shorter	widely spaced transverse	strong tubercules/lobes	round, tapering distally	strongly rounded, anastomose
[7] *P.* (*A.*) *candicans* (Meyer, 1974)	South Africa	subspatulate, on tubercles	distinctly shorter	widely spaced transverse	medially wide spaced longitudinal, remaining dashed	slightly incised	complex anastomose
[9] *P.* (*A.*) *concolor* Chaudhri, Akbar & Rasool, 1974	Pakistan	lanceolate	distinctly shorter	closely spaced transverse	weak transverse	deeply incised	anastomose
[9] *P.* (*A.*) *contiguus* (Chaudhri, Akbar & Rasool, 1974)	Pakistan	lanceolate	distinctly shorter	closely spaced transverse	dotted longitudinal	slightly incised	weak branched anastomose
[9] *P.* (*A.*) *daryaensis* Chaudhri, Akbar & Rasool, 1974	Pakistan	lanceolate	distinctly shorter	closely spaced transverse	irregular, weak, longitudinal, medially transverse	slightly incised	anastomose
[9] *P.* (*A.*) *glebulenta* (Meyer, 1974)	South Africa	lanceolate	distinctly shorter	Small tubercles making pattern		round	sausage anastomose
[2,9] *P.* (*A.*) *haloxylonia* Alatawi & Mirza, sp. n.	Riyadh, KSA	lanceolate	distinctly shorter	closely spaced transverse	weak, longitudinal	slightly incised	weak anastomose with few long thread like branches

Species	Distribution	Dorsal setae		Dorsal Striations		Stylophore anteriorly	Peritremes
		Shape	**Distance of dorso-central hysterosomal setae especially (c1, d1, e1) to the setae next in line**	**Hysterosoma medialy**	**Propodosomal shield**		
P. (*A.*) *harteni*(Meyer, 1996)	Yemen	lanceolate	distinctly shorter	closely spaced transverse	dashed, transverse	slightly incised	weakly anastomose with few branches
[1] *P.* (*A.*) *inornata* (Meyer, 1987)	South Africa	slender /setiform	distinctly shorter	widely spaced transverse	coarse longitudinal, plate dashed	incised	weak branched anastomose
[7] *P.* (*A.*) *prosopis* (Tuttle & Baker, 1964)	Miami USA, Marigat, Kenya	subspatulate	distinctly shorter	widely spaced transverse	longitudinal	-	anastomose
[2] *P.* (*A.*) *tabukensis* Kamran & Alatawi, sp. n.	Tabuk, KSA	narowly lanceolate	distinctly shorter	closely spaced transverse	weak, longitudinal	rounded	small, compact, anastomose
[9] *P.* (*A.*) *theroni* (Meyer, 1974)	South Africa	lanceolate, on tubercles	distinctly shorter	closely spaced transverse	dashed fine longitudinal	slightly incised	elongate anastomose
[8] *P.* (*A.*) *allionia* Baker & Tuttle, 1972	USA	slender/ setiform	as long as/ slightly longer	closely spaced transverse	strong tuberculate longitudinal	rounded	small, elongate anastomose bulb
[3] *P.* (*A.*) *calame* (Pritchard & Baker, 1955)	USA	slender/ setiform, on small tubercles	as long as/ slightly longer	widely spaced transverse	pebbled	rounded	three chambered branches
[5] *P.* (*A.*) *coldinae* (Tuttle & Baker, 1964)	USA	slender/setiform	much longer	-	-	rounded	anastomose
[7] *P.* (*A.*) *juliflorae* (Tuttle & Baker, 1968)	USA	subspatulate on small tubercles	longer	widely spaced tuberculate striate	tuberculate striate	rounded	Weak branched anastomose
[3] *P.* (*A.*) *artemisia* Baker & Tuttle, 1972	Mexico, USA	slender, blunt distally	as long as/slightly longer	widely spaced tuberculate fold, tranverse	broken, irregular, longitudinal	rounded	elongate anastomose bulb
[4] *P.* (*A.*) *berberis* Baker & Tuttle, 1972	USA	slender/setiform	as long as/ slightly longer	widely spaced broad folds with tubercules	small tubercules	rounded	small anastomose bulb
[6,7] *P.* (*A.*) *euphorbiae* (Tuttle & Baker, 1964)	Mexico, USA	subspatulate	slightly shorter/as long as	irregular transverse	basket weave	rounded	anastomose
[9] *P.* (*A.*) *tshipensis* (Meyer, 1974)	South Africa	spatulate, on tubercles	longer	closely spaced transverse	broken longitudinal	deeply incised	oval anastomose

Host Plant Family: 1. Acanthaceae, 2. Amaranthaceae, 3. Asteraceae, 4. Berberidaceae, 5. Boraginaceae, 6. Euphorbiaceae, 7. Fabaceae, 8. Nyctaginaceae, 9. Poaceae

Table2. Length of leg I and leg chaetotaxy of world species of subgenus *Anaplonobia* genus *Paraplonobia*.

Species		Length of leg I as compared to body length	Coxa				Trochanter				Femora				Genua				Tibia				Tarsus			
			I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
P. (A.) acharis		longer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13(1)	-	-	-	14(3)+2dup	-	-	-
P. (A.) algarrobicola		longer	2	2	0	0	1	1	1	1	5	5	3	3	4	4	3	2	10(2)	7	9	8	19	14	11	11
P. (A.) arabica	Tabook, Riyadh	longer	2	2	1	1	1	1	1	1	5	5	3	3	5	4	3	3	9(1)	9	9	9	14(2)+2dup	8(1)+1dup	8(1)	9(1)
	Jizan		2	2	1	1	1	1	1	1	5	5	3	3	5	4	3	3	9(1)	8	9	9	12(1)+2dup	9(1)+1dup	9(1)	9(1)
P. (A.) brickellia		longer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. (A.) daryaensis		longer	2	2	1	1	1	1	1	1	9	6	4	4	5	5	4	4	14	9	9	9	22	15	15	15
P. (A.) haloxylonia		longer	2	2	1	1	1	1	1	1	9	6	4	4	5	5	4	4	13(1)	9	9	9	15(2)+2dup	10(1)+1dup	12(1)	12(1)
P. (A.) harteni		longer	2	2	1	1	1	1	1	1	9	6	4	4	5	5	4	4	13(1)	9	9	9	18(2)+2dup	12(2)+1dup	14(1)	14(1)
P. (A.) prosopis	Tuttle and Baker 1964	longer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11(1)	8	-	9	15(1)+2dup	9(1)+1dup	-	10(1)
	Toroitich et al. 2009		-	-	-	-	-	-	-	-	5	4	3	3	4	4	3	2	9	7	8	7	13(2)	10(1)	9	8
P. (A.) tabukensis		longer	2	2	1	1	1	1	1	1	8	6	3	3	4	5	3	3	13(1)	9	8	8	10+2dup	7+1dup	9+1dup	9+1dup
P. (A.) allionia		longer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. (A.) artemisia		longer	-	-	-	-	1	-	-	-	6	-	-	-	4	-	-	-	9(1)	-	-	-	11(1)+2dup	-	-	-
P. (A.) berberis		longer	-	-	-	-	-		-	-	-	-	-	-	4	3	-	-	9(1)	5	-	6	8(1)+2dup	8	-	9
P. (A.) calame		longer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13(1)	-	-	-	13(2)+2dup	-	-	-
P. (A.) concolor		shorter	2	2	1	1	1	1	1	1	9	6	4	4	5	5	4	4	14	9	9	9	22	15	15	15
P. (A.) contiguus		shorter	2	2	1	1	1	1	1	1	9	6	4	4	4	5	4	4	14	9	9	9	20	15	15	15
P. (A.) tshipensis		shorter	-	-	-	-	-	-	-	-	9	6	4	4	5	5	4	4	13(1)	8	6	7	16(1)+2dup	13+1dup	12(1)	12(1)
P. (A.) ambrosiae		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13(1)	-	-	-	16(3)+2dup	(1)+2dup	-	-
P. (A.) boutelouae		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. (A.) candicans		-	-	-	-	-	-	-	-	-	11	8	6	5	8-9	9	7	7	13(1)	9	9	9	18(1)+2dup	15+1dup	14(1)	14(1)
P. (A.) glebulenta		-	-	-	-	-	-	-	-	-	9	6	4	4	5	5	3	3	12-13(1)	9	9	9	16(2)+2dup	12-13(2)+1dup	12(1)	12(1)
P. (A.) innornata		-	2	2	1	1	1	1	1	1	9	6	4	4	5	5	6	6	13(1)	9	9	9	18(2)+2dup	14+1dup	14(1)	14(1)
P. (A.) theroni		-	-	-	-	-	-	-	-	-	9	6	4	4	5	5	4	4	13(1)	9	6	9	18(1)+2dup	14+1dup	12-13(1)	14(1)
P. (A.) coldinae		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. (A.) euphorbiae		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. (A.) juliflorae		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Materials and methods

The mite specimens were collected by shaking the plant parts, especially leaves, onto a white sheet of paper. Mites found moving on paper were collected with the help of a camel hairbrush and preserved in small vials containing 70% ethanol. Preserved mite specimens were observed under a stereomicroscope (SZX10, Olympus, Tokyo, Japan) and mounted on glass slides in Hoyer's medium. The mounted specimens were examined under phase contrast microscope (DM2500, Leica, Wetzlar, Germany). Different body parts were pictured using an auto montage software system (Syncroscopy, Cambridge, UK), then drawn with Adobe Illustrator (Adobe System Inc., San Jose, CA, USA). All measurements are in micrometers. The terminology used in this paper follows that of Lindquist (1985). All type specimens were deposited at Acarology Laboratory, Department of Plant Protection, College of Food and Agricultural Sciences, King Saud University except one each of female and male paratypes of *Paraplonobia* (*Anaplonobia*) *haloxylonia* sp. n., female paratype each of *P.* (*A.*) *arabica* sp. n., and *P.* (*A.*) *tabukensis* sp. n., with Accession numbers, OSAL 0115769, OSAL 00115768, OSAL 0110333 and OSAL 0110332 respectively, that were deposited at Ohio State University Acarology Laboratory (OSAL), USA.

Results and discussion

Family Tetranychidae Donnadieu
Subfamily Bryobinae Berlese

Genus *Paraplonobia* Wainstein, 1960

Aplonobia (*Paraplonobia*) Wainstein, 1960: 140.

Paraplonobia: Tuttle and Baker 1968: 48, Meyer 1974: 119, Chaudhri et al. 1974: 28, Gutierrez 1985: 75, Bolland et al. 1998: 7.

Type species. *Aplonobia* (*Paraplonobia*) *echinopsili* Wainstein, 1960 by original designation.

Diagnosis. Based on Baker and Tuttle 1968, Gutierrez 1955, Meyer 1974, Meyer 1987, Bolland et al. 1998.

Body oval; prodorsum without lobes and with three pairs of setae; dorsal opisthosomal setae ten pairs. Dorsal setae not set on prominent tubercles; setae f_1 normal in position, coxal setal formula variable, most species with 2–2–1–1 except one species of the subgenus *Brachynychus* having 4–3–2–2 setae on coxae I–IV respectively; anal setae three pairs; peritremes simple/anastomosing; tarsus I with two sets of duplex setae, present close to distal end of tarsus; claws and empodium pad-like each with tenant hairs (Fig. 5A).

Subgenus *Anaplonobia* Wainstein

Diagnosis. Based on Gutierrez 1985, Bolland et al. 1998.

Peritremes anastomosed, coxal setal formula 2–2–1–1.

The subgenus *Anaplonobia* includes 22 species (Migeon and Flechtmann 2004). The species of the subgenus *Anaplonobia* can be grouped into two categories: 1) Eight species with dorsal body setae slightly shorter/as long as or longer than distances to the bases of consecutive setae (Tables 1 and 2), second group with dorsal body setae distinctly shorter than distances between their bases, contains 17 species including three new species (*P.* (*A.*) *arabica* sp. n., *P.* (*A.*) *haloxylonia* sp. n., and *P.* (*A.*) *tabukensis* sp. n.) reported in this study (Table 1, 2).

Shape of setae (spatulate, subspatulate, lanceolate or setiform), comparative length of setae with respect to the distance of setae next behind, shape of peritremes (compact anastomose, branched or weakly anastomosed), propodosomal shield (pebbled, lobbed, with longitudinal/transverse striations), hysterosoma (medially with closely/ widely spaced striations), comparative length of leg I with respect to body length (shorter/longer) and leg chaetotaxy are the major diagnostic characters vary among/ within the species of subgenus *Anaplonobia* (Table 1, 2).

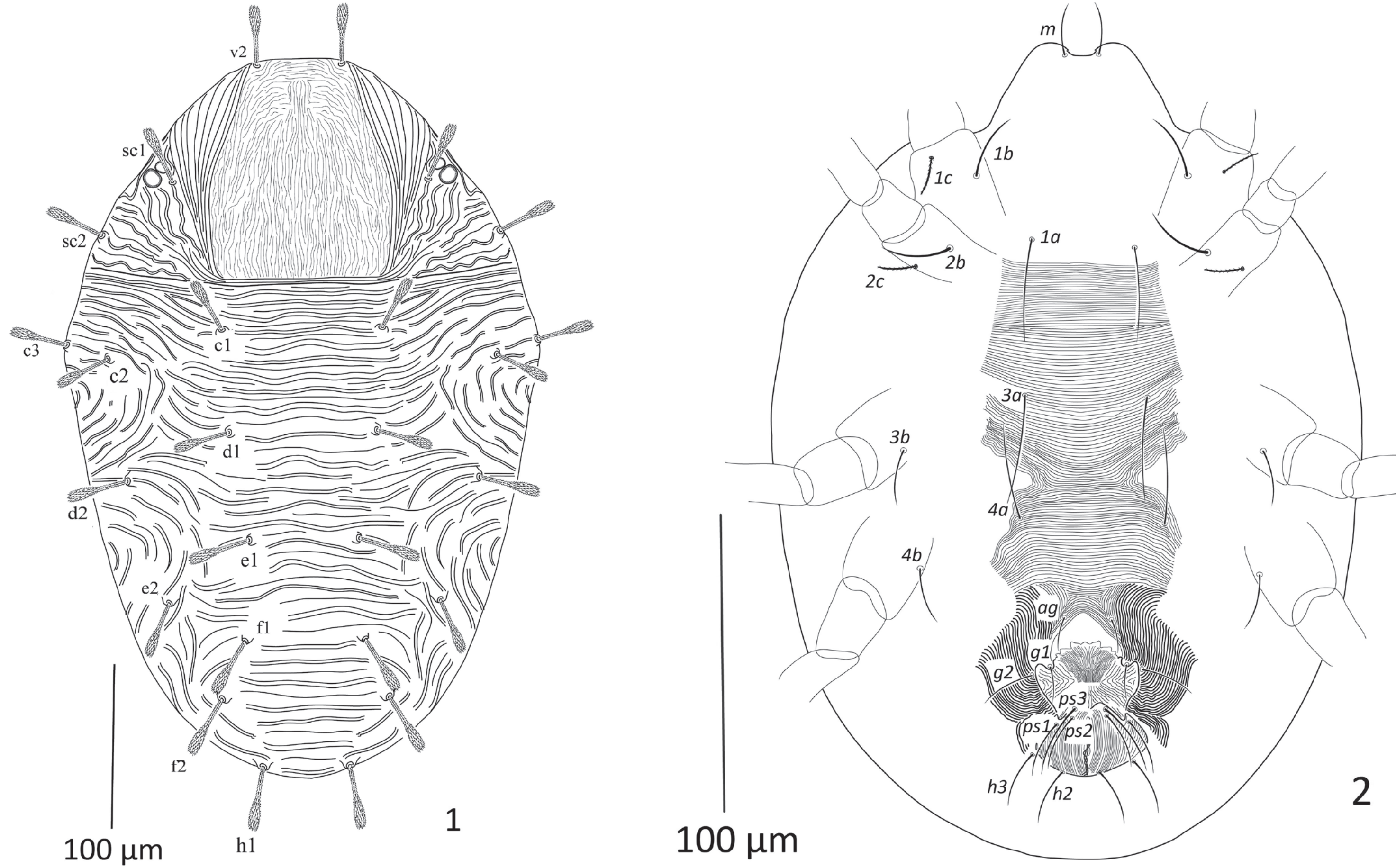
Most species of the subgenus *Anaplonobia* have been reported from USA, Mexico, South Africa and Pakistan and collected mostly from three host plants families Asteraceaea, Fabaceae and Poaceae (Bolland et al. 1998) (Table 1).

The specimens of new species *P.* (*A.*) *arabica* sp. n., collected from *Prosopis juliflora* from three different regions (Riyadh, Tabuk, and Jazan) of Saudi Arabia, are morphologically similar except for some variations in setal counts on Tibia II and Tarsus I–II–III. (Table 2). The variations in the setal count of leg I–II–IV (Tibia and Tarsus) in *P.* (*A.*) *prosopis* had been found also in the description made by Tuttle and Baker (1964) from USA and Toroitich and Ueckermann (2009) from Kenya (Table 2). However, in some other species of the subgenus *Anaplonobia*, setal variations on genua, tibiae and tarsi have been found among the different specimens collected from the same host and location within the same species. i.e. genua I (8–9) in *P.* (*A.*) *candicans*, tibia I (12–13) and tarsus II (12–13) of *P.* (*A.*) *glebulanta*, and tarsus III (12–13) of *P.* (*A.*) *theroni* (Table 2).

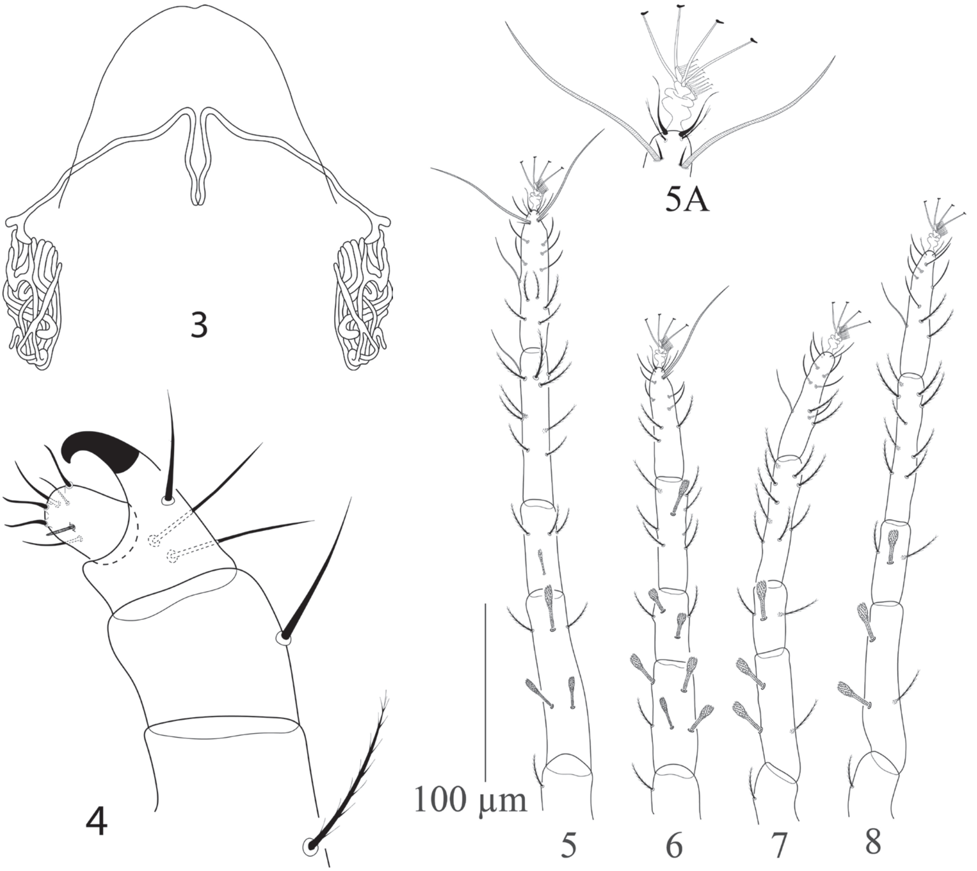
Paraplonobia (*Anaplonobia*) *arabica* sp. n.

http://zoobank.org/200D2E10-9324-4C31-8B04-F08C8F33EBD1
Figs 1–8

Diagnosis. Dorsal body setae subspatulate, serrate, expanded distally and distinctly shorter to the distances of setae next in line, first pair of dorsocentral setae c1 reaching 2/3 to the distance of setae d1, setae c1 almost 1.5 times widely spaced than setae f1, setae e_2, f_1, f_2 and h_1 set on small tubercles, dorsal hysterosomal striations widely spaced, propodosoma medially with longitudinal broken striations, stylophore with a



Figures 1, 2. *Paraplonobia* (*Anaplonobia*) *arabica* sp. n. adult female. **1** dorsum **2** venter.



Figures 3–8. *Paraplonobia* (*Anaplonobia*) *arabica* sp. n. adult female. **3** stylophore and peritremes **4** palp **5** leg I **5A** duplex setae, empodium and claws of tarsus I **6** legII **7** legIII **8** leg IV.

small mediocephalic emargination, peritremes branched tube like compact anastomosing, leg I shorter than body length.

Description of holotype female (n = 9). Measurement of holotype followed by 8 paratypes (in parenthesis) (Figs 1–8).

Dorsum (Fig. 1)**.** Body oval; length of idiosoma 439 (430–443), maximum width 282 (280–287), length of body (gnathosoma + idiosoma) 476 (472–480). Propodosoma medially with longitudinal broken striations, without anterior projections. Dorsal body setae subspatulate, serrate, expanded distally and distinctly shorter to the distances of setae next in line, first pair of dorsocentral setae c1 reaching 2/3 to the distance of setae d1, setae c1 almost 1.5 times widely spaced than setae f1, setae e_2, f_1, f_2 and h_1 set on small tubercles. Dorsal striations transverse on hysterosoma, without lobes and widely spaced. Length of dorsal setae: v_2 45 (42–46), sc_1 40 (38–41), sc_2 41 (40–43), c_1 45 (44–48), c_2 42 (40–44), c_3 40 (39–44), d_1 34 (32–38), d_2 44 (43–46), e_1 45 (44–48), e_2 44 (43–45), f_1 45 (44–45), f_2 44 (42–45), h_1 46 (45–48). Distance between dorsal setae: v_2–v_2 53 (51–55), v_2–sc_1 97

(95–98), sc_1–sc_2 56 (54–57), sc_1–sc_1 166 (162–167), sc_2–sc_2 263 (260–266), c_1–c_1 94 (92–96), c_1–c_2 82 (80–85), c_2–c_3 41 (39–44), c_2–c_2 261 (260–264), c_3–c_3 345 (340–346), d_1–d_1 82 (80–84), d_1–d_2 81 (80–82), d_2–d_2 226 (224–228), c_1–d_1 75 (74–78), c_3–d_2 97 (95–99), e_1–e_1 63 (61–65), e_1–e_2 78 (74–79), e_2–d_2 85 (83–86), e_2–e_2 79 (75–79), f_1–f_1 63 (60–65), f_2–f_2 107 (105–108), f_1–f_2 53 (50–54), f_1–d_1 69 (66–70), h_1–h_1 57 (55–59).

Venter (Fig. 2)**.** Idiosoma ventrally with transverse striations from setae 1a to 3a; most of the area between 3a to 4a is transverse with few V-shaped striations laterally; transverse posterior to setae 4a; striations transverse regular anterior to aggenital setae (ag). The intercoxal setae 1a slightly longer than the distance 1a–1a. The intercoxal setae 3a just equal to distance 3a–3a. The intercoxal setae 4a 4/5 to the distance 4a–4a. Length of intercoxal and coxal setae: 1a 39 (35–40), 3a 52 (51–55), 4a 50 (48–52), 1b 54 (52–56), 1c 18 (16–20), 2b 37 (35–38), 2c 21 (20–24), 3b 23 (21–25), 4b 38 (36–39); aggenital setae ag 48 (44–48), ag–ag 27 (25–28); genital setae two pairs, g_1 32 (30–24), g_2 40 (38–42), g_1–g_1 40 (39–44), g_2–g_2 56 (52–57), g_1–g_2 12 (10–14); anal setae three pairs, ps_1 21 (18–24), ps_2 37 (35–39), ps_3 58 (54–60), ps_1–ps_1 33 (30–34), ps_2–ps_2 26 (24–27), ps_3–ps_3 19 (18–22); para-anal setae two pairs, h_2 33 (31–34), h_2–h_2 17 (16–18), h_3 38(35–40), h_3–h_3 46 (45–48).

Gnathosoma (Figs 3–4)**.** Stylophore elongate, slender and slightly notched anteriorly. Peritremes branched tube like compact anastomosing (Fig. 3). Scapular setae m 36 (34–37), m–m 32 (31–35). Palp femur and genu each with one seta, palp tibia with three setae, tibial claw strongly curved; palp tarsus with three setae, three eupathidia, one solenidion (Fig. 4).

Legs (Figs 5–8)**.** Length of legs I–IV (without coxae) 336, 251, 276, 298 respectively. Leg I shorter than body length. Number of setae and solenidia (in parenthesis) on legs I–IV: coxae 2–2–1–1, trochanters 1–1–1–1, femora 5–5–3–3, genua 4–4–3–3, tibiae 9(1)–(8–9)–9–9; tarsi I with 12–14 tactile setae, two sets of duplex setae at distal end, two eupathidia and one/two solenidion; tarsi II with 8–9 tactile setae, one set of duplex setae, two eupathidia and one solenidion; tarsi III with 8–9 tactile setae and one solenidion; tarsi IV with 9 tactile setae and one solenidion. True claws pad like each with one pair of tenant hair; empodium pad–like with two rows of small tenant hairs.

Male. Not in collection.

Etymology. The specific epithet is derived from the region "Arabia" from where type specimens were collected.

Type material. Holotype and one paratype female, *P. juliflora* (Fabaceae), Deesa Valley, Dessa, Tabuk, SA, 27°36.048'N, 036°25.592'E, October, 18, 2015, coll. J.H. Mirza.; seven paratype females, *P. juliflora* (Fabaceae), Sharma, Near Red Sea, Tabuk, SA, 28°03.479'N, 035°17.186'E, October, 19, 2015, coll. M. Kamran.

Remarks. The *P.* (*A.*) *arabica* sp. n. relates to *P.* (*A.*) *prosopis* (Tuttle & Baker, 1964), *P.* (*A.*) *algarrobicola* (Gonzalez, 1977) and *P.* (*A.*) *boutelouae* Tuttle & Baker, 1968 because of sharing following similar characters: dorsal body setae spatulate and distinctly shorter to the distances of setae next behind and widely spaced dorsal hysterosomal striations. Also, the new species closely resembles *P.* (*A.*) *prosopis* by setae c_1 at least reaching half distance to the bases of setae d_1. However, the new species differs from all related species by having stylophore anteriorly with slight incision (notch). The new species is also distinguished from *P.* (*A.*) *prosopis* by setae c_1 reaching to the distance of setae d_1 (2/3 vs.1/2), setae c_1–c_1 almost 1.5 times widely spaced than setae f_1–f_1 vs. almost sub/equally spaced in *P.* (*A.*) *prosopis*. The new species can be separated from other related species *P.* (*A.*) *algarrobicola* and *P.* (*A.*) *bouteloua*e by the setae c_1 reaching 2/3 to the distance of d_1 vs. less than half as long as distances to the bases setae next behind in later species

***Paraplonobia* (*Anaplonobia*) *haloxylonia* sp. n.**

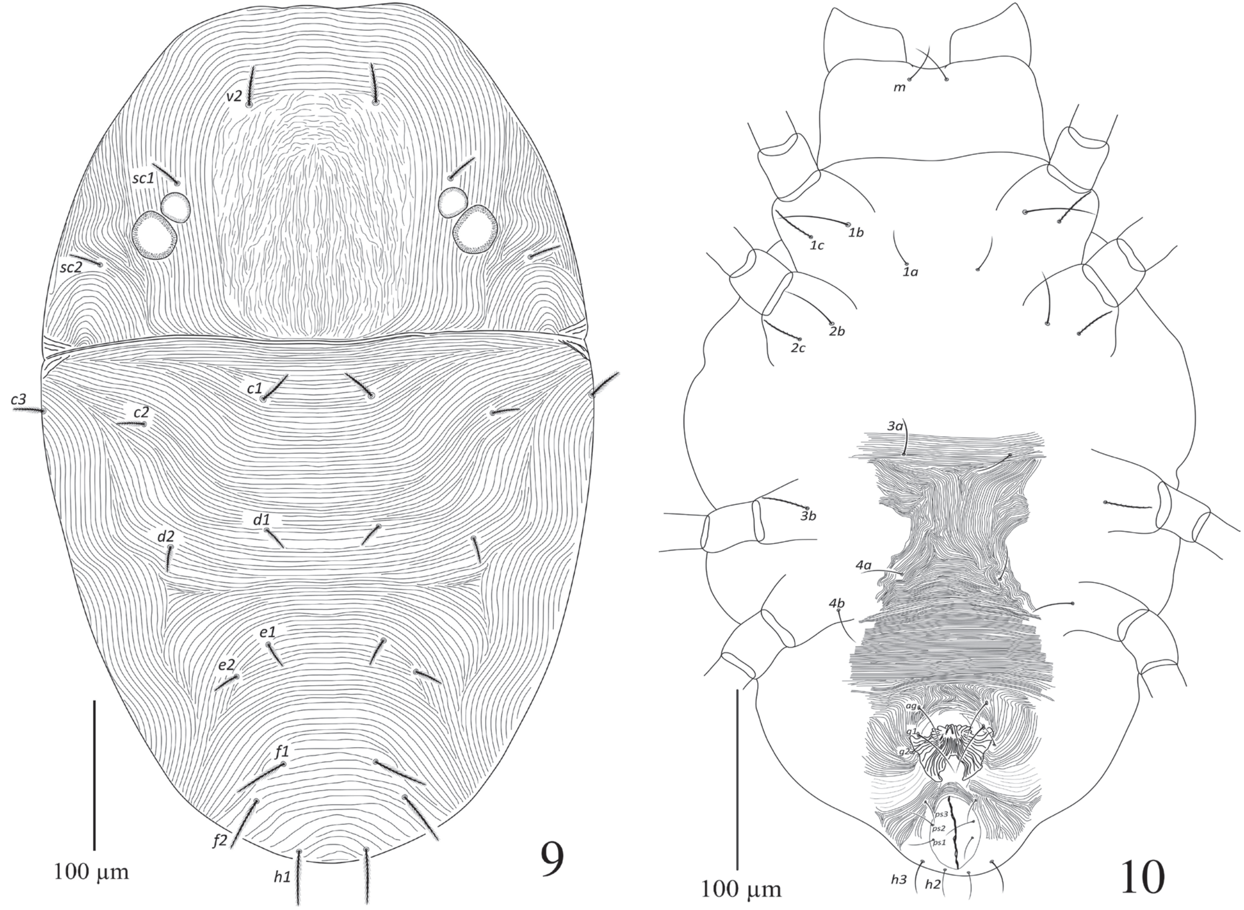
http://zoobank.org/09E8353-E635-4C38-B277-8D6DDC56C31A
Figs 9–28

Diagnosis. Dorsal setae lanceolate, densely serrate, not set on tubercles and distinctly shorter to the distances of setae next behind, dorsocentral setae (c1, d1 and e1) almost 1/3 to the distance of setae next behind, propodosoma medially with weak, longitudinal irregular striations, hysterosoma with transverse and closely spacedstriations medially, stylophore slightly notched anteriorly, peritremes anastomosed distally, with few long thread like branches, and hysterosomal striations closely spaced, leg I shorter than body.

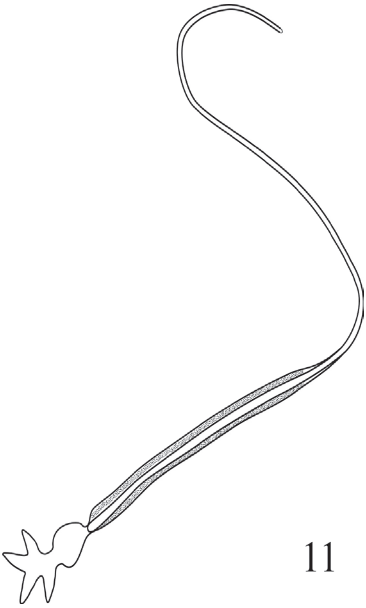
Description of holotype female (n = 39). Measurements of holotype followed by 38 paratypes (in parenthesis) (Figs 9–19).

Dorsum (Fig. 9)**.** Body oval; length of idiosoma 583 (578–585), maximum width 372 (369–378), length of body (gnathosoma + idiosoma) 658 (655–663). Propodosoma medially with weak, longitudinal irregular striations; laterally longitudinal regular striations; hysterosomal striations medially transverse and closely spaced, laterally longitudinal irregular. Dorsal setae lanceolate, densely serrate, not present on tubercles and distinctly shorter to the distances of setae next behind, dorsocentral setae (c1, d1 and e1) almost 1/3 to the distance of setae next behind, Length of dorsal setae: v_2 28 (26–29), sc_1 24 (23–25), sc_2 22 (21–24), c_1 19 (18–21), c_2 22 (21–23), c_3 25 (24–28), d_1 15 (12–16), d_2 18 (17–19), e_1 16 (15–17), e_2 20 (19–20), f_1 25 (24–28), f_2 31 (29–32), h_1 34 (32–35). Distance between dorsal setae: v_2–v_2 72 (70–73), v_2–sc_1 75 (72–78), sc_1–sc_2 66 (63–67), sc_1–sc_1 167 (163–172), sc_2–sc_2 254 (250–259), c_1–c_1 90 (88–92), c_1–c_2 75 (71–78), c_2–c_3 81 (78–85), c_2–c_2 231 (229–234), c_3–c_3 373 (372–375), d_1–d_1 91 (89–92), d_1–d_2 65 (62–69), d_2–d_2 204 (201–206), c_1–d_1 103 (100–104), c_3–d_2 160 (158–161), e_1–e_1 55 (53–57), e_1–e_2 53 (50–54), e_2–d_2 85 (82–86), e_2–e_2 150 (148–152), f_1–f_1 60 (59–62), f_2–f_2 80 (78–83), f_1–f_2 28 (25–29), f_1–d_1 93 (91–94), h_1–h_1 31 (28–32).

Venter (Figs 10–12). Idiosoma ventrally with transverse simple striations from setae 1a to 3a; longitudinal irregular between setae 3a and 4a; transverse posterior to setae 4a; striations longitudinal irregular anterior to aggenital setae (ag). Length of intercoxal and coxal setae: 1a 25 (24–26), 3a 19 (19–21), 4a 22 (21–23), 1b 33 (31–33), 1c 22 (21–24), 2b 24(23–25), 2c 22 (21–23), 3b 23 (22–24), 4b 27 (26–28); aggenital setae, ag 28 (27–28), ag–ag 32 (29–32); genital setae two pairs, g_1 31 (30–33), g_2 20 (19–21), g_1–g_1 32 (31–33), g_2–g_2 35 (34–36), g_1–g_2 10 (10–12); anal setae three pairs, ps_1 11 (10–12), ps_2



Figures 9, 10. *Paraplonobia* (*Anaplonobia*) *haloxylonia* sp. n. adult female. **9** dorsum **10** venter.



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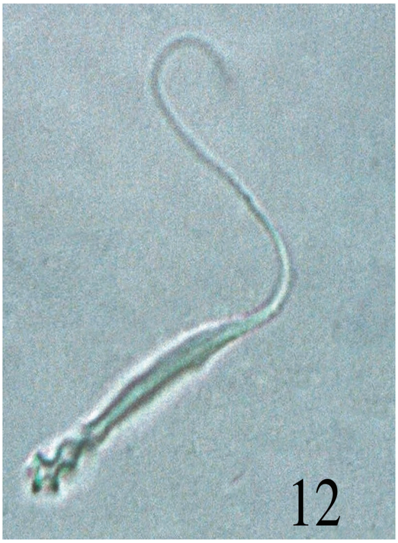

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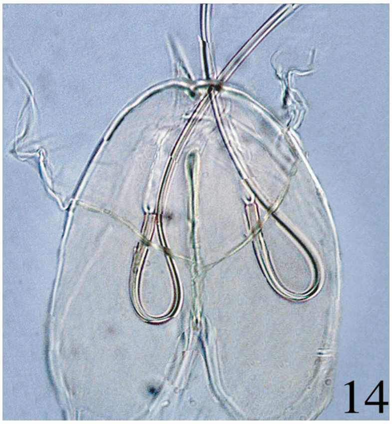

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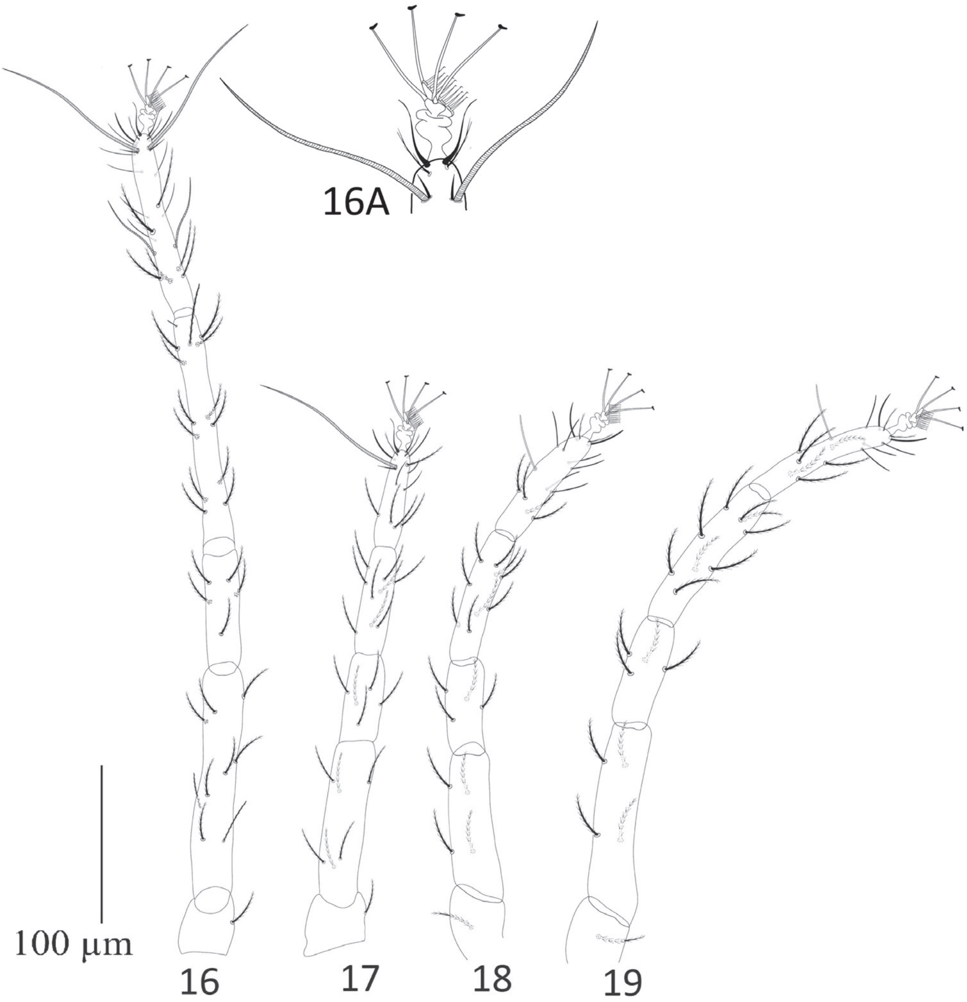
Figures 11–15. *Paraplonobia* (*Anaplonobia*) *haloxylonia* sp. n. adult female. **11, 12** spermatheca **13, 14** stylophore and peritremes **15** palp.

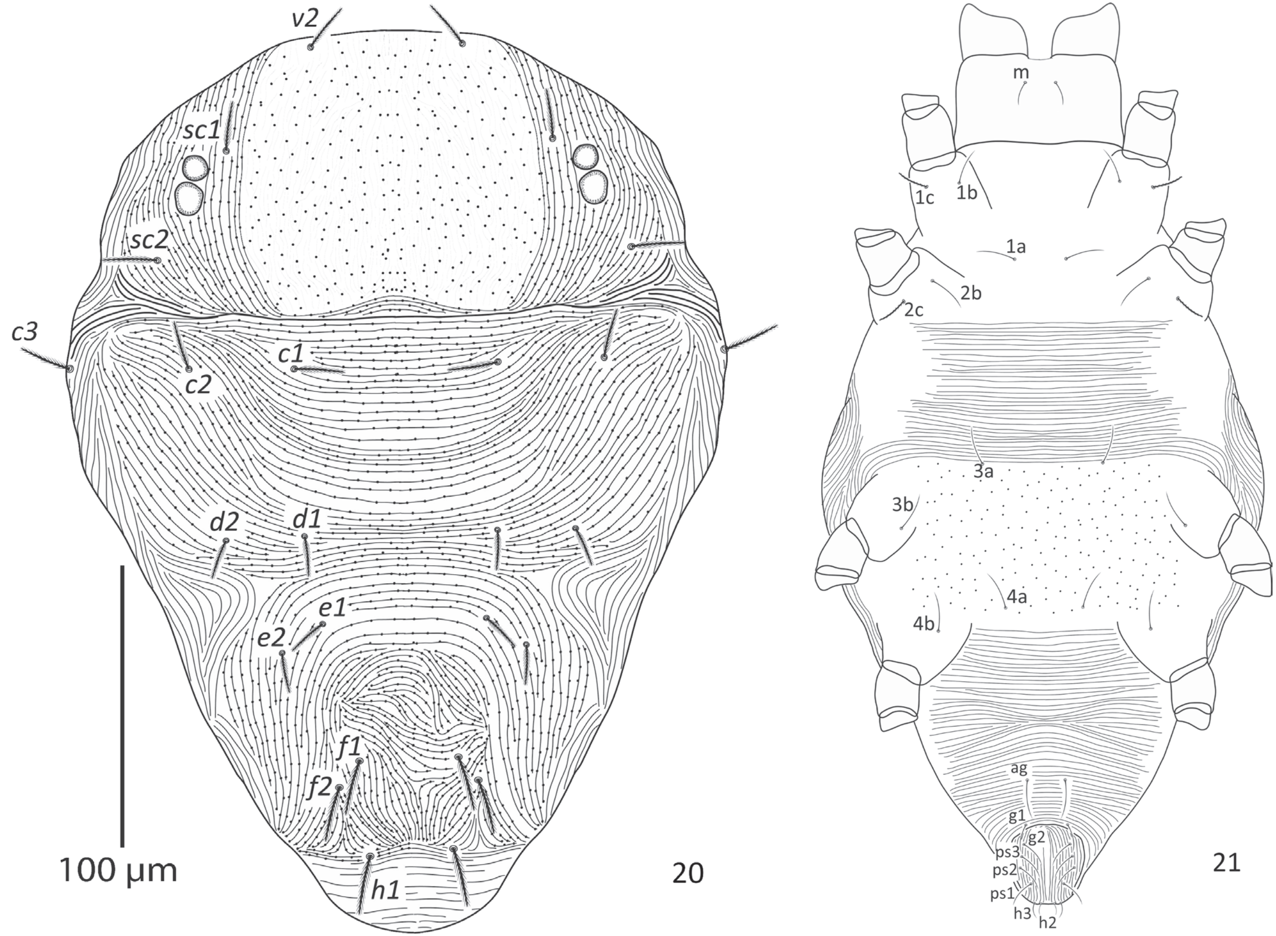


Figures 16–19. *Paraplonobia* (*Anaplonobia*) *haloxylonia* sp. n. **16** legI **16A** duplex setae, empodium and claws of tarsus I **17** leg II **18** leg III **19** leg IV.

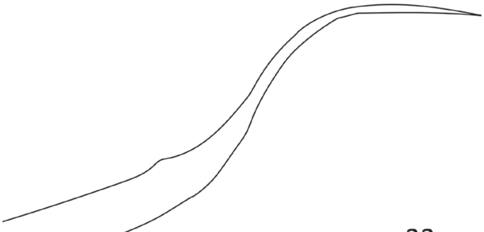
16 (15–17), ps_3 17(16–18), ps_1–ps_1 16 (15–18), ps_2–ps_2 22 (20–23), ps_3–ps_3 26 (25–26); para–anal setae two pairs, h_2 16 (15–17), h_{2-} h_2 14 (13–16), h_3 17 (15–17), h_3–h_3 31 (30–32) (Fig. 10). Spermathecae elongate, star shaped structure at distal end (Fig. 11–12).

Gnathosoma (Figs 13–15). Stylophore slightly notched anteriorly. Peritremes anastomosed distally, with few long thread like branches (Figs 13–14). Scapular setae m 22 (21–23), m–m 17 (16–18). Palp femur and genu each with one seta, palp tibia with three setae, tibial claw strongly curved; palp tarsus with three setae, three eupathidia, one solenidion (Fig. 15).

Legs (Figs 16–19). Length of legs I–IV (without coxae) 507, 328, 340, 400 respectively. Leg I shorter than body length. Number of setae and solenidia (in parenthesis)



Figures 20, 21. *Paraplonobia* (*Anaplonobia*) *haloxylonia* sp. n. adult male. **20** dorsum **21** venter.



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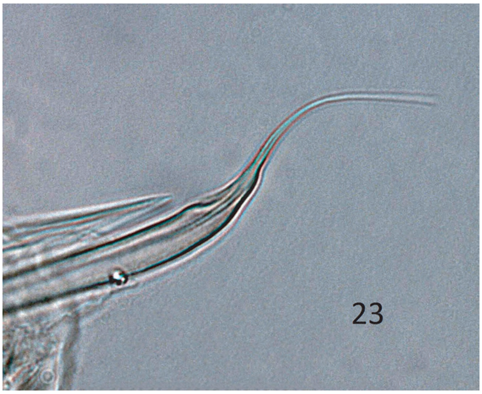

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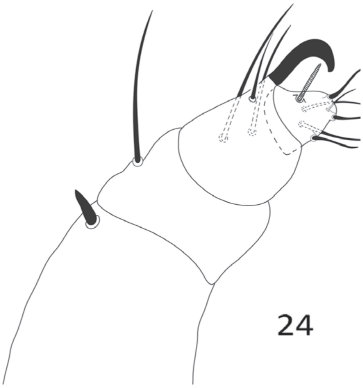


24

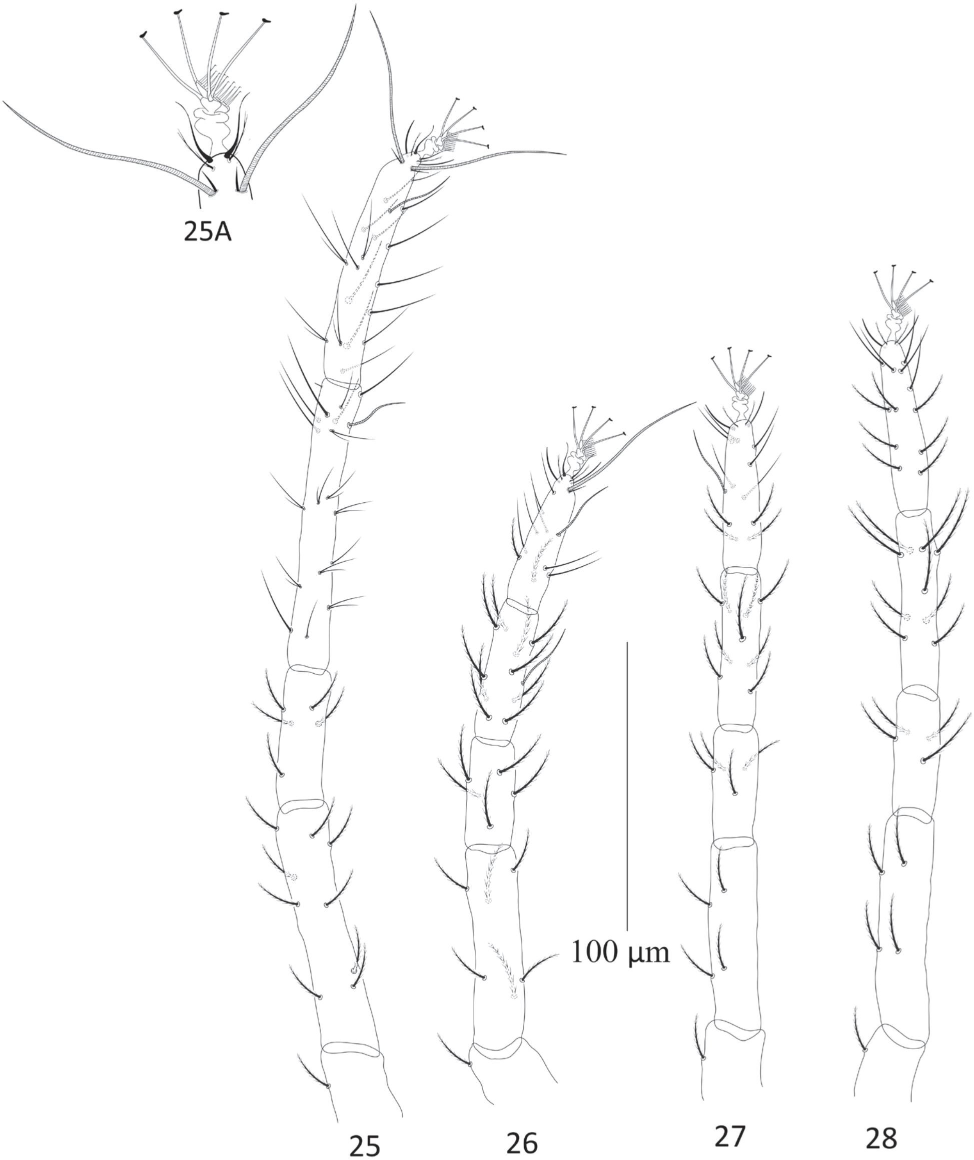
Figures 22–24. *Paraplonobia* (*Anaplonobia*) *haloxylonia* sp. n. adult male. **22–23** aedeagus **24** palp.

on legs I–IV: coxae 2–2–1–1, trochanters 1–1–1–1, femora 9–6–4–4, genua 5–5–4–4, tibiae 13(1)–9–9–9; tarsi I with 15 tactile setae, two sets of duplex setae at distal end, 11 tactile setae and two solenidia well proximal to duplex setae, two eupathidia; tarsi II with 10 tactile setae, one set of duplex setae, two eupathidia and one solenidion; tarsi III with 12 tactile setae and one solenidion; tarsi IV with 12 tactile setae and one solenidion. True claws pad like each with one pair of tenant hair; empodium pad-like with two rows of small tenant hairs.

Male (n = 11) (Figs 20–28). **Dorsum** (Fig. 20). Body almost oval, slightly tapering caudally; idiosoma 320–325 long, 190 wide; striations on dorsum entirely dotted; propodosomal striations same as in female, hysterosomal also same as in female except longitudinal/oblique or irregular in the area medially between dorsal setae e1 and h1, shape of setae also same as in female.

Venter (Figs 21–23). Idiosoma ventrally with transverse striations except in the area between ventral setae 3a and 4a and genito-anal area; the area between 3a and 4a with simple dots (without striations); genito-anal setae five pair, genital setae two pairs (g_1, g_2), anal setae three pairs (ps_1, ps_2, ps_3); para-anal setae two pairs (h_2, h_3); aedeagus up turned, broadly sigmoid, sharply tapering distally (Figs 22–23).

Gnathosoma. Stylophore and peritremes as in female; palp femur with small horn-like seta, palp genu with one dorsal seta, palp tibia with three setae and strongly curved tibial claw; palp tarsus thumb like with one solenidion, three eupathidia and three setae (Fig. 24).



Figures 25–28. *Paraplonobia* (*Anaplonobia*) *haloxylonia* sp. n. adult male. **25** leg I **25A** duplex setae, empodium and claws of tarsus I **26** leg II **27** leg III **28** leg IV.

Legs (Figs 25–28). Length of leg I–IV (without coxae) 366, 223, 250, 289 respectively. Setae with solenidion in parenthesis on legs I–IV as; coxae 2–2–1–1, trochanters 1–1–1–1, femora 9–6–4–4, genua 5–5–4–4, tibiae 9(2)+8duplex–10(1)–9–9, tarsus I with six pairs of duplex setae (two pairs distally, two pairs at mid and two pairs at proximal part of the tarsus), 15 tactile setae, two eupathidia, one solenidion, tarsus II with one duplex seta, nine tactile setae, two eupathidia, one solenidion, tarsus III with

12 tactile setae, one solenidion, tarsus IV with 13 tactile setae, one solenidion. True claws pad like each with one pair of tenant hair; empodium pad-like with two rows of small tenant hairs.

Etymology. The specific epithet is derived after the host plant, *Haloxylon salicornicum* from which some type specimens were collected.

Type material. Holotype female, one male and two female paratypes, *H. salicornicum* (Amaranthaceae), Salbookh Road, Dariyah, Riyadh, SA, 24°30.649'N, 46°46.615'E, September, 18, 2012, coll. M. Kamran; four males and 22 female paratypes, *Hilaria* sp. (Poaceae), Tashlia, Heyer Road, Riyadh, SA, 24°29.000'N, 46°47.890'E, January, 17, 2015, coll. J.H. Mirza; five males and four females paratypes, *Hilaria* spp. (Poaceae), Sanabal Farm, Kharaj, Riyadh, SA, 24°16.999'N, 47°11.854'E, January, 23, 2015, coll. M. Kamran.

Remarks. The *P.* (*A.*) *haloxylonia* sp. n. closely resembles *P.* (*A.*) *contiguus* (Chaudhri, Akbar and Rasool 1974) because both species sharing the following set of similar characters; peritremes distally with few branches, dorsal body setae short, subequal in length, lanceolate, prodorsal shield entirely with longitudinal striaitons and hysterosomal striations closely spaced. The new species, differs from *P.* (*A.*) *contiguus* by comparative length of leg I (shorter than body vs. longer than body), dorsocentral setae (c1, d1 and e1) almost 1/3 to the distance of setae next behind vs. more than half, number of setae on genu I (5 vs. 4) in *P.* (*A.*) *contiguus*.

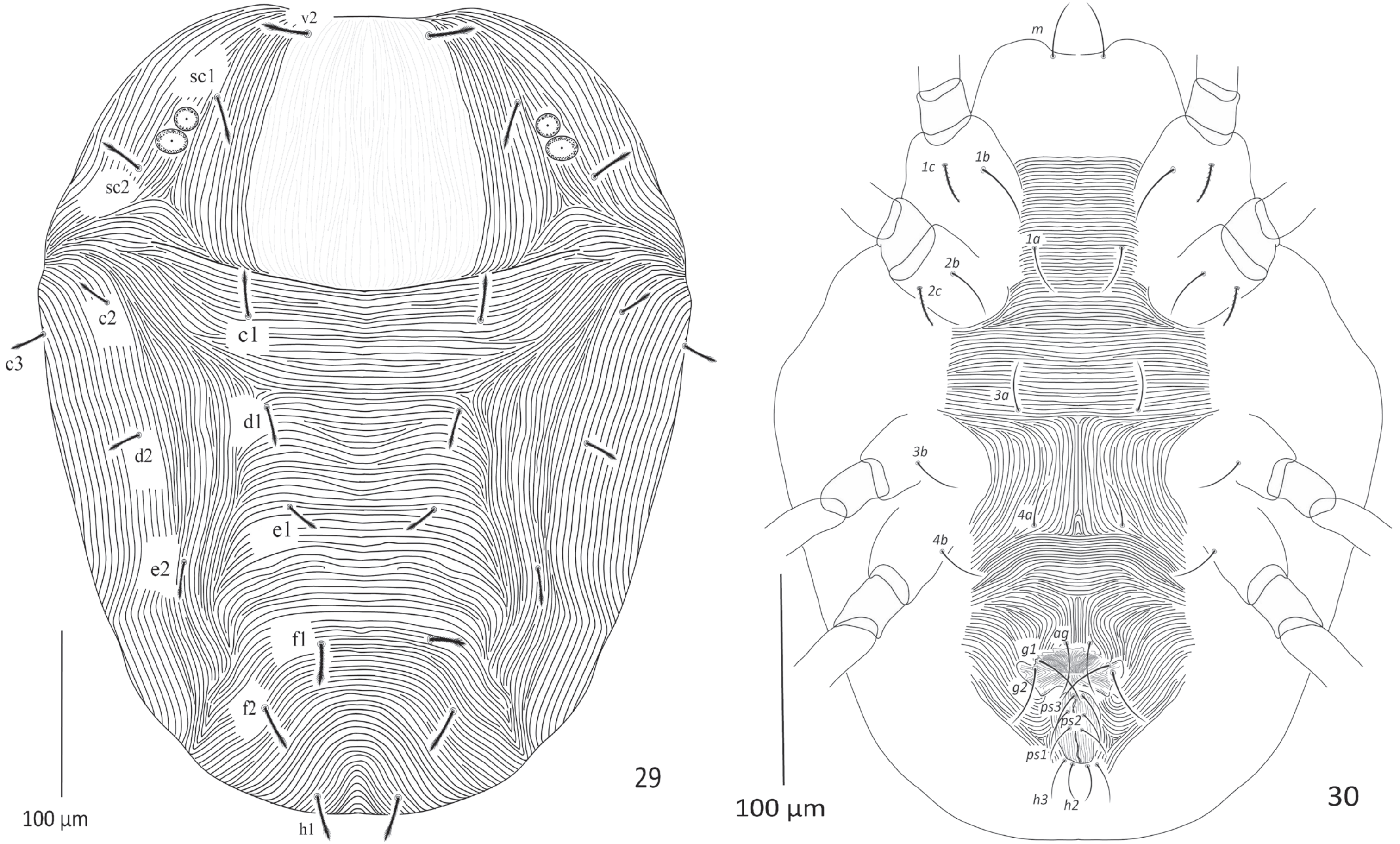
***Paraplonobia* (*Anaplonobia*) *tabukensis* sp. n.**

http://zoobank.org/57BF2D3A-80B0-4C7E-90CD-FACB4543B5FF
Figs 29–36

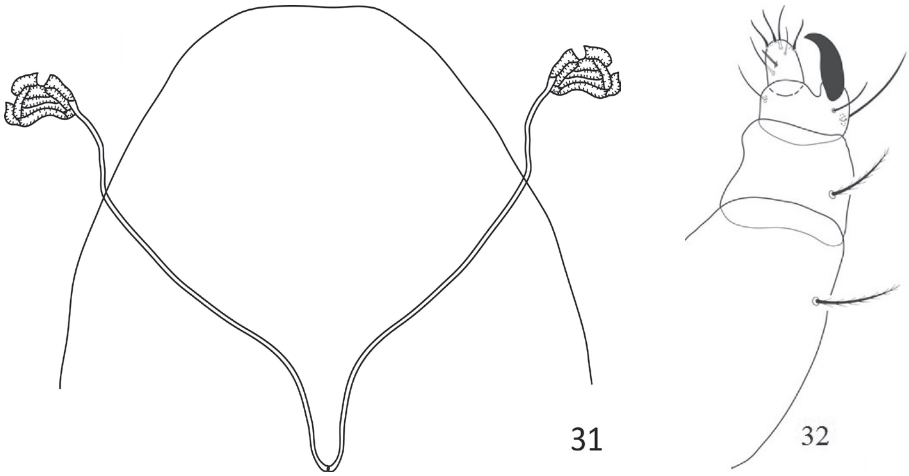
Diagnosis. Dorsal setae slightly lanceolate, densely serrate, not present on tubercles and distinctly shorter to the distances of setae next behind, prodorsum entirely with longitudinal striaitons, hysterosomal striations closely spaced, peritremes complex anastomosed distally, stylophore slightly rounded anteriorly, leg I shorter than body length, number of setae on femur I–IV 8–6–3–3, number of setae on genu I–IV 4–5–3–3.

Description of holotype female (n = 3). Measurements of holotype followed by 2 paratypes (in parenthesis) (Figs 29–36).

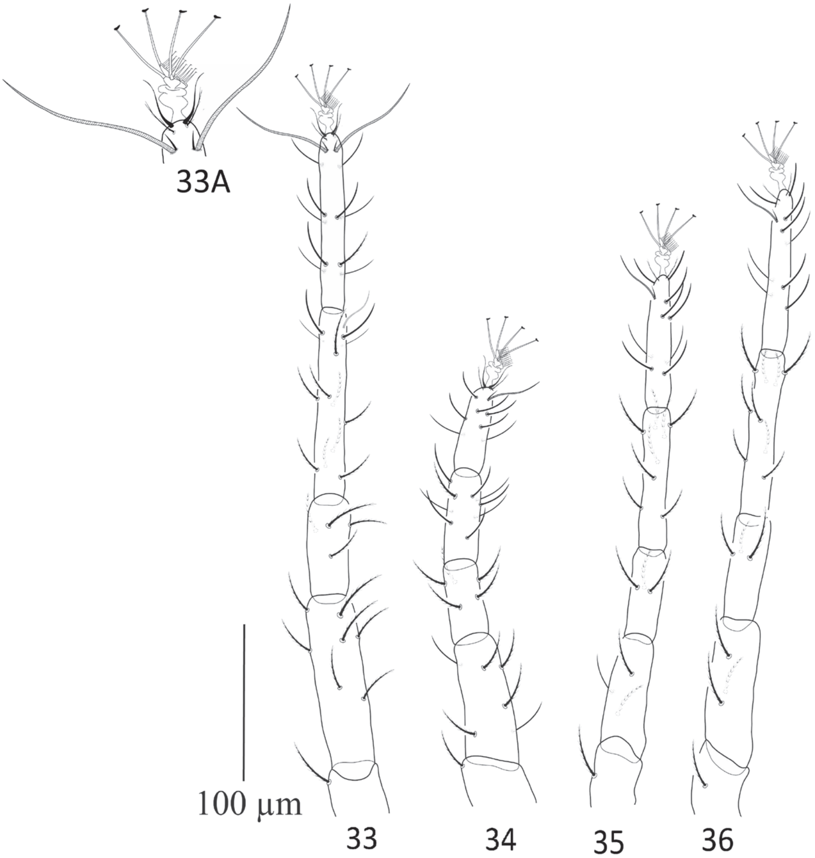
Dorsum (Fig. 29). Body rounded; length of idiosoma 483 (480–490), maximum width 445 (440–450), length of body (gnathosoma + idiosoma) 595 (590–610). Propodosoma medially with weak and laterally with strong longitudinal regular striations; hysterosomal striations medially transverse and closely spaced, laterally longitudinal irregular. Dorsal setae slightly lanceolate, densely serrate, not present on tubercles and and distinctly shorter to the distances of setae next behind, dorsocentral setae (c1, d1 and e1) almost 1/3 to the distance of setae next behind. Length of dorsal setae: v_2 34 (32–36), sc_1 29 (28–31), sc_2 30 (28–32), c_1 28 (26–30), c_2 26 (24–28), c_3 29 (28–32), d_1 23 (21–25), d_2 22 (21–24), e_1 21 (20–23), e_2 22 (21–24), f_1 23 (21–24), f_2 26 (24–27), h_1 27 (25–29). Distance between dorsal setae: v_2–v_2 89 (85–90), v_2–sc_1 68



Figures 29, 30. *Paraplonobia* (*Anaplonobia*) *tabukensis* sp. n. adult female **29** dorsum **30** venter.



Figures 31, 32. *Paraplonobia* (*Anaplonobia*) *tabukensis* sp. n. adult female. **31** Stylophore and peritremes **32** palp.



Figures 33–36. *Paraplonobia* (*Anaplonobia*) *tabukensis* sp. n. **33** leg I **33A** duplex setae, empodium and claws of tarsus I **34** leg II **35** leg III **36** leg IV.

(65–690), sc_1–sc_2 68 (67–70), sc_1–sc_1 204 (202–206), sc_2–sc_2 301 298–302), c_1–c_1 138 (135–140), c_1–c_2 91 (89–92), c_2–c_3 79 (75–80), c_2–c_2 327 (325–328), c_3–c_3 424 (422–426), d_1–d_1 119 (118–120), d_1–d_2 91 (89–92), d_2–d_2 295 (292–298), c_1–d_1 88 (86–89), c_3–d_2 110 (109–112), e_1–e_1 27 (25–28), e_1–e_2 85 (84–86), e_2–d_2 85 (84–86), e_2–e_2 229 (228–231), f_1–f_1 78 (76–80), f_2–f_2 113 (110–114), f_1–f_2 35 (33–36), f_1–d_1 82 (81–84), h_1–h_1 53 (52–56).

Venter (Fig. 30). Idiosoma ventrally with transverse simple striations from setae 1a to 3a; longitudinal regular between setae 3a and 4a; transverse posterior to setae 4a; striations longitudinal regular anterior to aggenital setae (ag). Length of intercoxal and coxal setae: 1a 40 (38–42), 3a 32 (31–34), 4a 32 (30–35), 1b 46 (44–47), 1c 32 (31–34), 2b 30 (29–34), 2c 29 (28–31), 3b 32 (31–34), 4b 32 (31–35); aggenital setae (ag) 42 (41–45), ag–ag 23 (21–25); genital setae two pairs, g_1 43 (40–44), g_2 39 (35–40), g_1–g_1 52 (50–55), g_2–g_2 60 (58–64), g_1–g_2 12 (10–13); anal setae three pairs, ps_1 20 (18–21), ps_2 26 (24–27), ps_3 28 (27–29), ps_1–ps_1 23 (20–24), ps_2–ps_2 32 (31–35), ps_3–ps_3 23 (21–26); para-anal setae two pairs, h_2 27 (26–28), h_{2-} h_2 11 (10–13), h_3 32 (31–34), h_3–h_3 28 (27–29).

Gnathosoma (Figs 31–32). Stylophore rounded anteriorly. Peritremes small compact anastomosed distally (Fig. 31). Scapular setae m 28 (27–29), m–m 37 (26–39). Palp femur and genu each with one seta, palp tibia with three setae, tibial claw strongly curved; palp tarsus with three setae, three eupathidia, one solenidion (Fig. 32).

Legs (Figs 33–36). Length of legs I–IV (without coxae) 450, 282, 345, 408 respectively. Leg I shorter than body length. Number of setae and solenidia (in parenthesis) on legs I–IV: coxae 2–2–1–1, trochanters 1–1–1–1, femora 8–6–3–3, genua 4–5–3–3, tibiae 13(1)–9–8–8; tarsi I with 10 tactile setae, two sets of duplex setae at distal end, all tactile setae well proximal to duplex setae, two eupathidia; tarsi II with 7 tactile setae, one set of duplex setae, two eupathidia; tarsi III with 11 tactile setae, one set of duplex setae,; tarsi IV with 11 tactile setae one set of duplex setae,. True claws pad like each with one pair of tenant hair; empodium pad-like with two rows of small tenant hairs.

Male. Not in collection.

Etymology. The specific epithet is derived from the region of Saudi Arabia, Tabuk, from where it was collected.

Type material. Holotype female, two paratype females, *H. salicornicum* (Amaranthaceae), 30 km Tabuk road, Sharma, Tabuk region, SA, 28°03.479'N, 035°17.186'E, October, 19, 2015, coll. M. Kamran and J.H. Mirza.

Remarks. The *P.* (*A.*) *tabukensis* sp. n. closely resembles *P.* (*A.*) *theroni* (Meyer 1974) because both species share the following set of similar characters; dorsal body setae, lanceolate and distinctly shorter to the distances of setae next behind, prodorsum entirely with longitudinal striaitons, hysterosomal striations closely spaced, peritremes complex anastomosed distally (Meyer 1974, 1987). The new species differs from *P.* (*A.*) *theroni* by shape of stylophore anteriorly (rounded vs. slightly indented), number of setae on femur I–IV (8–6–3–3 vs. 9–6–4–4), number of setae on genu I–IV (4–5–3–3 vs. 5–5–6–6), number of setae on tibia III (8 vs. 6) and on tarsi I–II excluding duplex setae and solenidia (10–7 vs. 18–14) in *P.* (*A.*) *theroni*.

Key to the world species of the genus *Paraplonobia* (Prostigmata: Tetranychidae) (after Meyer 1987).

1 Coxal formula not exceeding 3–3–1–1 .... **2**

– Coxal formula 4–3–2–2, dorsal body setae serrate pointed at the tip not set on tubercles, peritremes simple, empodial pad and true claws equal in length .... **subg. *Brachynychus*, species *P.* (*B.*) *cousiniae* (Mitrofanov & Strunk.)**

2 Peritremes anastomosed .... **subg. *Anaplonobia*, 11**

– Peritremes simple .... **subg. *Paraplonobia*, 3**

3 Stylophore rounded anteriorly **4**

– Stylophore notched anteriorly **5**

4 Dorsal body setae slightly lanceolate, leg I shorter that body .... ***P.* (*P.*) *edenvillensis* Meyer**

– Dorsal body setae slender, leg I about as long as body .... ***P.* (*P.*) *myops* (Pritchard & Baker)**

5 Dorsal body setae generally slender or slightly lanceolate and pointed distally **6**

– Dorsal body setae broadly lanceolate **9**

6 First three pair of dorsocentral setae c_1, d_1 and e_1 about half as long as distance between bases of consecutive setae **7**

– First three pair of dorsocentral setae c_1, d_1 and e_1 minute about a third to a fourth as long as the distance between bases of consecutive setae **8**

7 Length of body 466 µm (530 µm including gnathosoma), leg I as long as body, posterior opisthosomal setae longer than longitudinal distance between their bases .... ***P.* (*P.*) *hilariae* Tuttle & Baker**

– Length of body 380 µm, leg I 160 µm long, shorter than body, posterior opisthosomal setae shorter than longitudinal distance between their bases .... ***P.* (*P.*) *herniariae* (Bagdasarian)**

8 Body elongate, length of body 345 µm, length of leg I 191 µm (without coxa and trochanter) .... ***P.* (*P.*) *boutelouae* Baker & Tuttle**

– Body oval, length of body 570 µm, length of leg I 419 µm (without coxa and trochanter) .... ***P.* (*P.*) *dactyloni* Smiley & Baker**

9 Dorsocentral setae (c1, d1, e1 and f1) more than half as long as distances between consecutive setae, leg I shorter than body **10**

– Dorsocentral setae (c1, d1, e1 and f1) almost half as long as distances between consecutive setae, leg I shorter than body .... ***P.* (*P.*) *tridens* Tuttle & Baker**

10 Peritremes terminating in a ball-like rounded structure; prodorsum with a wellmarked punctate shield; tibia IV with 8 setae .... ***P.* (*P.*) *penicillatus* Chaudhri et al.**

– Peritremes terminating in oval shaped structure; prodorsum without a well-marked punctate shield; tibia IV with 7 setae .... ***P.* (*P.*) *echinopsili* (Wainstein)**

11 Dorsal body setae slightly shorter/as long as/ longer than distances between their bases **28**

– Dorsal setae distinctly shorter than distances between their bases **12**

12 Dorsal integument striated, without tubercles or lumps **13**

– Dorsal integument provided with tubercles or lumps forming a distinct pattern along with striation .... ***P.* (*A.*) *glebulenta* (Meyer)**

13 Dorsal body setae slender, setiform **14**

– Dorsal body setae broadly spatulate, subspatulate or lanceolate **15**

14 Stylophore indented anteriorly, dorsocentral setae c_1, d_1 and e_1 about 2/3 of the distance between their basis, peritremes weakly anastomosed .... ***P.* (*A.*) *inornata* (Meyer)**

– Stylophore rounded anteriorly, dorsocentral setae c_1, d_1 and e_1 about half the distance between, peritremes strongly anastomosed, stylophore rounded anteriorly. .... ***P.* (*A.*) *ambrosiae* (Tuttle et al.)**

15 All dorsal body setae spatulate, subspatulate, expanded distally **16**

– Most of dorsal body setae lanceolate, not expanded distally **19**

16 First pair of dorsocentral setae c_1 less than half as long as distances to the bases setae next behind **17**

– First pair of dorsocentral setae c1 at least reaching 1/2 or 2/3 of distance to the bases of setae next behind **18**

17 Prodorsum medially with irregular broken striations................................. .... ***P.* (*A.*) *boutelouae* Tuttle & Baker**

– Prodorsum medially with regular longitudinal striations .... ***P.* (*A.*) *algarrobicola* (Gonzalez)**

18 First pair of dorsocentral setae c1 reaching one half to the distance of setae next behind, setae c1 and f1 almost sub/equally spaced............................... .... ***P.* (*A.*) *prosopis* (Tuttle & Baker)**

– First pair of dorsocentral setae c1 reaching 2/3 to the distance of setae next behind, setae c1 almost 1.5 times widely spaced than setae f1 .... ***P.* (*A.*) *arabica* sp. n.**

19 Hysterosomal setae d1and e1 lanceolate and about half as long as f1, setae f1 spatulate .... ***P.* (*A.*) *brickellia* Baker & Tuttle**

– Dorsocentral setae subequal in length, lanceolate serrate **20**

20 Prodorsum entirely with longitudinal striations **21**

– Median area of prodorsum entirely/partially with transverse striations...... **26**

21 Peritremes ending with few irregular branches **22**

– Peritremes distally with complex anastomosed.. **24**

22 Stylophore slightly indented anteriorly, dorsum with closely spaced striations **23**

– Stylophore rounded anteriorly, dorsum with widely spaced striations .... ***P.* (*A.*) *acharis* (Pritchard & Baker)**

23 Leg I distinctly longer than the body, first pair of dorsocentral setae c1 more than half to the distance of setae next behind .... ***P.* (*A.*) *contiguus*** **(Chaudhri et al.)**

– Leg I shorter than body, first pair dorsocentral setae c1 1/3 to the distance of setae next behind .... ***P.* (*A.*) *haloxylonia*** **sp. n.**

24 Dorsum with widely spaced striaitons, femora I with 11 setae .... ***P.* (*A.*) *candicans*** **(Meyer)**

– Dorsum with closely spaced striations, femora I with 8 or 9 setae **25**

25 Stylophore rounded anteriorly, setae on femora I–IV 8–6–3–3, setae of genua I–IV 4–5–3–3 .... ***P.* (*A.*) *tabukensis*** **sp. n.**

– Stylophore indented anteriorly, setae on femora I–IV 9–6–4–4, setae of genua I–IV 5–5–6–6 .... ***P.* (*A.*) *theroni*** **(Meyer)**

26 Propodosomal shield medially with two distinct bands of transverse striations .... ***P.* (*A.*) *daryaensis*** **Chaudhri et al.**

– Propodosomal shield entirely with transverse strations **27**

27 Leg I shorter than body, peritremes weakly anastomosed .... ***P.* (*A.*) *harteni*** **(Meyer)**

– Leg I longer than body, peritremes with complex anastomose...................... .... ***P.* (*A.*) *concolor*** **Chaudhri et al.**

28 Stylophore anteriorly rounded **29**

– Stylophore anteriorly deeply notched.................. ***P.* (*A.*) *tshipensis*** **(Meyer)**

29 Dorsal body setae slender/setifrom...**31**

– Dorsal body setae spatulate/subspatulate..**30**

30 Dorsal body setae set on tubercles, longer than the distances of setae next behind, propodosoma with broken striations .... ***P.* (*A.*) *juliflorae*** **(Tuttle & Baker)**

– Dorsal body setae not set on tubercles, as long as or slightly shorter to the distances of setae next behind, propodosoma medially with basket weaved pattern .... ***P.* (*A.*) *euphorbiae*** **(Tuttle & Baker)**

31 Opisthosomal setae much longer than the distance to the setae next in line .. .. ***P.* (*A.*) *coldeniae*** **(Tuttle & Baker)**

– Opisthosomal setae as long as the distance to the setae next in line **32**

32 Prodorsal shield pebbled, most of opisthosomal setae set on tubercles........... .... ***P.* (*A.*)*calame*** **(Pritchard & Baker)**

– Prodorsal shield tuberculate/striate, opisthosomal setae not set on tubercles...... **33**

33 Opisthosomal striations closely spaced with fine lobes .... ***P.* (*A.*) *artemisia*** **Baker & Tuttle**

– Prodorsal shield tuberculate 34

34 Opisthosomal striations mostly broad folds and covered with tubercles, peritreme small bulb like anastomosing .... ***P.* (*A.*) *berberis*** **Baker & Tuttle**

– Opisthomosal striations comparatively closely spaced with fine lobes, peritremes elongate anastomose .... ***P.* (*A.*) *allionia*** **Baker & Tuttle**

Genus *Neopetrobia* Wainstein, 1956

Monoceronychus: Pritchard and Baker 1955: 77.
Neopetrobia: Wainstein 1956: 151, Wainstein 1960a: 128, Tuttle and Baker 1968: 57, Meyer 1974: 93–94.

Type species. *Neopetrobia dubinini* Wainstein, 1956.

Diagnosis. Based on Baker and Tuttle 1968, Gutierrez 1955, Meyer 1974, Meyer 1987, and Bolland et al. 1998.

True claws pad like, each bearing a pair of tenant hairs; empodial pad longer than true claws, bearing a row of tenant hairs, distally not coalescent; dorsum with 3 pairs of prodorsal setae which are short and spindle shaped or spatulate; setal tubercles small or nonexistent; fourth pair of dorsocentral setae (f_1) widely spaced, not normal as c_1; peritremes anastomosing distally.

Subgenus *Neopetrobia* Wainstein

Diagnosis. Based on Gutierrez 1985, and Bolland et al. 1998.

Integument without tuberculate or reticulate pattern; dorsal setae rounded or spindle-shaped.

Neopetrobia mcgregori (Pritchard & Baker)

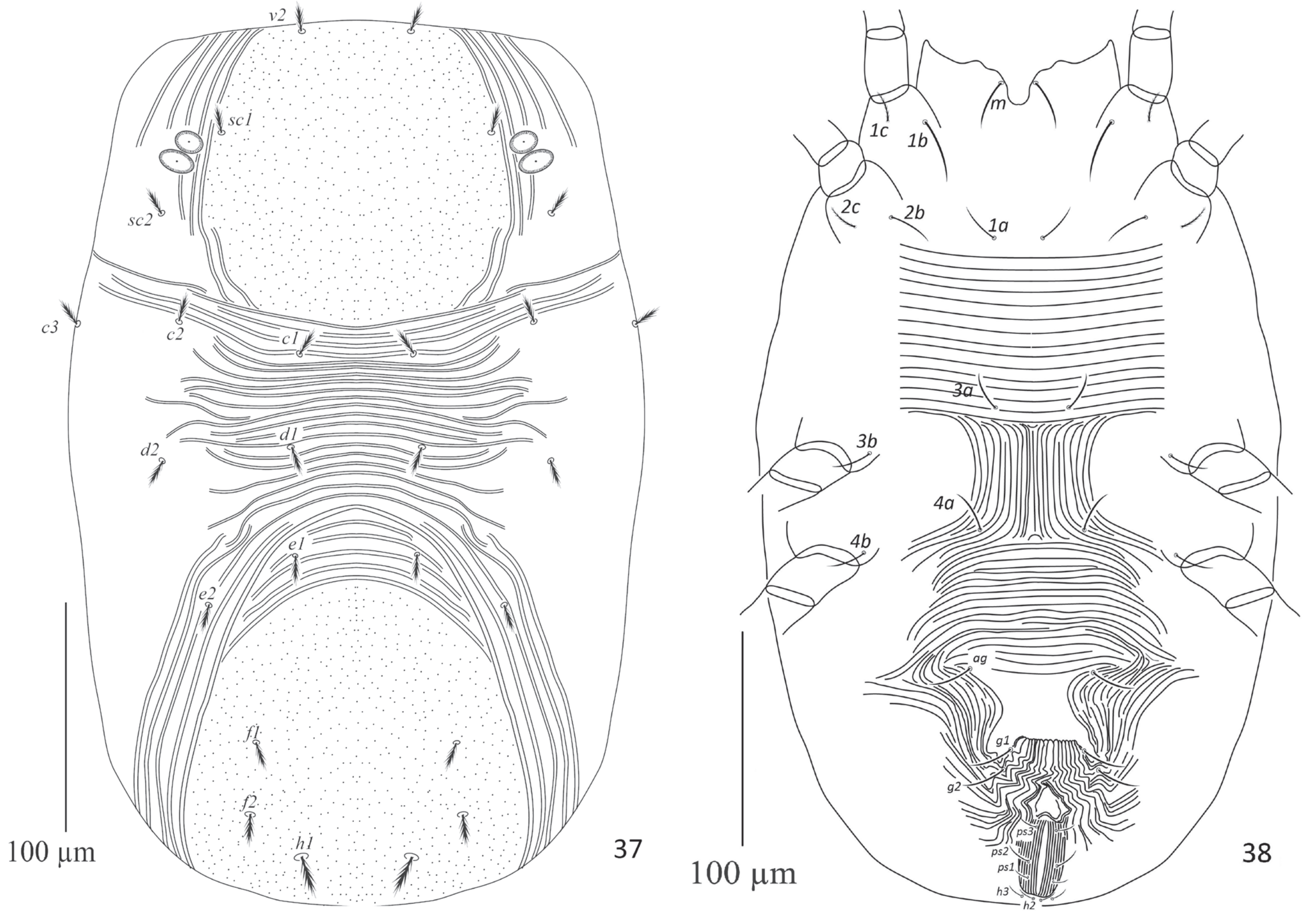
Figs 37–44

Monoceronychus mcgregori Pritchard & Baker, 1955.
Neopetrobia mcgregori (Pritchard & Baker) Meyer, 1987. Bolland et al. 1998.

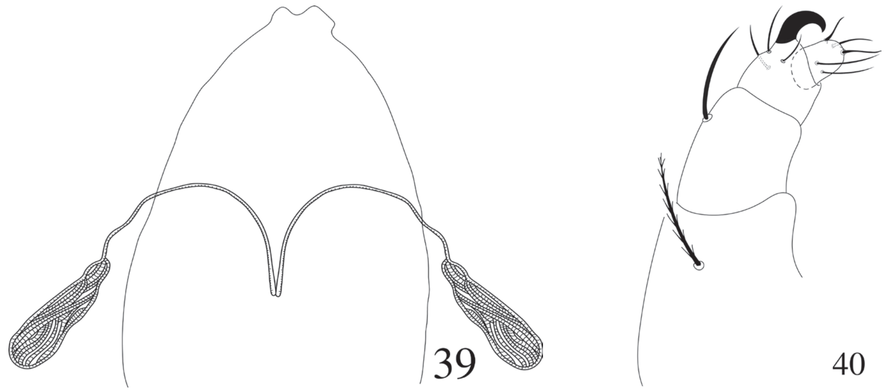
Redescription. Female (n=9). Body oval; length of idiosoma 369–372, maximum width 238–241, length of body (gnathosoma + idiosoma) 430–433.

Dorsum (Fig. 37)**.** Propodosoma without anterior projections. Dorsum of opisthosoma and most of opisthosoma with nearly smooth integument, metapodosomal dorsum with widely spaced strong striations. Dorsal body setae minute, lanceolate, densely serrate, not present on tubercles. Length of dorsal setae: v_2 13–14, sc_1 14–15, sc_2 13–14, c_1 13–14, c_2 12–13, c_3 10–11, d_1 11–12, d_2 12–13, e_1 10–11, e_2 12–13, f_1 11–12, f_2 15–16, h_1 16–17. Distance between dorsal setae: v_2–v_2 54–56, v_2–sc_1 48–50, sc_1–sc_2 47–50, sc_1–sc_1 113–114, sc_2–sc_2 165–167, c_1–c_1 57–58, c_1–c_2 50–52, c_2–c_3 41–42, c_2–c_2 161–162, c_3–c_3 234–236, d_1–d_1 57–58, d_1–d_2 56–57, d_2–d_2 160–161, c_1–d_1 57–58, c_3–d_2 79–80, e_1–e_1 54–56, e_1–e_2 45–47, e_2–d_2 64–66, e_2–e_2 135–136, f_1–f_1 80–82, f_2–f_2 86–88, f_1–f_2 31–32, f_1–d_1 79–80, h_1–h_1 38–40.

Venter (Fig. 38)**.** Idiosoma ventrally with transverse simple widely spaced striations from setae 1a to 3a; longitudinal regular between setae 3a and 4a; transverse pos-



Figures 37, 38. *Neopetrobia* (*Neopetrobia*) *mcgregori* (Pritchard & Baker) adult female. **37** dorsum **38** venter.



Figures 39, 40. *Neopetrobia* (*Neopetrobia*) *mcgregori* (Pritchard & Baker) adult female. **39** stylophore and peritremes **40** palp.

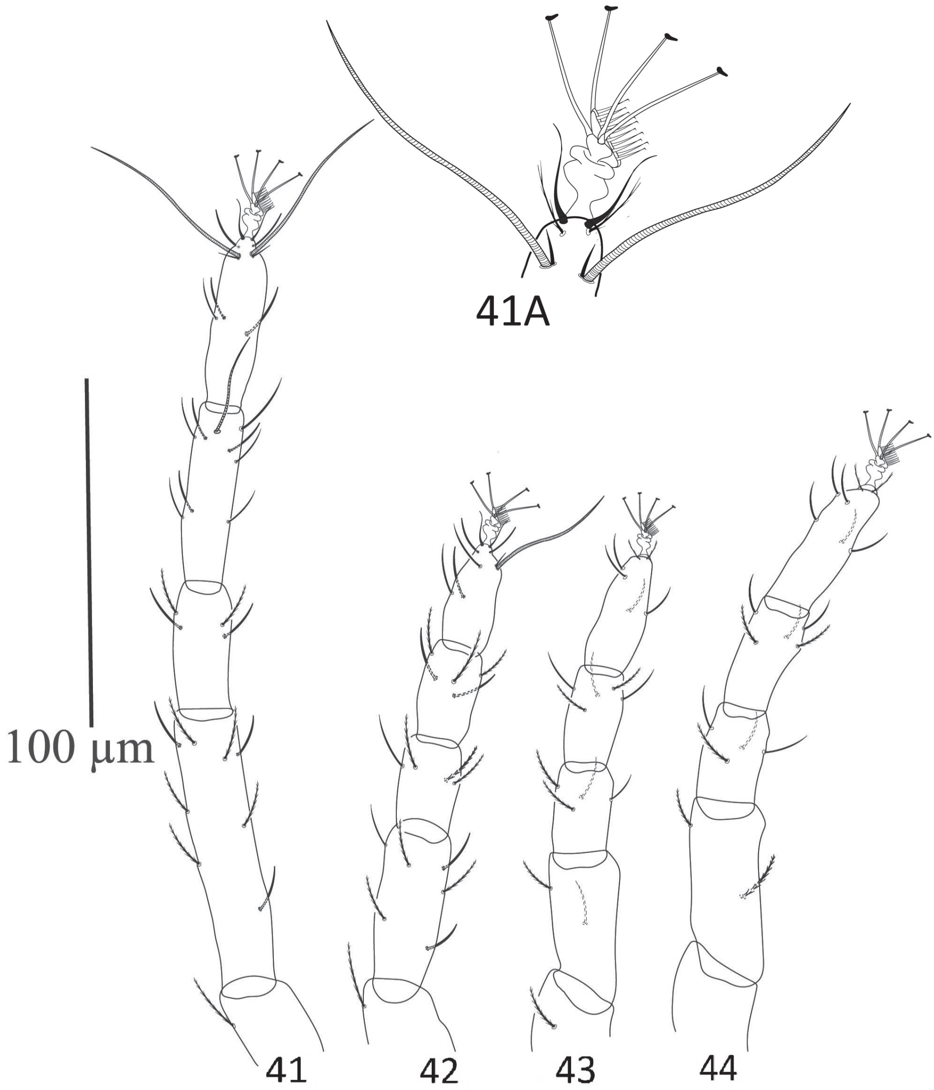
terior to setae 4a; striations transverse regular anterior to aggenital setae (ag). Length of intercoxal and coxal setae: 1a 18–19, 3a 19–20, 4a 15–16, 1b 30–31, 1c 13–14, 2b 16–17, 2c 10–13, 3b 15–17, 4b 11–12; aggenital setae (ag) 26–27, ag–ag 38–39; genital setae two pairs, g_1 17–18, g_2 21–22, g_1–g_1 41–42, g_2–g_2 76–78, g_1–g_2 21–22; anal setae three pairs, ps_1 11–12, ps_2 10–11, ps_3 12–13, ps_1–ps_1 11–13, ps_2–ps_2 16–18, ps_3–ps_3 11–13; para-anal setae two pairs, h_2 11–13, h_{2-} h_2 7–9, h_3 7–8, h_3–h_3 17–19.

Gnathosoma (Figs 39–40). Stylophore slender, the sides angularly converging anteriorly and with a small mediocephalic emargination. Peritremes anastomosing with distal enlargement slender. Scapular setae m 17–18, m–m 19–21. Palp femur and genu each with one seta, palp tibia with three setae, tibial claw strongly curved; palp tarsus with two setae, two eupathidia, one solenidion.

Legs (Fig 41–44). Length of legs I–IV (without coxae) 240, 150, 148, 180 respectively. Number of setae and solenidia (in parenthesis) on legs I–IV: coxae 2–2–1–1, trochanters 1–1–1–0, femora 8–6–2–2, genua 4–4–4–4, tibia 8(1)–9–9–9; tarsi I with 11 tactile setae, two sets of duplex setae at distal end, three setae proximal to duplex setae, two eupathidia and one solenidion; tarsi II with nine tactile setae, one set of duplex setae, two setae proximal to duplex setae, one setae in line with duplex setae, two eupathidia and one solenidion; tarsi III with six tactile setae; tarsi IV with seven tactile setae. True claws pad like each with one pair of tenant hair; empodium pad-like with two rows of small tenant hairs.

Materials examined. 12 females, *Cynodon dactylon* (Poaceae), near exit10, King Abdullah Road, Riyadh, SA, 24°45.826'N, 46°45.470'E, September 07, 2015, coll. M. Kamran and E. M. Khan.

Remarks. *Neopetrobia mcgregori* was originally described very briefly under the genus *Monoceronychus* and has been only reported from Miami shores of Florida, USA (Pritchard and Baker 1955). Later, it was moved to the genus *Neopetrobia* on the basis of widely spaced fourth pair of dorsocentral setae (f_1) (Bolland et al. 1998). Worldwide, this is the second report of this species and no obvious differences have been observed in Saudi Arabian specimens from the original description.



Figures 41–44. *Neopetrobia* (*Neopetrobia*) *mcgregori* (Pritchard and Baker) adult female. **41** leg I **41A** duplex setae, empodium and claws of tarsus I **42** leg II **43** leg III **44** leg IV.

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RESEARCH ARTICLE

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Redescription of *Gammarus pseudosyriacus* (Karaman & Pinkster, 1977) and description of a new subspecies from southern Iran (Crustacea, Amphipoda, Gammaridae)

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Abstract

The present study focused on redescription of *Gammarus pseudosyriacus* (Karaman & Pinkster, 1977) based on new materials from Zagros Mountains and describes a new subspecies of freshwater amphipod, *Gammarus pseudosyriacus issatisi* **subsp. n.**, from the southern Zagros Mountains. The work is based on morphological and morphometric comparisons. This new subspecies has features similar to *Gammarus pseudosyriacus*. The distinct features that distinguish *Gammarus pseudosyriacus issatisi* subsp. n. from *Gammarus pseudosyriacus* are the smaller eyes, shorter body length, and shorter flagellum of antenna 1 and 2.

Keywords

Taxonomy, amphipod, Zagros, Yazd, Fars, invertebrate, freshwater, *Gammarus pseudosyriacus*

Introduction

Gammarus Fabricius, 1775 is the largest genus among the amphipod genera and is widespread throughout the northern hemisphere (Karaman and Pinkster 1977). By 2008 more than 200 species of *Gammarus*, which have the highest diversity in Palearctic region, especially in the Mediterranean mountains and Near East, had been described (Väinölä et al. 2008). Until now 18 species of *Gammarus* have been reported from the freshwater regions of Iran (Zamanpoore et al. 2011).

Gammarus pseudosyriacus Karaman & Pinkster, 1977 is distributed in Syria (surroundings areas of Damascus), Turkey, Afghanistan (Karaman and Pinkster 1977). This species is also distributed in all parts of the Zagros Mountains in Iran: northern, central and southern Zagros (Zamanpoore et al. 2011). This species has a wide tolerance range to temperature (usually 5–21 °C), so it seems that this adaptation to different water temperatures is the main reason for its wide distribution (Zamanpoore et al. 2011).

Gammarus pseudosyriacus was described in Karaman and Pinkster (1977); however, because of the high number of new species described in their publication, all descriptions including that of *G. pseudosyriacus* are minimal. Likewise, few illustrations of body parts were provided. This may cause problems in identification, especially in the case of *G. pseudosyriacus* due to its wide range of distribution and hence the probability of high morphological variation which requires detailed descriptions. Therefore, a redescription of *G. pseudosyriacus* based on new materials is given here. Due to the various catchment basins in the southern Zagros region, many isolated populations of aquatic organisms exist, and consequently, the probability of forming new subspecies and species is high (Zamanpoore et al. 2010). This paper presents results of the investigation of samples of two endemic populations from springs, one in Fars province, and the second from Yazd province, Iran. Each spring is surrounded and separated by desert plains and these plains provide geographical barriers between the two populations.

The aim of this study is to prepare a redescription of *G. pseudosyriacus* based on materials in its more central distribution range inside the Zagros Mountains, and to describe a new subspecies from a population in the adjacent marginal range.

Methods

Specimens were collected by hand nets. Washed and cleaned specimens were preserved in 70% ethanol in the field. Thirty adult male specimens of each population were stained with Lignin Pink, dissected under a stereomicroscope (Zeiss, Stemi SV11), and dissected parts were mounted on a temporary slide with glycerine for examination under a compound microscope (Zeiss, Stemi IV6). Digital microphotographs of body parts were taken by a digital camera (Oculer, 3MPCCD). These photos were used for measuring all body parts of two populations with IMAGE TOOL software (V.3.0, 2002, UTHSCSA) and also to make digital drawings in CORELDRAW (V.11.633, 2002, Corel Corporation). By using the word spine in all parts of this paper, we mean "spinniform setae" as defined by Oshel and Steele (1988).

All specimens are stored in the amphipod collection of the Museum of Fars Research Centre of Agriculture and Natural Resources Aquatic Invertebrate Collection (FAIC), Shiraz, Iran and in the Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz, Iran (ZM–CBSU).

Some environmental factors that were measured in both locations include salinity, pH, electrical conductivity, water temperature and water depth.

Taxonomy

Gammarus pseudosyriacus pseudosyriacus Karaman & Pinkster, 1977

G. pseudosyriacus Karaman & Pinkster, 1977: 55–58, fig. 22

Type locality. The type locality of *G. pseudosyriacus* Karaman & Pinkster, 1977 is small pools in surroundings of Damascus. The samples were collected from springs and qanats of Zagros Mountains in October 2012. Location was Eghlid station (Rasoul Spring, Eghlid, Fars province, Iran, 30°53'27.6"N; 52°40'18.3"E, Altitude 2167 m) (Fig. 1). Leg. M. Semsar-Kazerooni.

Material examined. The description is based on new material collected by the authors from Eghlid, Fars, Iran, a locality inside the distribution range of the species (Turkey, Iran, Afghanistan, Israel and Syria). Eight males were completely dissected and examined in detail, and compared to another 22 males (FAIC 111300, ZM–CBSU #3210). One male, with genitalia in a separate microvial. Original label: "FAIC 111300, Eghlid, Rasoul Spring, 30°53'27.6"N; 52°40'18.3"E, 15 October 2012". As well as to samples from previously collected material from winter, spring, and summer.

Description. Maximum body length 22 mm; kidney-shaped and medium-sized eyes (the length of which are equal to the diameter of the first peduncular article of antenna 1) (Fig. 2C); sharp epimeres (Fig. 2F–H) and clearly elevated urosome segment (Fig. 3G). *Antenna 1*: Longer than antenna 2; peduncular articles 1>2>3; main and accessory flagella with 22–35 and 2–5 articles, armed with short simple setae (Fig. 2A). *Antenna 2*: Gland cone is shorter than the third peduncle article; peduncle articles 4 and 5 about equal length and armed with groups of short setae; flagellum with 10–18 articles and also armed with short simple setae; calceoli present (Fig. 2B). *Mandible*: All parts include incisor processes, *lacinia mobilis* and ridged molar process well developed, also a plumose long spine row is present (Fig. 3B). *Mandible palp*: First article without setae; second article with ventral setae, 3–6 proximal setae and 9–13 closely placed distal setae; inferior margin of the third article armed with a comb-like row of 30–36 D-setae, 5–6 long E-setae, one group of B-setae and one group of A-setae (Fig. 3A). *Maxilla 1*: Inner basal lobe with plumose setae; stout serrate spines on outer lobe; palps asymmetric; right palp with 4 robust tooth-like spines on apical margin, one

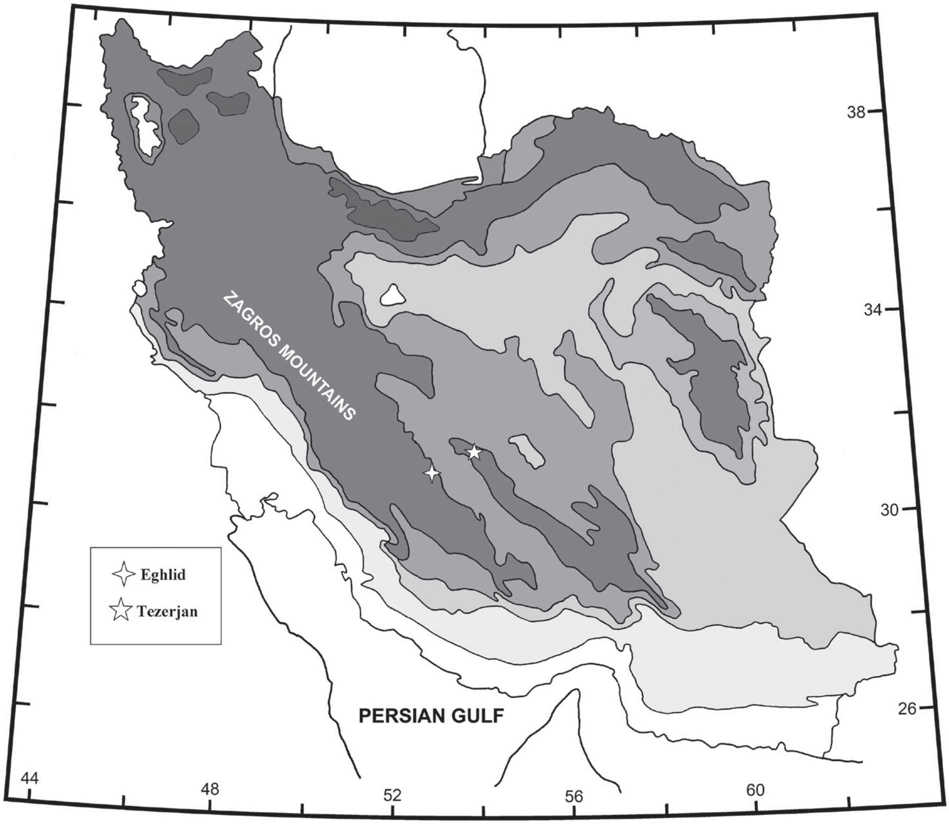

Figure 1. Map of sampling sites, Eghlid and Yazd stations.

longer separate subapical spine on its outer margin with one seta (Fig. 3D). Left palp with 5 apical spines accompanied by 2 median setae, one longer separate subapical spine on outer corner (Fig. 3C). *Maxillipeds*: Exopodite with a row of 3 strong teeth and 6 longer setae on distal margin, a row of setae at distal sub-margin which becomes plumose from the middle and continues towards the inferior margin to join to 8–10 long plumose setae, a single spine with a distance at sub-marginal interior corner, a row of three setae parallel to the long axis close to the single spine (Fig. 3E).

Gnathopod 1: Coxal plate distally slightly wider than proximal, rounded corners with a seta at the postero-ventral corner and 1–2 setae at antero-ventral corner; basis with a few long setae on both anterior and posterior margins; ischium with a postero-distal row of setae; merus and carpus with groups of short setae which are plumose at posterior margin of carpus; propodus pyriform with groups of spines and setae, 6–7 groups of small spines at posterior palmar margin; dactylus long (Fig. 2D). *Gnathopod 2*: Coxal plate distally slightly narrower than proximal, rounded corners with a seta at the postero-ventral corner and 1–3 setae at antero-ventral corner; basis with a few long setae on both anterior and posterior margins; ischium with a postero-distal row

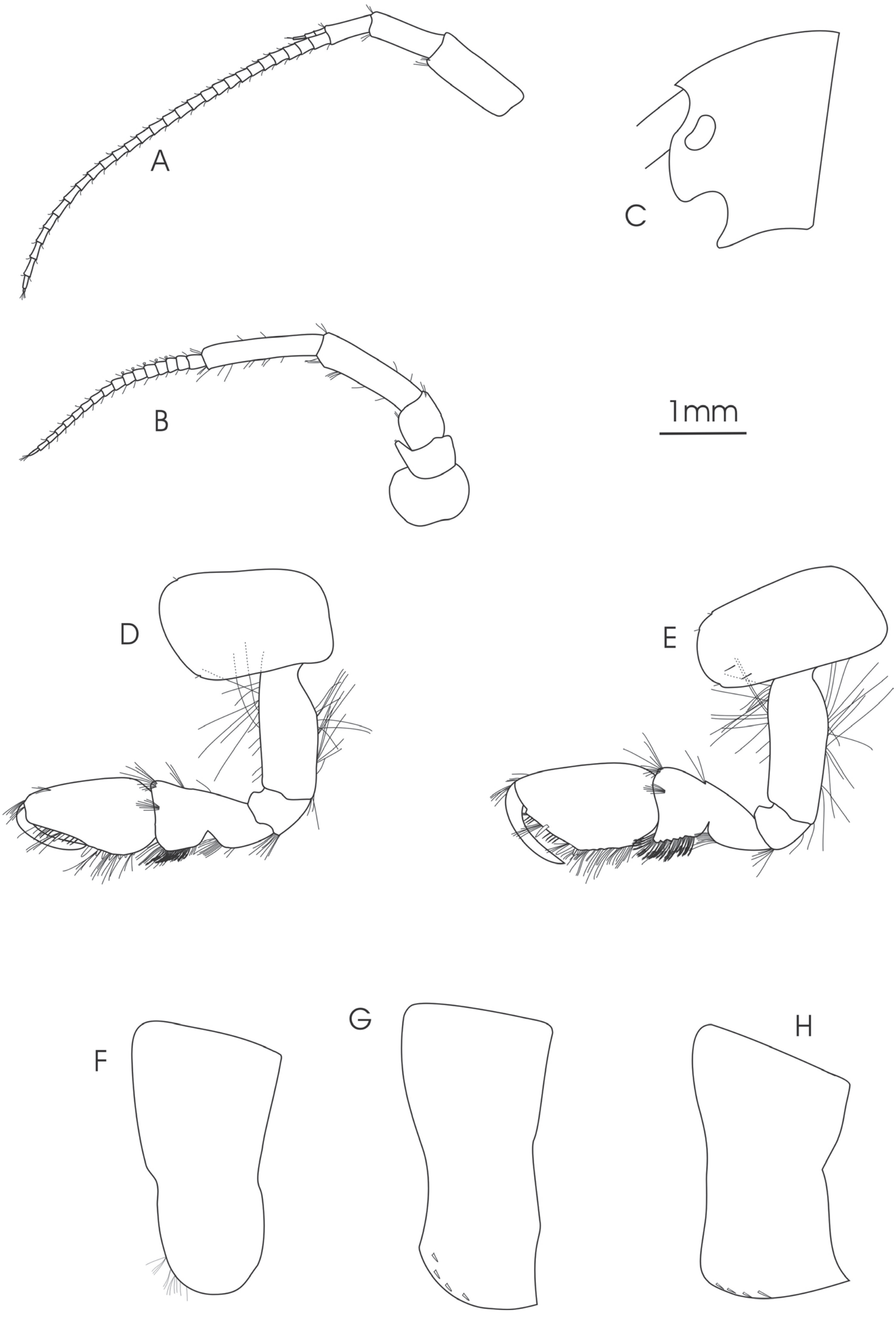

Figure 2. *Gammarus pseudosyriacus pseudosyriacus*, ♂, 20 mm. **A** antenna 1 **B** antenna 2 **C** head **D** gnathopod 1 **E** gnathopod 2 **F–H** epimeral plates1–3.

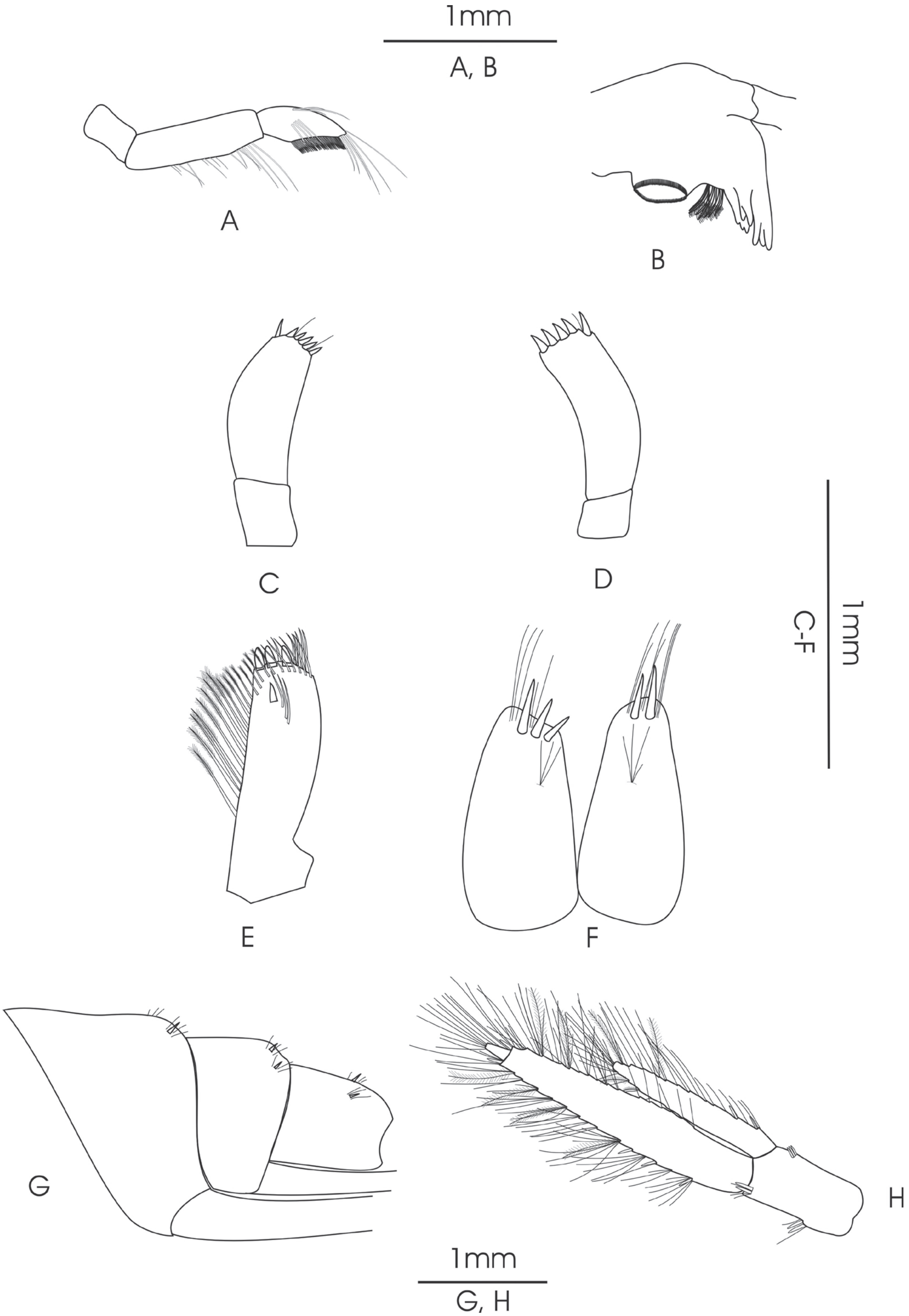

Figure 3. *Gammarus pseudosyriacus pseudosyriacus*, ♂, 20 mm. **A** mandible palp **B** mandible **C** palp of left maxilla **D** palp of right maxilla **E** exopodite of maxilliped **F** telson **G** urosomites **H** uropod 3.

of setae; merus and carpus with groups of short setae which are plumose at posterior margin of carpus; propodus trapezoid-shaped (subrectangular) with 3 groups of spines, and also groups of dense setae on palmar surface (Fig. 2E). *Pereopod 3*: Coxal plate rectangular and rounded distally, with two short setae at antero-distal corner and one at postero-distal corner; anterior and posterior margins of basis bear some long simple setae; posterior margins of merus and carpus densely setose; merus with several groups of dense setae on posterior margin about 1 to 1.5 times as long as the diameter of the article, anterior margin of merus with 2 groups of short spine that intermixed with short setae and a group of long setae and a spine at anterior tip; posterior margin of carpus with several groups of dense setae 2 times longer than the diameter of the article, a long spine and a group of longer setae are implanted on both its anterior and posterior tip; posterior margin of propodus with 6–7 groups of small spines and some long setae (Fig. 4A). *Pereopod 4*: Coxal plate with a small seta implanted at antero-distal margin and 1–4 at postero-distal margin; articles similar to pereopod 3, but setae are shorter and the number of setae is lower; anterior margin of merus with just one group of short setae and spines, long spines implanted at anterior tip among a group of setae; posterior margin of carpus with several groups of setae and spines; posterior margin of propodus with 6–8 groups of small spines and some long setae (Fig. 4B). *Pereopod 5*: Basis subrectangular, postero-distal lobe well developed, posterior margin with 2–5 very short setae, anterior margin with 4–6 spines mixed with a fine seta; merus and carpus with small spines and setae; propodus having 6–7 transverse rows of spines (Fig. 4C). *Pereopod 6*: Longer than pereopod 5; basis slender and posterior margin with 6–10 setae and anterior margin with 4–6 spines; other articles are similar to pereopod 5 (Fig. 4D). *Pereopod 7*: Basis wider proximally, postero-distal protruding lobe less developed than pereopod 6, posterior margin with 5–11 setae and anterior margin with 4–6 spines; anterior margin of merus and carpus with spines and longer setae; merus with two spines mixed with short setae at posterior margin; carpus with 2–3 spines at posterior margin; propodus with spines and setae which are as long as spines, 6–7 transverse rows of spines on anterior margin of propodus, two longer spines at posterior tip of propodus intermixed with a group of longer setae (Fig. 4E). *Uropod 3*: Endopodite length is about two-thirds of the exopodite; setae on outer and inner margin of both exopodite and endopodite are plumose (Fig. 3H).

Telson: Length of the lobes about twice their widest width; 2–5 long and robust spines and 5–8 long setae on distal margin; groups of setae at the dorsal surface of the lobes (Fig. 3F). *Epimeral plate 1*: Rounded with 12–14 long setae on antero-distal corner (Fig. 2F). *Epimeral plate 2*: Posterodistal corner pointed; distal margin with 2–7 short spines (Fig. 2G). *Epimeral plate 3*: Posterodistal corner sharply pointed; 3–5 short spines intermixed with short setae on distal margin (Fig. 2H). *Urosomites 1–3*: With clear dorsal elevation; each urosomite bears a dorsomedian and dorsolateral groups of short setae mixed with short spines on their posterior margins (Fig. 3G).

Distribution. The species is dispersed from Israel to Syria, Turkey, Iran and Afghanistan (Karaman and Pinkster 1977, Zamanpoore et al. 2011). In Iran, it is widespread in the inner parts of the Zagros Mountains, extending from northwest to southeast.

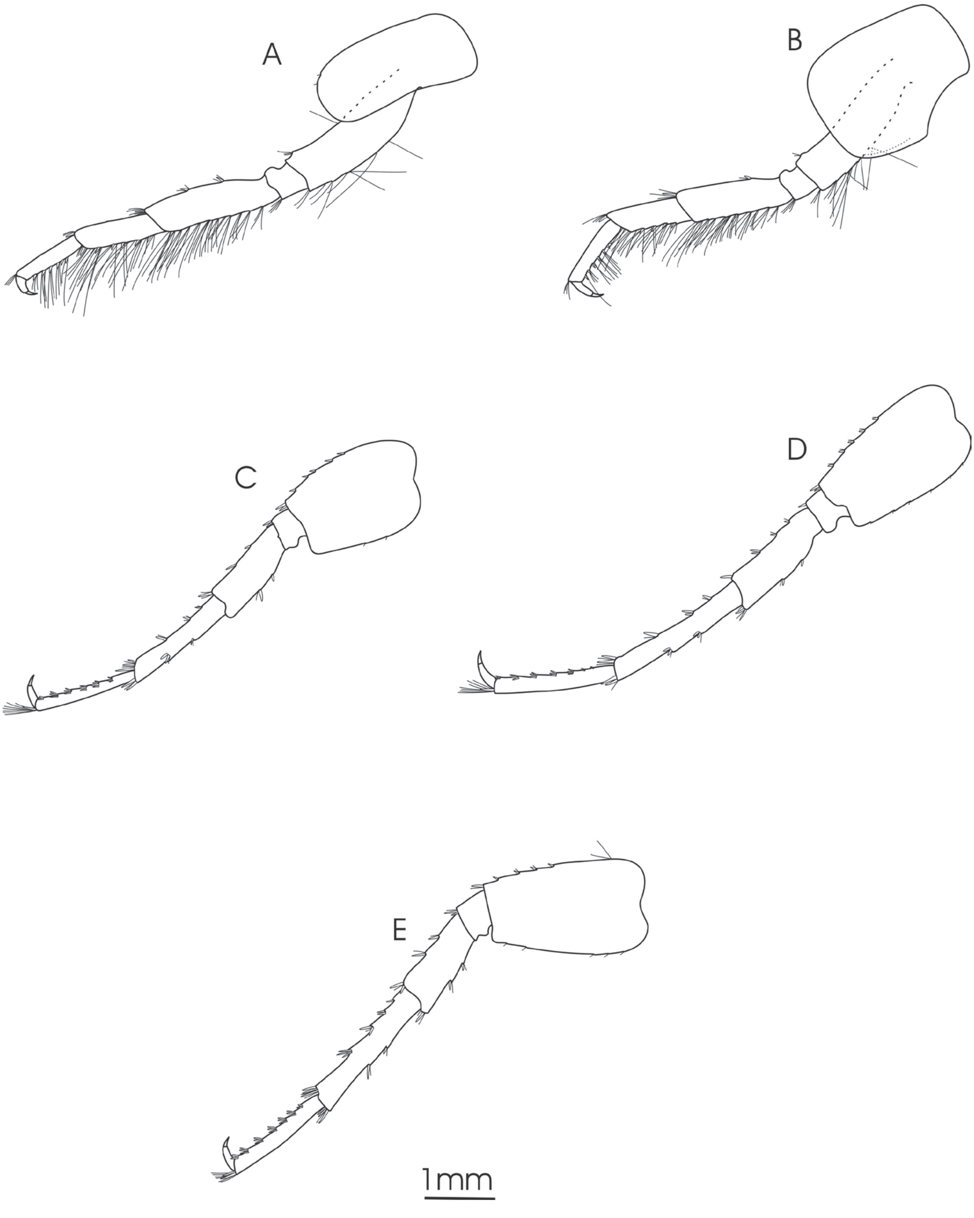

Figure 4. *Gammarus pseudosyriacus pseudosyriacus*, ♂, 20 mm. **A** pereopod 3 **B** pereopod 4 **C** pereopod 5 **D** pereopod 6 **E** pereopod 7.

Ecology. Rasoul Spring is covered by a gravel bed and some submersed aquatic plants. Ecological factors include salinity (0.19 g/lit), pH (6.5), electrical conductivity (350 µS/cm), water temperature (15 to17 °C), and water depth (25 cm).

Gammarus pseudosyriacus issatisi **subsp. n.**

http://zoobank.org/9353F82E-30E5-4657-90C9-96A17AABF7C4

Type locality. The samples were collected from springs and qanats of Zagros Mountains in May 2013. Location was in Yazd station (Qanat-e-Hojjat Abad, Tezarjan, Yazd province, Iran, 31°36'20.9"N; 54°10'43.4"E, Altitude 2162 m) (Fig. 1). leg. M. Semsar-Kazerooni.

Material examined. Holotype male, 16.9 mm, Qanat-e-Hojjat Abad, Yazd, Iran, many paratypes, eight males were completely dissected and examined in detail, and compared to another 22 males (FAIC 111299, ZM–CBSU #3209).

Type specimen. Holotype male, with genitalia in a separate microvial. Original label: "FAIC 111299, Yazd, Tezerjan Qanat, 31°36'20.9"N; 54°10'43.4"E, 12 May 2013".

Diagnosis. Small body (maximum length 17 mm), small eyes (smaller than diameter of first peduncular article of antenna 1) with a wider appearance, shorter flagellum of antenna 1 and 2, wider uropod 3, wider telson, wider merus in pereopods 3–6, wider carpus in third, fifth and sixth pereopods, wider basis in pereopod 4, longer basis in pereopod 6 and pereopod 7.

Description. Maximum body length 17 mm; small, kidney-shaped eyes (smaller than diameter of first peduncular article of antenna 1) (Fig. 5C). *Antenna 1*: Longer than antenna 2; peduncular articles 1>2>3; main and accessory flagella with 17–31 and 3–5 articles, armed with short simple setae (Fig. 5A). *Antenna 2*: Gland cone is shorter than the third peduncle article; peduncle articles 4 and 5 approximately equal length and armed with groups of short setae; flagellum with 11–15 articles armed with short simple setae; calceoli present (Fig. 5B). *Mandible*: Incisor processes, *lacinia mobilis* and ridged molar process well developed, a plumose long spine row exist (Fig. 6B). *Mandible palp*: First article without setae; second article with ventral setae, 4–5 proximal setae and 6–11 closely placed distal setae; inferior margin of the third article armed with a comb-like row of 20–26 D-setae, 5–6 long E-setae, a groupof B-setae and a group of A-setae (Fig. 6A). *Maxilla 1*: Long plumose setae on inner lobe; outer lobe with stout serrate spines; palps asymmetric; right palp with 4 robust tooth-like spines, one longer separate subapical spine with one long seta on its outer margin (Fig. 6D). Left palp with 6 apical spines accompanied by 2 median setae and a long subapical seta on inner corner, one longer separate subapical spine on outer corner (Fig. 6C). *Maxillipeds*: Distal margin of exopodite with a row of three strong teeth and 8 longer setae, a row of setae at distal sub-margin which becomes plumose from the middle and continues towards the inferior margin to join to 6–7 long plumose setae, a single spine with a distance at sub-marginal interior corner, a row of three setae parallel to the long axis close to the single spine (Fig. 6E).

Gnathopod 1: Coxal plate distally slightly wider than proximal, rounded corners with a seta at the postero-ventral corner and 1–3 setae at antero-ventral corner; basis with a few long setae on both anterior and posterior margins; ischium with a postero-distal row of setae; merus and carpus with groups of short setae which are plumose at

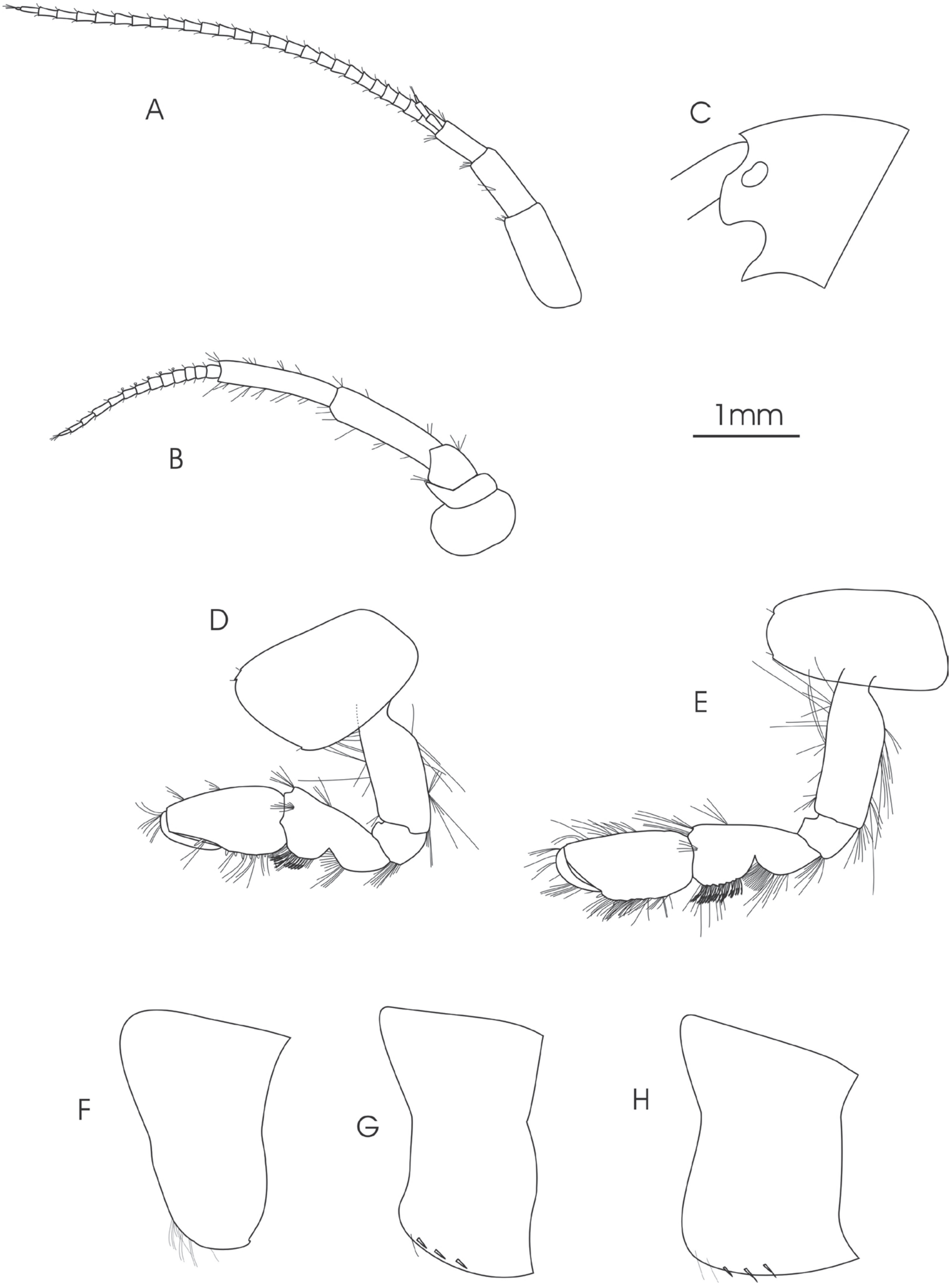

Figure 5. *Gammarus pseudosyriacus issatisi* subsp. n., ♂, 16.9 mm. **A** antenna 1 **B** antenna 2 **C** head **D** gnathopod 1 **E** gnathopod 2 **F–H** epimeral plates 1–3.

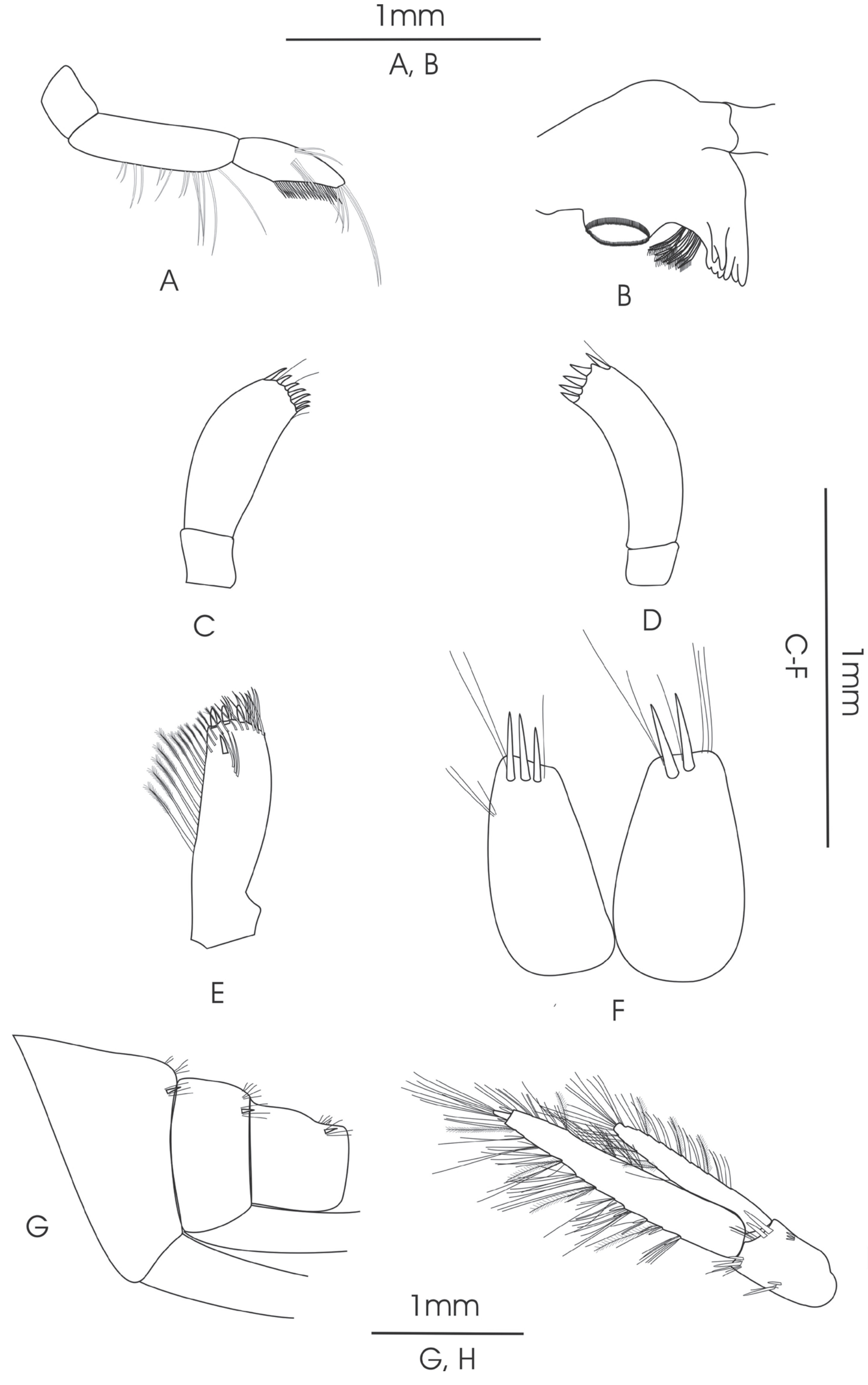

Figure 6. *Gammarus pseudosyriacus issatisi* subsp. n., ♂, 16.9 mm. **A** mandible palp **B** mandible **C** palp of left maxilla **D** palp of right maxilla **E** exopodite of maxilliped **F** telson **G** urosomites **H** uropod 3.

posterior margin of carpus; propodus pyriform with groups of spines and setae, 5–6 groups of small spines at posterior palmar margin; dactylus long (Fig. 5D). *Gnathopod 2*: Coxal plate distally slightly narrower than proximal, rounded corners with a seta at the postero-ventral corner and 1–3 setae at antero-ventral corner; basis with a few long setae on both anterior and posterior margins; ischium with a postero-distal row of setae; merus and carpus with groups of short setae which are plumose at posterior margin of carpus; propodus Trapezoid-shaped (subrectangular) with 2–3 groups of spines and also groups of dense setae on palmar surface (Fig. 5E). *Pereopod 3*: Coxal plate rectangular and rounded distally, with 2–3 very short setae at antero-distal corner and one at postero-distal corner; anterior and posterior margins of basis bear some long simple setae; posterior margins of merus and carpus densely setose; posterior margin of merus with several groups of dense setae about 1 to 1.5 times as long as the diameter of the article and anterior margin with 2–3 groups of short spine mixed with short setae and a group of long setae with a spine at anterior tip, mean ratio of merus length/width 3.1; posterior margin of carpus with several groups of setae about 2 times longer than the diameter of the article, a long spine and a group of longer setae are implanted on both its anterior and posterior tip, mean ratio of carpus length/width 3.3; posterior margin of propodus with 6 groups of small spine and some long setae (Fig. 7A). *Pereopod 4*: Coxal plate with 2–3 small setae implanted at antero-distal margin and 6–7 at postero-distal margin; articles similar to pereopod 3, but setae are shorter and the number of setae and groups is lower; mean ratio of basis length/width 3.2; anterior margin of merus with just one group of short setae and one spine, two long spines among a group of setae implanted at anterior tip of merus, mean ratio of merus length/width is 2.9; posterior margin of carpus with several groups of setae and spines; posterior margin of propodus with 5–6 groups of one small spine and some long setae (Fig. 7B). *Pereopod 5*: Basis subrectangular, postero-distal lobe well developed, posterior margin with 10–11 very short setae, anterior margin with 4–5 spines; merus and carpus with small spines and setae, mean ratio of merus length/width 2.5; mean ratio of carpus length/width 5.4; propodus having 6 transverse rows of spines (Fig. 7C). *Pereopod 6*: Longer than pereopod 5; basis slender and posterior margin with 10–11 setae and anterior margin with 4–5 spines, mean ratio of basis length/width 1.8; other articles are similar to pereopod 5; mean ratio of merus length/width 2.8; mean ratio of carpus length/width 6.1 (Fig. 7D). *Pereopod 7*: Basis wider proximally, postero-distal protruding lobe less developed than pereopod 6, posterior margin with 11–16 setae and anterior margin with 4–5 spines, mean ratio of basis length/width 1.9; anterior margin of merus and carpus with spines and longer setae; merus with two spine and some short setae at posterior margin; carpus with 1–3 spines at posterior margin; propodus with spines and setae which are as long as spines, 6–7 transverse rows of spines on anterior margin of propodus, two longer spines at posterior tip of propodus intermixed with a group of longer setae (Fig. 7E). *Uropod 3*: Endopodite length is about two-thirds of the exopodite; setae on outer and inner margin of both exopodite and endopodite are plumose; mean ratio of exopodite length/width 5.7 (Fig. 6H).

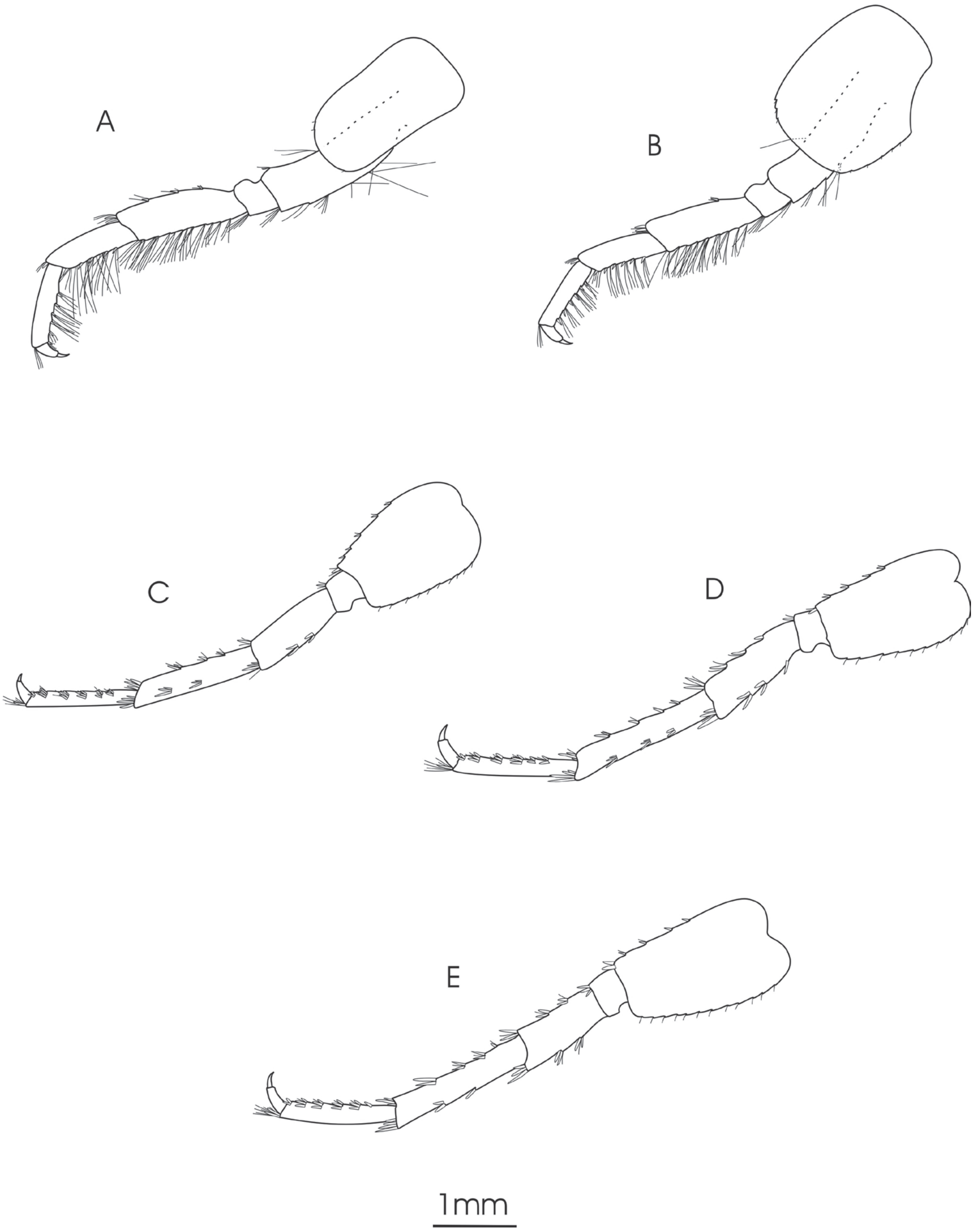

Figure 7. *Gammarus pseudosyriacus issatisi* subsp. n., ♂, 16.9 mm. **A** pereopod 3 **B** pereopod 4 **C** pereopod 5 **D** pereopod 6 **E** pereopod 7.

Telson: Length of the lobes about twice their widest width; two to three long and robust spines and 5–6 long setae on distal margin; groups of setae at the dorsal surface of the lobes; mean ratio of telson lobe length/width 1.7 (Fig. 6F). *Epimeral plate 1*:

Rounded with 9–12 long setae on antero-distal corner (Fig. 5F). *Epimeral plate 2*: Posterodistal corner pointed; distal margin with 1–4 short spines that mixed with setae (Fig. 5G). *Epimeral plate 3*: Posterodistal corner sharply pointed; 2–4 short spines intermixed with short setae on distal margin (Fig. 5H). *Urosomites 1–3*: With clear dorsal elevation; each urosomite bears a dorsomedian and dorsolateral groups of setae, mixed with short spines on their posterior margins (Fig. 6G).

Etymology. The specific name *issatisi* is an adjective that comes from "Issatis", which was the previous name of Yazd during the time of the Median Empire. Yazd is an ancient city with a 3,000 year history. The type locality is in the vicinity of Yazd city.

Distribution. *Gammarus pseudosyriacus issatisi* subsp. n. is distributed in several springs and qanats in Yazd Province, in the south of Iran.

Ecology. Qanat–e–Hojjat Abad showed pebbles and a sandy bed. Ecological factors include salinity (0.4 g/lit), pH (7.7), electrical conductivity (733.3 μS/cm), water temperature (13 °C) and water depth (less than 20 cm).

Discussion

The first record of *G. pseudosyriacus* from Iran (Charmahal-Va-Bakhteyari province) was reported by Khalaji-Pirbalouti and Sari in 2004. In addition, this species was found in other provinces including Markazi Province and Isfahan Province (Naghib 2002) and Kerman Province (Pourmohammadi-Sarbanani 2002) in the far margin of southern Zagros Mountains. These studies show distribution of this species along the Zagros Mountains from the northwest to the southeast (Khalaji-Pirbalouty and Sari 2004, Zamanpoore et al. 2011, Ebrahimnezhad et al. 2005).

A morphological redescription and complete illustrations of *Gammarus pseudosyriacus* are presented. This species shows a high morphological variation across its distribution range (Khalaji-Pirbalouty and Sari 2004, Zamanpoore et al. 2011, Özbek 2011). The original description of this species was presented in an extensive volume (Karaman and Pinkster 1977) describing a large number of new species, so that, as the authors emphasized, "… it was not possible to illustrate all morphological details of every taxon mentioned" (Karaman and Pinkster 1977, p.1), including *G. pseudosyriacus*. For better evaluation of this species for future taxonomic studies, a description of this species in greater detail was prepared.

All major body parts were described, as well as those which were not previously described. These include mandible, maxilla 1(left and right), maxillipeds, first and second gnathopods, and third and fourth pereopods. Complete illustrations are provided, including antenna 1, mandible, maxilla 1 (left and right), maxillipeds, first and second gnathopods, third, fourth and sixth pereopods which were not present in the original paper (except for propodus of gnathopod 1 and 2).

In addition, the Yazd population is introduced as a new subspecies of *G. pseudosyriacus* because of its obvious differences such as smaller eyes, shorter body length, and shorter flagellum of antenna 1 and 2 from the originally described species which we hereby refer to as *G. pseudosyriacus pseudosyriacus*. According to the data from morphometrical study, this subspecies has significant morphological differences from *G. p. pseudosyriacus* in one or several parts of its body organs. It must be noted that there were no seasonal variations in any body parts of the Eghlid population, so it is concluded that these differences are not related to seasonal morphological changes.

In the Zagros Mountains there are many aquatic habitats that were occupied by different populations of *G. pseudosyriacus* and it seems that these populations inhabiting the inner parts of the Zagros are temporally or permanently connected to each other. Eghlid population is one of these Zagros populations which is consistent with the first descriptions of holotype (Karaman and Pinkster 1977), and is therefore considered as a source population. Considering that the source populations have the best and most fit ecological situations, usually the main phenotypes of each species are found in these populations (Mayr 1970). It is suggested that over time the sub-populations of the source population dispersed through the common methods and occupied peripheral habitats. Yazd Province lies in a hot and dry desert, with very few sources of running water, and no existing connection to surface waters of Eghlid and the rest of the Zagros. So the Yazd population can be considered as a sink population. The two populations are established in 2 different catchment basins surrounded by mountains and hills. On the other hand, populations of *G. pseudosyriacus* in Eghlid and Yazd are surrounded and separated by desert plains. These highlands and vast deserts between two habitats have acted as strong geographical barriers which led to long disconnection between two populations and decrease of gene flow.

In terms of ecological characteristics, there are also significant differences between two habitats, including the salinity and electrical conductivity which were much higher in Yazd station (twice). In addition, morphological divergence could have increased as a result of environmental pressures acting in different ways. These factors, along with genetic drift (and even the founder effect) may have led to the formation of the new characters independently. Therefore, it can be concluded that these morphological and probably genetic differences have occurred after separating from the main population.

There are records of isolated populations which are considered as subspecies in different species of amphipods. Cole (1970) described a new subspecies *Gammarus minus pinicollis*, this subspecies in some features such as lack of calceoli, dorso-lateral armature of the urosomites and the ratio of endopod-exopod (exceed 0.67 commonly) differs from *Gammarus minus* Say, 1818. Sutcliffe (2010) introduced two subspecies, *G. duebeni duebeni* and *G. duebeni celticus* based on differences in ratios of merus width to length of pereopod 7. Özbek and Rasouli (2014) described a new subspecies, *Gammarus komareki aznavensis*, that has some features that are different from *Gammarus komareki* Schäferna, 1922 including shorter flagellum of antenna 1, kidney-shaped and bigger eyes, more setose outer margin of the exopodite of uropod 3 and shorter endopodite of uropod 3. Based on our findings on the differences described in the present article, we propose Yazd population to be recognized as a new subspecies, *G. pseudosyriacus issatisi* subsp. n.

Conclusions

Based on previous studies, *G. pseudosyriacus* is distributed from the northwest to the southeast of the Zagros Mountains. In this study we considered Eghlid population in the inner parts of the Zagros Mountains as a source population. It is connected to other populations of the species, so that it shows no clear variation with the first descriptions of holotype (Karaman and Pinkster 1977). Hereby, we assume it as *Gammarus pseudosyriacus pseudosyriacus* subsp. n. Based on our morphological and morphometric study, revealing significant variations (such as smaller eyes, shorter body length, and shorter flagellum of antenna 1 and 2) between Yazd population and the rest of the populations of *G. pseudosyriacus*, we introduced Yazd population as a new subspecies *G. pseudosyriacus issatisi* subsp. n.

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RESEARCH ARTICLE

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Morphological features of larvae of *Drusus plicatus* Radovanović (Insecta, Trichoptera) from the Republic of Macedonia with molecular, ecological, ethological, and distributional notes

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Abstract

A description of the larva of *Drusus plicatus* Radovanović is given for the first time. The most important diagnostic characters enabling separation from larvae of the other Drusinae from the southeast Europe are listed. Molecular, ecological, and ethological features and distribution patterns of the species are given. Additionally, information on the sympatric caddisfly species of the three springs where larvae and adults of *D. plicatus* were found and presented.

Keywords

Caddisfly, Drusinae, southeast Europe, larval description, fauna

Introduction

Drusus plicatus Radovanović (Limnephilidae, subfamiliy Drusinae), was described by Radovanović based on specimens collected in Labunište village situated in the southwest part of the Republic of Macedonia (Radovanović 1942) in southeast Europe (Fig. 1A). This region (southeast Europe) is delimited by the Croatia on the west and north, by the Serbia on the north, by the Bulgaria on the east and by the Greece on the south (Ecoregions: 5, 6, 7, 11, 12; Graf et al. 2008). In the area delimited in this way, 46 *Drusus* species have been recorded (e.g., Malicky 2004, 2005, Oláh 2010, 2011, Oláh and Kovács 2013, Kučinić et al. 2014, Ibrahimi et al. 2015, 2016, Vitecek et al. 2015a, 2015b, 2015c), from which six species are widely distributed (e.g., *D. biguttatus* Pictet, *D. chrysotus* Rambur, *D. croaticus* Marinković-Gospodnetić, *D. discolor* Rambur). The remaining 40 species are endemics of southeast Europe. Most species of *Drusus* from southeast Europe are reported from Bulgaria, Albania, Macedonia, and Bosnia and Herzegovina (e.g., Marinković-Gospodnetić 1979, Kumanski 1988, Malicky 2004, Oláh 2010, 2011, Oláh and Kovács 2013, Vitecek et al. 2015a, 2015b, 2015c), while the lowest number of species is recorded in Croatia (Kučinić et al. 2014). In recent years intensive research focussing on caddisfly diversity in southeast Europe has resulted in the description of 16 new species from the subfamily Drusinae (Oláh 2010, 2011, Oláh and Kovács 2013, Previšić et al. 2014a, Ibrahimi et al. 2015, 2016, Vitecek et al. 2015b, 2015c).

The subfamily Drusinae consists of eight genera with some 110 described species (Hickin 1967, Oláh 2010, 2011, Malicky 2004, Oláh and Kovács 2013, Oláh et al. 2015, Previšić et al. 2014a, Ibrahimi et al. 2015, 2016, Vitecek et al. 2015a, 2015b, 2015c), from which 95 belong to the genus *Drusus*, which is one of the largest genera in the European Trichoptera fauna (Malicky 2004).

Faunistic, phylogenetic, and phylogeographic characteristics of Drusinae have been studied extensively (e.g., Pauls et al. 2006, 2008, 2009; Previšić et al. 2009, 2012, 2014a, 2014b, Previšić and Popijač 2010, Ibrahimi et al. 2012, 2014, Stanić-Koštroman et al. 2012, 2015, Vitecek et al. 2015a). Additionally, taxonomic interest in the group was demonstrated by a number of studies focussing on the delineation of new species (Sipahiler 1992, Urbanič et al. 2002, Oláh 2010, 2011, Oláh and Kovács 2013, Previšić et al. 2014a, Ibrahimi et al. 2015, 2016, Vitecek et al. 2015b, 2015c) and larval taxonomy (e.g., Waringer et al. 2007, 2011, 2015, 2016, Kučinić et al. 2008, 2015, Vitecek et al. 2015a, 2015c). Larval morphology of all widely distributed species (e.g., *D. biguttatus*, *D. chrysotus*. *D. discolor*) of this genus recorded in southeast Europe is well known (Lepneva 1966, Waringer and Graf 1997, Previšić et al. 2012, Vitecek et al. 2015a); this is also valid for 16 of the southeast Europe endemic species (Kučinić et al. 2008, 2010, 2011a, 2011b, 2015, Vitecek et al. 2015a, 2015c, Waringer et al. 2015, 2016).

The present study has three main objectives: 1. present the morphological features of the final larval instar of *Drusus plicatus*; 2. present molecular and ecological features and new data on the distribution of *D. plicatus*; 3. provide information on the caddisfly fauna in three springs in which larvae and adults of *D. plicatus* (Fig. 2) were found. Two of the springs are located in Mavrovo National Park, highlighting the importance of these data for the continued conservation of the protected areas of the Republic of Macedonia.

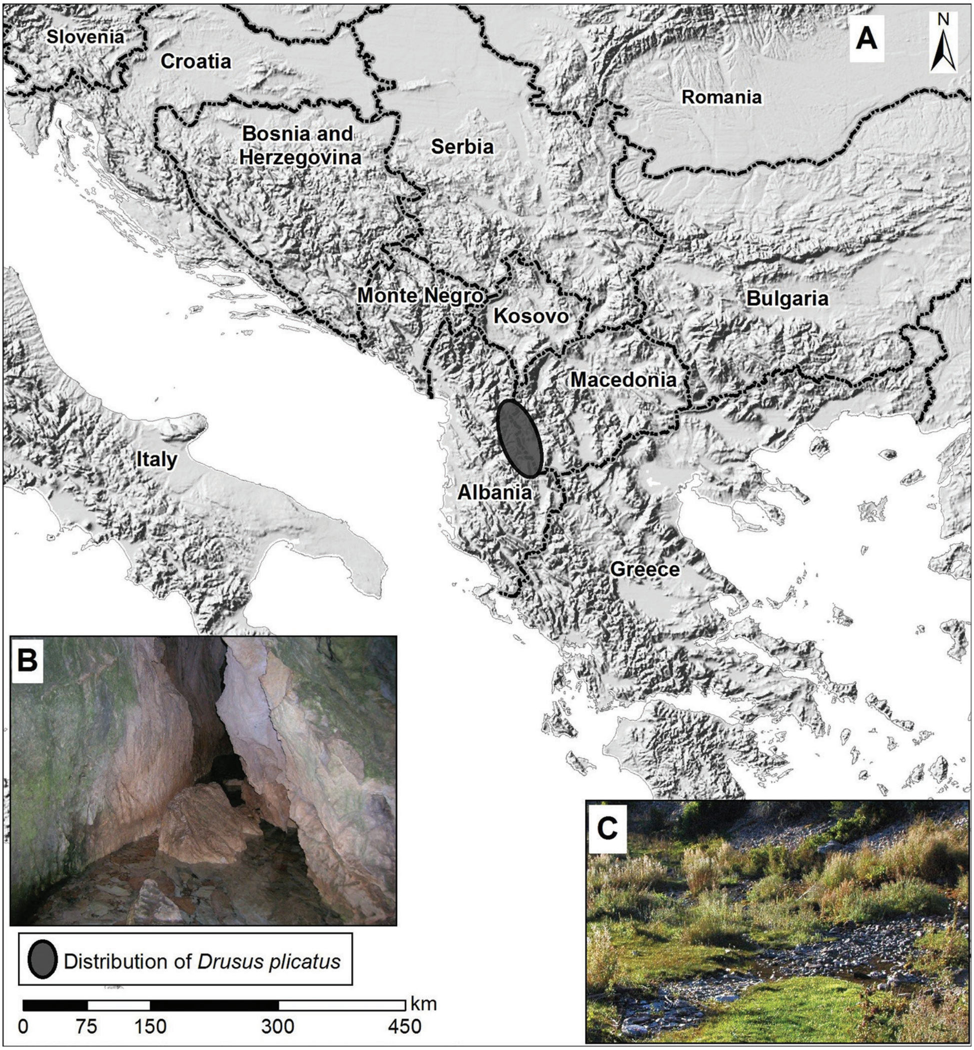

Figure 1. A Southeast Europe with distribution of *Drusus plicataus* (grey) **B** Spring Vevčani **C** Spring of the River Galička reka.

Material and methods

Fieldwork and sampling

The material studied comprises 7 larvae of *Drusus plicatus* collected on 23 August 2009 from the spring Vevčani (Fig. 1B), 12 larvae collected on 25 August 2009 (4th and 5th instar larvae), 24 larvae collected on 2 July 2010 (4th and 5th instar larvae), 4 larvae collected on 29 May 2013 from the spring of the River Galička reka (Fig. 1C), Mavrovo National Park, and 5 larvae of the same species collected on 2 July 2010 from the spring of the River Strežimirska reka, Mavrovo National Park (Table 1). Larvae were collected by handpicking and adults with an entomological net during the day. Collected specimens were stored in containers with 80% and 96% EtOH for morphological and molecular analysis, respectively.

Additionally, adult caddisfly communities in three springs in Macedonia (Vevčani spring, spring of the River Strežimirska reka, and the spring of the River Galička reka) were sampled using light traps. Identification of the adults was conducted using the works of Malicky (2004) and Kumanki (1988). The larval morphological terminology follows Wiggins (1996) and the systematics follow Morse (2015). Most of the collected specimens of larvae and adults are deposited in the collections of the first (Croatian Natural History Museum in Zagreb) and second authors (Faculty of Science, University of Zagreb). Some adults are deposited in the Macedonian Museum of Natural History in Skopje (collection Trichoptera Kučinić, Mihoci & Krpač).

We have included literature data for caddisfly species collected in Vevčani spring (*Rhyacophila trescavicensis* Botosaneanu, *Wormaldia occipitalis* Pictet, *Tinodes rostocki* McLachlan, *Ecclisopteryx keroveci* Previšić, Graf & Vitecek, *Potamophylax luctuosus* Piller & Mitterpacher) (Oláh and Kovács 2014) which were not found during our investigation of this spring.

DNA extraction and PCR amplification

DNA was extracted from two adult males and two larvae of *D. plicatus* from the spring of the River Galička reka and one adult male and two larvae from Vevčani spring to confirm the association of the larvae with the adults. DNA extraction, amplification of the 541–bp–long fragment of the mitochondrial cytochrome oxidase I (mtCOI) using primers S20 and Jerry (Simon et al. 1994, Pauls 2004) were accomplished as outlined by Previšić et al. (2009). Sequences were edited manually using the program BioEdit v7.0.9 (Hall 1999) and aligned using ClustalX (Thompson et al. 1997). Sequences were deposited in GenBank under accession numbers listed in Table 2. Intraspecific *p*-distances were calculated using the software Mega 4.0.1 (Tamura et al. 2007).

Electron microscopy, macrophotography and biometry

Electron microscopy of larvae of *D. plicatus* (specimens from Vevčani spring) was carried out using a Tescan TS 5136 variable pressure scanning electron microscope (SEM). Samples were mounted with graphitic adhesive tape on the SEM stub and coated with carbon. The samples were examined by SEM operating in secondary electron (SE), or back-scattered (BSE) mode, at an accelerating voltage of 20 kV, running current of 110 pA, and variable pressure of 30 Pa to 5”10-1 Pa; sometimes the pressure was increased to 10 Pa to eliminate sample charging. Macrophotography and assessment of morphometric characteristics of pupae, larvae and larval cases were carried out using a Leica Wild MZ8 stereomicroscope and Olympus SP-500 UZ digital camera;

Table 1. Sampling sites of *Drusus plicatus* in Republic of Macedonia and literature data of *D. plicatus* in Albania (Oláh and Kovács 2013).

Locality	Country	Altitude	Longitude (E)	Latitude (N)
Vevčani spring	Macedonia	ca 950 m	20.5844	41.2396
Modrič	Macedonia	ca 960 m	20.3425	41.2156
Tresonče	Macedonia	ca 1030 m	20.7223	41.5606
Mavrovska reka	Macedonia	ca 1290 m	20.4465	41.3843
spring of the river Galička reka	Macedonia	ca 1410 m	20.6646	41.5934
spring Sveta voda, Ničpur, river Radika	Macedonia	ca 980 m	20.4034	41.4435
spring - Rosočka Reka, Rosoki village	Macedonia	ca 1200 m	20.6933	41.5694
spring of River Reč	Macedonia	ca 1280 m	20.6348	41.7902
Mt Kaptinë, brooks	Albania	ca 1600 m	20.2889	41.3866
Cermenikë Mts, Zalli and Steblevës streams	Albania	ca 1270 m	20.4425	41.3083

photographs were processed with the software Olympus Quick Photo Camera 2.2. In the larvae of *D. plicatus* the following features were measured (in mm): head width, total body length, length of the anterior sclerites, their width at the widest median part and the distance between them, and also the length of the posterior sclerites. The following characters of cases were measured: total length, width of the anterior part, and width of the posterior part.

Results

Description of the fifth instar larva of *Drusus plicatus*

Larval case constructed of mineral particles (Figs 3, 4), slightly curving, total length 9.97–19.19 mm, width of anterior part 2.30–2.70 mm, width of posterior part 1.64–2.01 mm. Overall body shape eruciform (Fig. 5).

Head capsule hypognathous (width 1.40–1.46 mm, n = 5) (Figs 5, 6, 7), in lateral view rounded in posterior dorsal part. Head (dark) brown to black, dorsally darker and laterally lighter (Fig. 6), with granular surface sculpturing and dark muscle attachment spots posteriorly. Genae reddish-brown to yellow with lighter (yellow) ring around each eye (Fig. 6). Frontoclypeal suture bell-shaped with narrow central region (Fig. 8). Antennae short, brown to dark brown (black), each positioned on small prominences (Fig. 6). Other primary setae positioned as shown in Fig. 8. Spinules (Figs 9, 10) present in small numbers, positioned around and between primary setae 15 and 16 (Fig. 8). Labrum symmetrical, brown to yellowish, with setal brush at anterolateral margins. Anterior part of labrum usually lighter. Mandibles black (Fig. 11), mesal part reddish. Typical for grazers, mesal margin with yellowish setal brush. Two setae present laterobasally on each mandible (Fig. 11). Labium and maxillae light-brown (yellowish). Each maxillary palp 5-segmented.

Thorax. Pronotum brown to black with granular surface sculpturing (Figs 6, 7, 12). Posterior margin rounded, both posterior and lateral margins thick and darkly

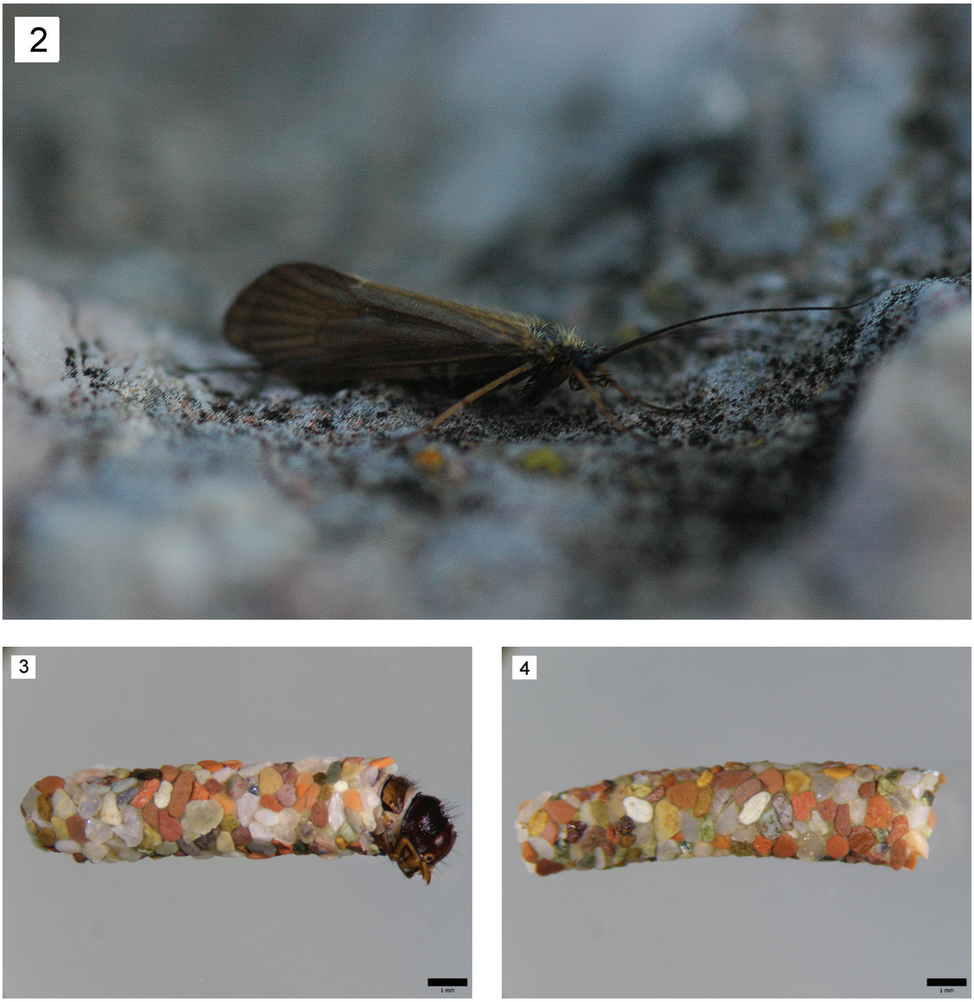

Figure 2–4. *Drusus plicatus* Radovanović. **2** Adult (spring of the River Galička reka) **3** Larva in case, 5th instar, right lateral view **4** Case, right lateral view.

sclerotized. In lateral view, anterior half of pronotum slightly concave, almost flat, posterior half slightly rounded (Figs 6, 7, 12). Pronotum bearing dark setae, especially laterally and on anterior margin, some of them long and conspicuous. Dorsal and lateral regions of pronotum bearing short, white, recumbent setae (Fig. 9).

Mesonotum sclerites brown, lighter than pronotum, with dark muscle attachment spots, dark setae and irregular surface (Fig. 13). Posterior and lateral margins thick and darkly sclerotized (Fig. 13).

Metanotum with 3 pairs of dorsal sclerites: anterior sclerites, posterior sclerites and lateral sclerites (Figs 5, 13). Anterior sclerites (*sa*1) elongated, triangular with rounded apices (Fig. 13), covered by setae, mainly in anterior part, color similar to mesonotum. Length of anterior sclerites 0.45–0.52 mm; width of anterior sclerites 0.25–0.31 mm;

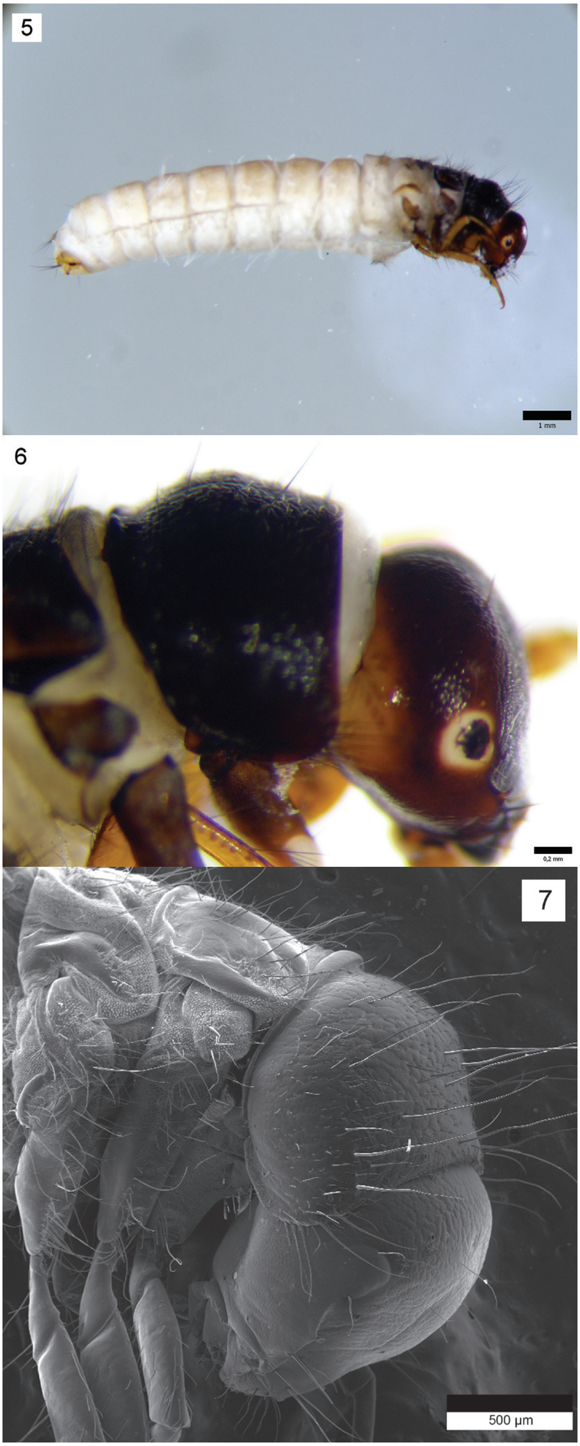

Figure 5–7. *Drusus plicatus* Radovanović, 5th instar larva. **5** Larva, right lateral view **6** Larva, head and pronotum, right lateral view **7** Head, pronotum and mesonotum, right lateral view.

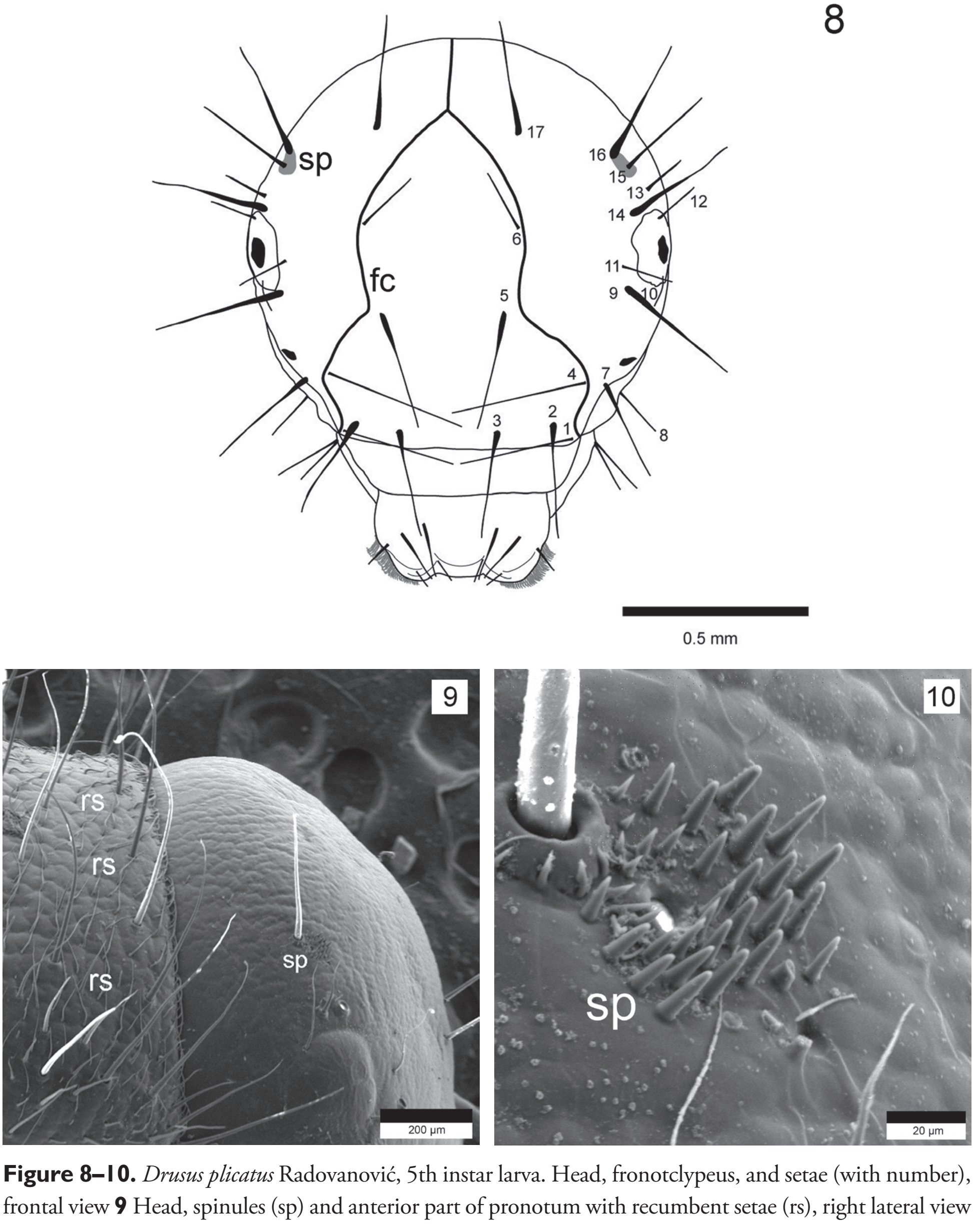

Figure 8–10. *Drusus plicatus* Radovanović, 5th instar larva. Head, fronotclypeus, and setae (with number), frontal view **9** Head, spinules (sp) and anterior part of pronotum with recumbent setae (rs), right lateral view **10** Spinules (sp) on the head, right lateral view.

distance between anterior sclerites 0.07–0.11 mm. Posterior sclerites (*sa*2) smaller and lighter than *sa*1 sclerites (Fig. 13), triangularly or irregularly ellipsoid and with many setae. Length of posterior sclerites 0.26–0.31 mm. Lateral sclerites (*sa*3) (Fig. 5) longitudinally prolonged, sickle-shaped, lighter brown with dark median region, and group of setae anteriorly.

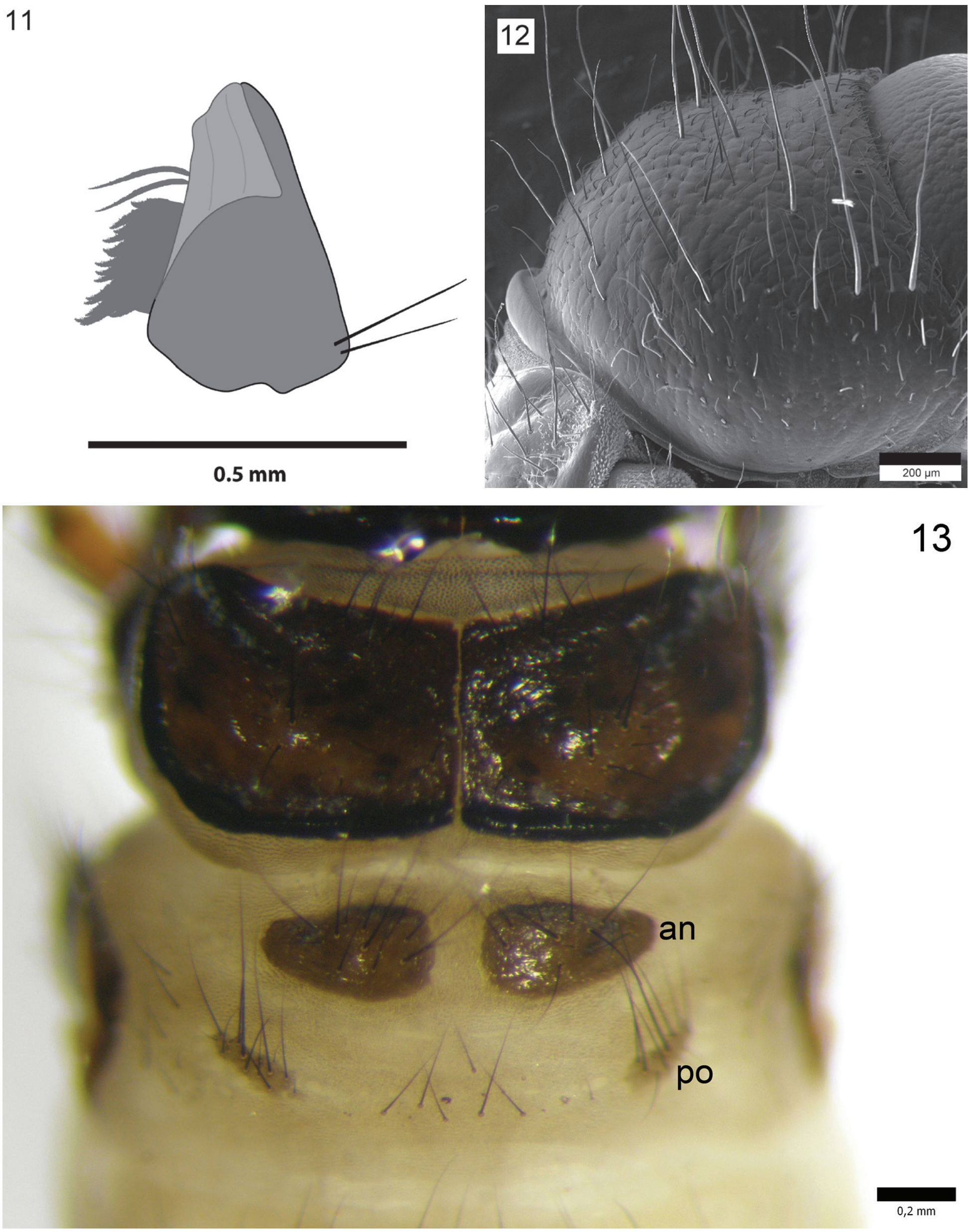

Figure 11–13. *Drusus plicatus* Radovanović, 5th instar larva. **11** Right mandible **12** Pronotum, right lateral view **13** Mesonotum, metanotum with anterior (an) and posterior sclerites (po), dorsal view.

Legs (Figs 14, 15, 16) yellow-brown to brown or black, with dark ventral and dorsal margins. Foreleg coxae with dark setae on ventral and dorsal edges. Foreleg trochanters without dorsal setae, each with few light yellow setae on ventral margin, trochanteral brush present (Fig. 14). Mid- and hind leg coxae and femora (Figs 15, 16)

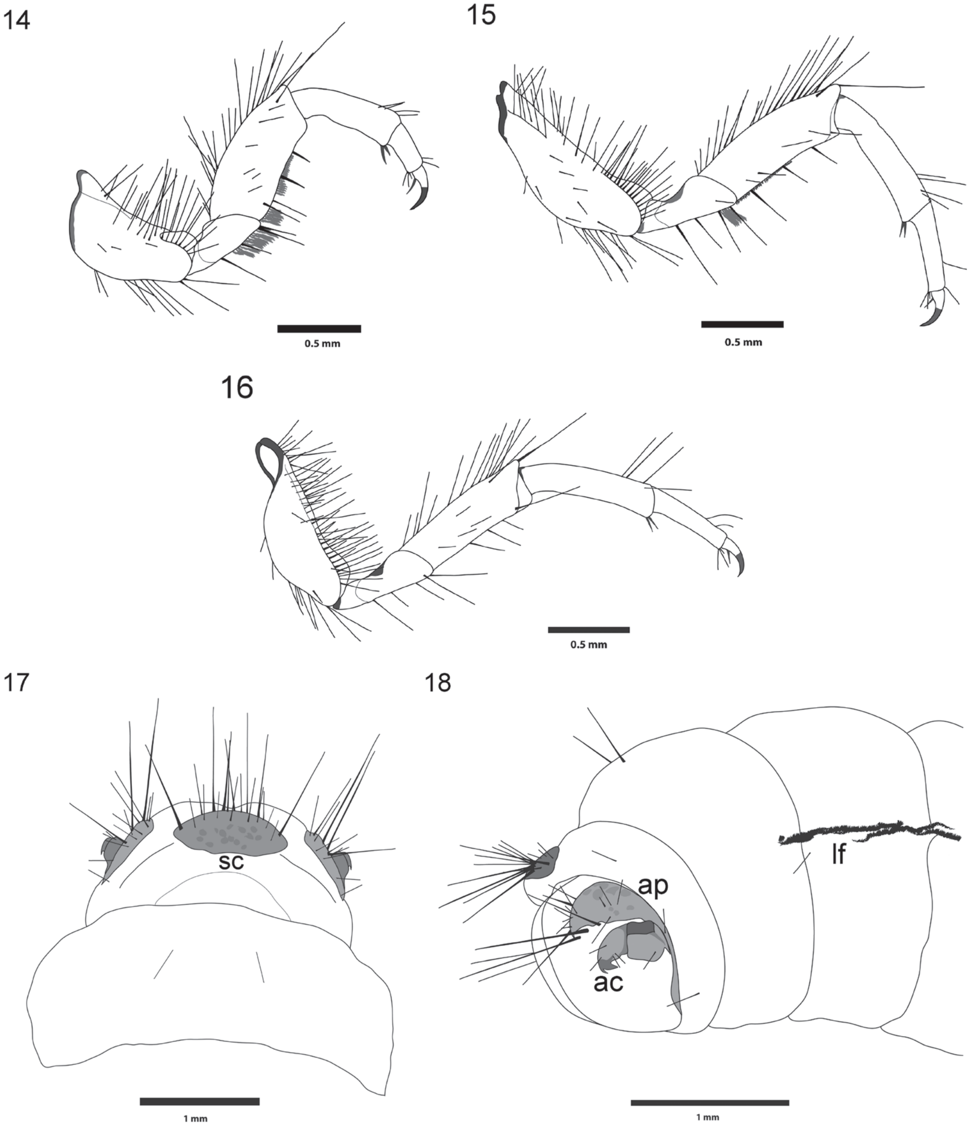

Figure 14–18. *Drusus plicatus* Radovanović, 5th instar larva. **14** Left foreleg **15** Left midleg **16** Left hindleg **17** Sclerites (sc) on segment IX, dorsal view **18** Anal proleg (ap), anal claws (ac), and lateral fringe (lf), right lateral view.

with dark setae on both ventral and dorsal edges. Additional setae present on anterior and posterior faces of all femora. Setae on dorsal edges of tibiae present only distally on all legs. Foreleg coxae and femora wide compared to those of mid- and hind legs (Figs 14, 15, 16). Mid- and hind legs similar in shape and size (Figs 15, 16), with slender coxae, trochanters, femora and tibiae.

Abdomen. Abdominal segment I with well-developed dorsal and lateral humps (protuberances) with numerous ventral setae, some of them with small sclerites at bases. Lateral protuberances with few setae. Some of them (1-2) with small sclerites at bases. Single-filament gills (Fig. 5) present on segments II–VII. Lateral gills present on segments II-V (on segment V only pre-segmental gills are present). Lateral fringe extending from second half of segment III to first half of segment VIII (Fig. 18).

Segment IX bearing irregular, semicircular, light brown dorsal sclerite, with few long dark setae on posterior margin (Fig. 17). The anal prolegs typical of limnephilids (Fig. 18). Each with lateral sclerite longitudinally prolonged, sickle-shaped, yellowish, with small setae and 2 large, dark setae posteriorly (Fig. 18). Anal claws brown to dark brown.

Ecology, ethology and distribution of *Drusus plicatus*

Mandible morphology of the larvae and observations during fieldwork suggest *Drusus plicatus* is a member of the Drusinae grazer clade (Previšić et al. 2014b). Species of this clade feed on epilithic algae and biofilms and can be found on stream bottoms, generally on cobbles, small pebbles and moss.

Based on the number of adults observed during the day, the most abundant population of *D. plicatus* was present in the spring of the River Galička reka (Fig. 1C). In this spring we observed two emergence peaks in spring and in late summer/autumn periods.

We collected *D. plicatus* larvae, adults or both in eight localities in the Republic of Macedonia (Table 1). Altitudes of locations where *D. plicatus* were collected range between approx. 950 m and 1410 m a.s.l. (Table 1).

Sympatric caddisfly communities in three springs

We collected adult caddisflies at the three springs inhabited by *Drusus plicatus*. In the Vevčani spring the following species were recorded: *Rhyacophila balcanica* Radovanović, *R. trescavicensis* (literature data), *Wormaldia occipitalis* (literature data), *Tinodes rostocki* (literature data), *Tinodes* sp. (female), *Ecclisopteryx keroveci* (literature data), *D. tenellus* Klapálek, *D. plicatus*, *Potamophylax latipennis* Curtis, *P. luctuosus* (literature data), in the spring of River Strežimirska reka: *R. balcanica*, *R. laevis* Pictet, *Synagapetus iridipennis* McLachlan, *Tinodes* sp. (female), *Hydropsyche* sp. (females), *Philopotamus montanus* Donovan, *Annitella* cf. *triloba* Marinković-Gospodnetić, *D. plicatus*, *Potamophylax pallidus* Klapálek, *Allogamus* sp. (male), *Thremma anomalum* Mclachlan and in the spring of the River Galička reka the following species: *R. balcanica*, *D. plicatus*, *Philopotamus montanus*, *Thremma anomalum*, *D. plicatus* and *Potamophylax lemezes* Oláh & Graf.

Discussion

Association of larvae and adults of *D. plicatus*

Association of larvae and adults of *D. plicatus* is supported by the similarity of partial COI haplotypes. Since the association of larvae and adults is not completely reliable based solely on comparisons of sequences of a single gene from one specimen each (e.g., Zhou et al. 2007), we analysed specimens from two different populations. At each locality some adult males of *D. plicatus* and unassigned larvae shared identical COI haplotypes (Table 2). Observed variability in COI haplotypes within populations (Table 2) is in line with the variability of the same COI fragment in populations of some other *Drusus* species (e.g., Pauls et al. 2009, Previšić et al. 2009). Variability between populations in *D. plicatus* (Table 2), however, seems to be lower than observed in some other Dinaric *Drusus* endemics (e.g., *D. croaticus*, Previšić et al. 2009, *D. krusniki* Malicky, Previšić et al. 2014b).

Moreover, additional data, such as larvae and adults of *D. plicatus* recorded in 3 springs in Republic of Macedonia (Vevčani spring, spring of the River Galička reka, and the spring of the River Strežimirska reka), confirm our association of larvae and adults of *D. plicatus*. In these springs *D. plicatus* is sympatric with the following Drusinae species: *Drusus tenellus*, *D. botosaneanui* Kumanski and *D. biguttatus*, and larvae of these species exhibit different morphological characteristics from those observed in larvae of *D. plicatus* (Waringer and Graf 1997, Waringer et al. 2015).

Separation of larvae of *Drusus plicatus* from other European Trichoptera larvae

Morphological features of the known larvae from the subfamily Drusinae are usually species specific and stable, enabling separation and identification of the species (e.g., Hickin 1967, Waringer and Graf 1997, Waringer et al. 2010, 2015). This is not the case for some other groups of Trichoptera in which larvae of many species are still not described or for which the separation of known larvae of some genera (e.g., *Hydroptila* Dalman, *Chaetopteryx* Stephens, *Rhyacophila* Pictet) is either very difficult or generally not possible (Waringer and Graf 1997).

Larvae from the subfamily Drusinae can be separated from other European Trichoptera larvae by the following morphological features (e.g., Waringer and Graf 1997, Graf et al. 2005, Kučinić et al. 2015): 1. A fully sclerotized pronotum and mesonotum; 2. Metanotum with six sclerites; 3. Gills with one filament; 4. Additional setae present on anterior and posterior faces of mid- and hind leg femora.

From the total of 49 Drusinae species recorded in southeast Europe, larval descriptions and taxonomic tools exist for the following 25 species: *Drusus balcanicus* Kumanski, *D. biguttatus*, *D. botosaneanui*, *D. bosnicus* Klapálek, *D. chrysotus* Rambur, *D. crenophylax* Graf & Vitecek, *D. croaticus*, *D. discolor*, *D. klapaleki* Marinković-Gospodnetić, *D. krpachi* Kučinić, Graf & Vitecek, *D. krusniki*, *D. macedonicus*

Table 2. Intraspecific uncorrected pairwise distances (*p*) of partial mitochondrial cytochrome oxidase I (mtCOI) sequences observed in *Drusus plicatus* (shown as percent). Abbreviations are used to denote life stages; IM (M) = adult male, L = larva.

Locality	Specimen codes	Stage	DpMAIM1	DpMAIM2	DpMAL1	DpMAL2	DpVEIM1	DpVEL1	DpVEL2	GenBank accession nos
Spring of Galičnka reka, Mavrovo National Park	DpMAIM1	IM (M)								KT598014
	DpMAIM2	IM (M)	0.7							KT598015
	DpMAL1	L	0.7	0.0						KT598016
	DpMAL2	L	0.7	0.0	0.0					KT598017
Vevčani	DpVEIM1	IM (M)	1.1	1.1	1.1	1.1				KC881523
	DpVEL1	L	1.1	1.1	1.1	1.1	0.0			KT598018
	DpVEL2	L	0.6	0.9	0.9	0.9	0.9	0.9		KT598019

Schmid, *D. medianus* Marinković-Gospodnetić, *D. meridionalis* Kumanski, *D. radovanovici* Marinković-Gospodnetić, *D. ramae* Marinković-Gospodnetić, *D. septentrionis* Marinković-Gospodnetić, *D. serbicus* Marinković-Gospodnetić, *D. siveci* Malicky, *D. tenellus*, *D. vernonensis* Malicky, *D. vespertinus* Marinković-Gospodnetić, *Ecclisopteryx dalecarlica* Kolenati, *E. ivkae* Previšić, Graf & Vitecek and *E. keroveci* (Kučinić et al. 2008, 2010, 2011a, 2011b, 2015, Previšić et al. 2014a, Vitecek et al. 2015a, 2015c, Waringer et al. 2010, 2015, 2016).

Drusus plicatus larvae can be easily distinguished from larvae of these species by the following morphological features:

- *D. chrysotus*, *D. discolor*, *D. krpachi D. meridionalis* and *D. siveci* have mandibles with terminal teeth and filtering bristles on legs and the first abdominal sternite, *D. plicatus* does not have any of the listed morphological features;
- *D. chrysotus*, *D. discolor*, *D. krpachi*, *D. meridionalis* and *D. siveci* have a head capsule concavity, a typical characteristic for larvae of these species, which is absent in *D. plicatus* larvae;
- Larvae of *D. plicatus*, *D. bosnicus* and *D. ramae* differ in head capsule shapes in lateral view. In *D. bosnicus* and *D. ramae* the head vertex is flat, while in *D. plicatus* the vertex is slightly rounded;
- *D. ramae* has a specific shape of the pronotum with two prominent acute humps on the posterior part, while the posterior part of the pronotum in *D. plicatus* is rounded; *D. plicatus* has areas of spinules on the head capsule that are absent in *D. ramae*;
- Larvae of *D. bosnicus*, *D. klapaleki*, *D. krusniki*, *D. medianus*, *D. septentrionis* and *D. vespertinus* have a pronounced hump in the central part of the pronotum in lateral view which is absent in *D. plicatus*, in which the pronotum is flat in the anterior part and slightly rounded in the posterior part;
- Larvae of *D. serbicus* have a recognizable shape of the pronotum in lateral view with an annular crest highest at dorsal center and gradually declining laterally, while the pronotum of *D. plicatus* larvae has a different shape (flat in the anterior part and slightly rounded in the posterior part);
- Larvae of *D. serbicus* lack lateral gills on the abdomen, *D. plicatus* has lateral gills on abdominal segments II throughout V;
- Larvae of *D. croaticus* lack prominent, long median setae dorsally on the anterior border of the pronotum and spinule areas on the head, which can be found in *D. plicatus*;
- Larvae of *D. radovanovici* and *D. vernonensis* have the dorsal part of the pronotum covered with numerous thin long, yellow (yellowish) setae, which are lacking in *D. plicatus*;
- Larvae of *D. botosaneanui*, *D. tenellus*, *Ecclisopteryx dalecarlica*, *E. ivkae* and *E. keroveci* have distinct parietal spines on the head, which are absent in *D. plicatus*;

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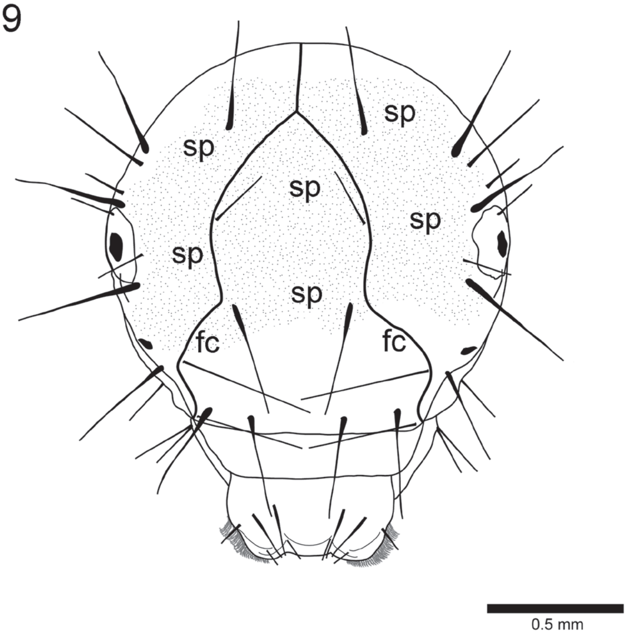
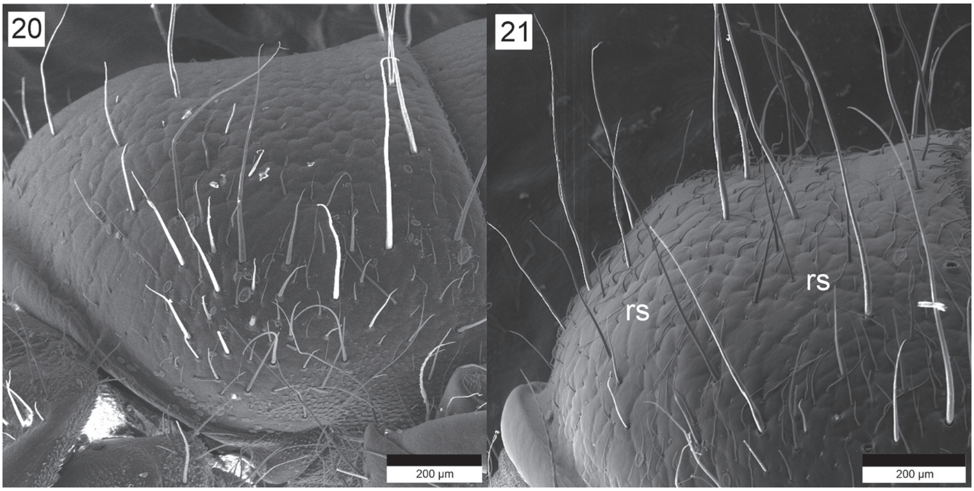


Figure 19. *Drusus plicatus* Radovanović, 4th instar larva. Head, frontoclypeus (fc) and area with spinules (sp), frontal view.

- The whole pronotum of *D. plicatus* larvae is covered in white recumbent setae, *D. crenophylax* lacks these setae in a semicircular area anterior to the pronotal ridge, *D. biguttatus* generally lacks these recumbent setae on the whole pronotum;
- Larvae of *D. balcanicus* and *D. biguttatus* lack spinule areas on the head, which can be found in *D. plicatus*.

Interestingly, the last larval instar of *D. plicatus* differs from the earlier larval stages not only in head capsule width, but also in the larger extent of spinule fields (Fig. 19, fourth instar larva). So far, this feature was noticed only for the earlier larval stages of *D. bosnicus* (M. Kučinić, unpublished data) and for last instars of *D. vernonensis* (Waringer et al. 2016).

Faunistic research conducted in western Macedonia, for the last eight years recoverd besides *D. plicatus*, eight more species from the genus *Drusus*: *D. biguttatus*, *D. vernonensis*, *D. botosaneanui*, *D. discolor*, *D. discophorus* Radovanovic, *D. macedonicus*, *D. krpachi* and *D. tenellus* (Radovanović 1942, Botosaneanu 1960, Vitecek et al. 2015a, 2015b, Waringer et al. 2015, 2016). From all the above listed species only larva of *D. discophorus* was not described yet. Of these species only *D. biguttatus* and *D. plicatus* larvae cannot be easily distinguished (Figs 20, 21). Differentiation of *D. biguttatus* larvae from *D. plicatus* larvae can be done by careful examination of morphological features on the pronotum (Figs 20, 21) and on the head.



Figures 20–21. 20 *Drusus biguttatus* Pictet. Pronotum, right lateral view **21** *Drusus plicatus* Radovanović. Pronotum, showing recumbent setae (rs), right lateral view.

Drusus discophorus larvae have not been described yet, but this species seem to be limited to the type locality consists of a spring and little mountain stream at Labuniško Lake (Jablanica Mt.). In this locality we never found larvae or adults of *D. plicatus* during several years of repeated collections. Radovanović described both species from the Jablanica Mt. and stated that *D. discophorus* inhabits higher elevations (1900 m a.s.l.), while *D. plicatus* inhabits lower altitudes (approx. up to 900 m a.s.l., Labunište village) (Radovanović 1942). In this investigation we recorded *D. plicatus* in localities at higher elevation (approx. 1410 m a.s.l., spring of the River Galička reka), and Oláh and Kovács (2013) found this species in one location in Albania at an elevation of approx. 1600 m a.s.l. (Table 1). However, the morphology of male genitalia of *D. plicatus* and *D. discophorus* is very similar (Radovanović 1942, Malicky 2004), and a comprehensive study using morphology and molecular genetic data is necessary to enable clear separation of all stages of these two species.

Ecological and ethological aspects and distribution of *Drusus plicatus*

Based on shared morphological (dark coloring of the imago, morphology of genitalia), and behavioral features (diurnal activity), *Drusus plicatus* could be closely related to the *Drusus bosnicus* group that is represented by a great number of species in southeast Europe (Marinković-Gospodnetić 1976, 1978, Kučinić et al. 2014, Vitecek et al. 2015c). Most *Drusus bosnicus* group species exhibit highly similar male genital morphology (Mariković-Gospodnetić 1978, Malicky 2004, Kučinić et al. 2011a, 2011b, Vitecek et al. 2015c). Analysis of the molecular data of *D. plicatus,* as well as of the other *Drusus* species (Malicky 2004, 2005, Oláh 2010, 2011, Oláh and Kovács 2013, Kučinić et al. 2014, Vitecek et al. 2015c), could show which species belong to the *Drusus bosnicus* group and clarify their phylogenetic and evolutionary relationships.

The subfamily Drusinae has been shown to comprise 3 groups differing in larval feeding ecology and morphology (Pauls et al. 2008). Also, these groups represent distinct evolutionary lineages (Pauls et al. 2008; Vitecek et al. 2015a). Based on the morphology of the larvae mandibles of *Drusus plicatus* are grazers. In addition to species with grazing larvae (e.g., species from *Drusus bosnicus* group, *D. plicatus*) (Kučinić et al. 2014, Viteck et al. 2015c), southeast Europe, along with western Alps, is a center of diversity for species with different larval feeding behaviors, for example, carnivorous filters (*D. meridonalis*, *D. macedonicus*, *D. krpachi*, *D. siveci*) (Vitecek et al. 2015a, 2015b). The mandibles of grazers are morphologically different from larvae that have carnivorous filtering feeding behavior (Pauls et al. 2008, Kučinić et al. 2011a, 2011b, 2015, Vitecek et al. 2015a). Molecular data from grazers and carnivorous filterers indicate a closer phylogenetic relationship among species in each group and also suggest certain evolutionary processes of speciation that probably happened in the ancestors of each feeding group (Marinković-Gospodnetić 1978, Kučinić et al. 2011a, Pauls et al. 2008, Vitecek et al. 2015a). Data suggest greater similarity for species that are geographically closer and have a similar feeding behaviour (Previšić et al. 2014b, Vitecek et al. 2015a) with *Drusus plicatus* grouping with grazers from Albania, for example *D. arbanios* Oláh, *D. dacothracus* Oláh, *D. illyricus* Oláh and *D. pelasgus* Oláh (Previšić et al. 2014b). Speciation of these and other *Drusus* is driven not only by the allopatric distribution caused by distinct geological and hydrological processes (e.g., karstification) in the past (Previšić et al. 2014b), but also by specific biologies that also condition this type of distribution, such as limited dispersal ability of adults (Kučinić et al. 2014, Geismar et al. 2015).

According to Schmid (1956), species of the *Drusus bosnicus* group are distributed in southeast Europe and the Alps. Generally, all are endemics or micro-endemics with small distribution areas and known only one or a few populations per species (Marinković-Gospodnetić 1979, Kučinić et al. 2008, Oláh 2010, 2011, Oláh and Kovács 2013, Vitecek et al. 2015c). *Drusus krusniki* is an exception, as more populations of this species are known (Previšić et al. 2014b). We collected *D. plicatus* at 8 localities in the Republic of Macedonia and the species is further reported from two localities in Albania (Oláh and Kovács 2013) (Table 1), rendering this also one of the more widely distributed endemic *Drusus bosnicus* group species in the southeast of Europe. We did not find *D. plicatus* at the type locality in Labunište village (Radovanović 1942), but we collected larvae and adults of this species in Vevčani spring (Table 1, Fig. 1B), several kilometres from Labunište village. Type locality in Labunište village was destroyed by anthropogenic influence: high level of urbanisation, pollution, stream canalisation.

The distance between the southern-most (Vevčani spring) (Fig. 1B) and the northern-most sampling location (spring of the River Strežimirska reka) of *D. plicatus* is about 100 km (Fig. 1A). Compared to the other species of the *Drusus bosnicus* group in the southeast Europe, this is a relatively large distance (Marinković-Gospodnetić 1978, 1979, Kučinić et al. 2014).

Drusus plicatus inhabits the creanal zone of streams and rivers with adults day-active at or near the spring. Diurnal activity is reported for several *Drusus* species in southeast Europe, e.g., *D. krusniki*, *D. vespertinus*, *D. medianus*, *D. klapaleki*, *D. radovanovici* (Kučinić et al. 2014, M. Kučinić, A. Previšić, unpublished data). However, a small number of *D. plicatus* specimens were collected also during the night using UV light traps at the spring of the River Galička reka, which is an exception for dark colored species of caddisflies that generally are active during day (Kučinić et al. 2014). At this locality, the highest abundance of *D. plicatus* has been recorded, with several hundreds of adults, during the day.

A similar mass emergence of adults has been previously recorded in *D. septentrionis* at two localities in Bosnia and Herzegovina (springs of the rivers Bistrica and Sturba, Kučinić et al. 2008, M. Kučinić, unpublished data) and in *D. krusniki* at Alipaša's springs in Montenegro (A. Previšić unpublished data). We observed two peaks in the emergence of *D. plicatus* at the spring of the River Galička reka, the first one in spring (May - June) and the second one in autumn (September). The same emergence pattern was recorded for some other *Drusus* species in the Balkan Peninsula, e.g. *D. croaticus* and *D. septentrionis* (Kučinić 2002, Kučinić et al. 2008).

Caddisfly species richness

Among the three springs encompassed in this study the highest biodiversity (species richness) of caddisflies was recorded in the spring of the River Strežimirska reka, and the lowest in the spring of the River Galička reka. Only two species, *Rhyacophila balcanica* and *Drusus plicatus*, were recorded in all three springs. Also during this study, *Synagapetus iridipennis* was recorded for the first time for the Trichoptera fauna of the Republic of Macedonia.

Potamophylax lemezes was described based on specimens collected in the spring of the River Galička reka (Oláh et al. 2013). The exact taxonomic status of this population would ideally be assessed using molecular methods for a comparison of this population with some other populations of *Potamophylax nigricornis* Pictet, from which *P. lemezes* was delineated (Oláh et al. 2013).

According to the literature *Wormaldia occipitalis* was recorded from Vevčani spring (Oláh and Kovács 2014). During our investigation we did not collect specimens of any *Wormaldia* from this locality. The taxonomic status of this species will be evaluated in future studies following Neu (2015), because this species is not present in the Republic of Macedonia.

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New species of *Goniocolletes* and *Trichocolletes* (Hymenoptera, Colletidae) from southern Australia

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Abstract

Goniocolletes comatus Maynard, 2013 is redescribed. *G. wanni* **sp. n.** and the male of *Trichocolletes luteorufus* Batley & Houston, 2012 are described.

Keywords

Colletid bees, Bush Blitz survey

Introduction

This paper reports on bee species that were collected on Bush Blitz surveys in remote locations of Australia. Bush Blitz is a partnership between the Australian Government, BHP Billiton and Earthwatch Australia to document fauna and flora on selected national reserves. These surveys regularly result in the discovery of new invertebrate species (e.g. true bugs: Symonds and Cassis 2014; spiders: Baehr et al. 2013, bees: Hogendoorn et al. 2015).

The Australian native bee species are still relatively unknown. Close to 60% of the known species are not in identification keys and with each generic revision numerous new species are added (Batley and Hogendoorn 2009). Therefore, species identification and recognition of new species is most straightforward for those genera that have recently been revised and where species identification keys have been included in the revision (Hogendoorn et al 2015). Here, we describe species in two genera of Colletidae that were revised recently (*Trichocolletes*: Batley and Houston 2012; *Goniocolletes*: Maynard 2013). The descriptions cover related genera and involve one new species, one redescription and a description of a male for a species of which hitherto only the female was known.

Methods

Bee species were caught on flowering plants using a hand net. Specimens of *Trichocolletes* and *Goniocolletes* were identified using identification keys in recent revisions of these genera by Batley and Houston (2012) and Maynard (2013) respectively. The identified specimens were compared to type specimens and other reliably named material at the Queensland Museum, the Western Australian Museum and the South Australian Museum.

For descriptions of the new species the terminology used by Michener (2007) was followed. Using a stereomicroscope with step-less zoom and an eyepiece micro-meter measurements were taken relative to the head width, whereby head width was set to 50 units (following Houston 1990). Abbreviations for these relative measurements are as follows: AOD antennocular distance; ASD antennal socket diameter; BMW basal width of mandible; DMA distance between anterior mandibular articulations; FL flagellum length; HL head length; HVO height of vertex above lateral ocelli; HW head width; IAD interantennal distance; LID lower interorbital distance; ML mandible length; MOD diameter of median ocellus; MSL malar space length; OOD ocellocular distance; SL scape length; SW scape width; UFW upper width of face; UID upper interorbital distance; WOC width of ocellar cluster. Other abbreviations used are: T1, T2 etc. for first, second metasomal tergite; S1, S2 for first, second metasomal sternite, etc.

Some of the specimens treated here were also submitted to BOLD (Barcode of Life Database) for DNA barcoding using the cytochrome c oxidase subunit 1 gene. Specimen details, including DNA sequence, collecting dates and locality information can be accessed in BOLD under the project Australian Bee Survey, e.g. http://www.boldsystems.org/index.php/Public_RecordView?processid=AUSBS310-13

AUSBS-numbers are presented under material examined.

Under material examined, collection data are presented as copied from the specimen labels. Therefore, data formats for locality coordinates could vary. For specimens collected by Leijs et al. the locality coordinates are in decimal degrees.

Repositories

ANIC	Australian National Insect Collection
SAMA	South Australian Museum, Adelaide

QM Queensland Museum, which now also contains the former collections of the University of Queensland Insect Collection (UQIC)

Systematics

Goniocolletes Cockerell, 1907

Remarks. Two species of *Goniocolletes* were collected during a Bush Blitz survey at Hiltaba Station circa 130 km East of Ceduna, South Australia, November 2012. A single specimen of *G. abdominalis* (SAMA32-032978 / AUSBS 313-13) was collected on the same flowering *Eucalyptus* as where a number of other *Goniocolletes* males were caught. Using the key and information provided in Maynard's (2013) revision, these other collected specimens of *Goniocolletes* initially keyed out to *G. comatus* Maynard 2013. However, unlike the colour characters mentioned in Maynard (2013), our specimens had orange legs, with dark coxae and trochanters. Comparison to the type specimen showed that these colour characters seem to have been switched both in the identification key (Maynard 2013, pg. 98) and species description (Maynard 2013, pg. 104). Locating the holotype of *G. comatus* and other species examined specimens by Maynard (2013) was not straightforward, because none of the type specimens in SAMA and QM had been labelled as such. The male (holo)-type of *G. comatus* could be identified and located on the basis of its unique locality data, but this was impossible for the female type, because multiple specimens had the same label information as the type (Maynard 2013), and their repository was not stated. Examination of all specimens associated with *G. comatus* in the collections of QM and SAMA, indicated the existence of two species, with differences in pubescence and characters associated with the male genitalia and hidden sternites. We associated the sexes by using series of males and females collected from the same date and locality for each of the species. Here, we redescribe the male and female of *G. comatus* and describe the additional species on the basis of fresh material that was collected during a Bush Blitz survey at Hiltaba Station, South Australia.

As only a single species is added to this genus, we do not produce a completely new key, but suggest modifying the key produced by Maynard (2013, pg. 97) as follows:

For males:

9(8) Legs all black .... ***Goniocolletes albopilosus* (Rayment)**
– Legs orange with black coxae and trochanters **9a**
9a(9) Pubescence on T1 not dense and less than twice as long as on T2 (Fig. 1A, 1B), tip of S8 with long hairs pointed backwards (Fig. 3D) ....
.... ***Goniocolletes comatus* Maynard**

– Pubescence on T1 dense and more than twice as long as on T2 (Fig. 2A, 2B), tip of S8 with a sidewards directed patch of dense stiff long dark hairs on each side (Fig. 4D) .... ***Goniocolletes wanni*** **sp. n.**

For females:

24(23) Median area of supraclypeal area punctate ... ***Goniocolletes anthedonus*** **Maynard**
– Median area of supraclypeal area polished and impunctate **25**
25(24) Horizontal part of propodeal triangle gradually sloping, first flagellomere similar in length to third .... ***Goniocolletes comatus*** **Maynard**
– Horizontal part of propodeal triangle defined by sharp carinae, first flagellomere longer than third .... ***Goniocolletes wanni*** **sp. n.**

Goniocolletes comatus Maynard, 2013

Figs 1, 3, 5

Material examined. *Holotype*: ♂, Lake Gilles NP, 3 Feb 1975, C.A. & T.F. Houston, on *Eucalyptus* blossom (SAMA 32-032610).

Specimen used for the description of the female. ♀, Lake Gilles NP, SA, 8 Mar 1976, C.A. & T.F. Houston, on *Eucalyptus* (SAMA 32-033291).

Additional material examined 17♂. **SAMA:** 4♂, Lake Gilles NP, SA, 8 Mar 1976, C.A. & T.F. Houston, on *Eucalyptus*;

1♂, Orroroo, SA, 22 Apr 1944. **ANIC:** 10♂, Angorichina Hotel, 31.08S 138.34E, SA, 8 Nov 1987, I. Nauman & J. Cardale, on *Eucalypt* flowers; 2♂, Brachina, 31.01S 138.33E, 9 Nov 1987, I. Nauman & J. Cardale.

Diagnosis. This species is distinguished from other species in this genus by males with black metasoma, simple orange legs with brown coxae and trochanters, scape black, pubescence on T1 as long as on T2 and S8 with evenly and openly placed long radiating setae. Females with propodeal triangle gradually sloping downwards, horizontal area not defined by a sharp carina.

Redescriptions. *Holotype:* Male (SAMA 32-032610), body length 8.8 mm, head width 2.6 mm.

Relative head measurements. HW 50, HL 42, UID 30, UFW 33, LID 27, DMA 28, HVO 0, WOC 15, MOD 4, OOD 8, IAD 8, ASD 3, AOD 7, ML 21, BMW 8, MSL 1, SL 13, SW 3, FL 52.

Structure. Head: face longer than wide, clearly converging below; malar space circa 0.13× basal mandibular width; clypeus protuberant; vertex not elevated; gena about 0.8× eye width viewed laterally; flagellum as long as head width; flagellomeres 3-11 1.4× as long as wide. Legs not modified: hind tarsus longer than hind tibia; hind basitarsus 7× as long as wide; hind coxa without posterior ventral procession; S7 (Fig. 3C), inner lobes almost straight broadly rounded proximally; S8 (Fig. 3D) with evenly and

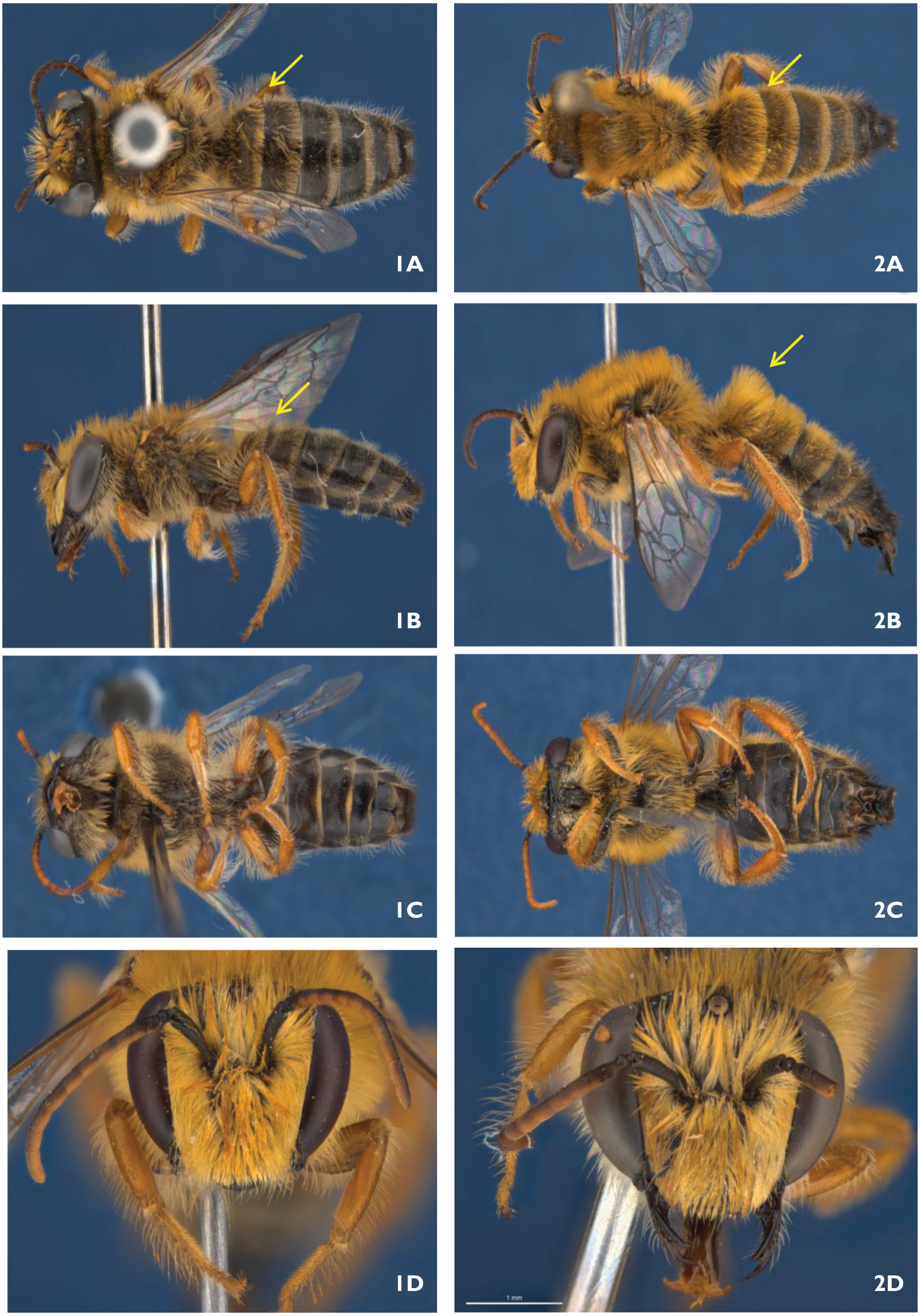

Figure 1,2. 1 Male holotype *G. comatus* (SAMA 32-032610): **A** dorsal **B** lateral **C** ventral **D** head **2** Male holotype *G. wanni* sp. n. (SAMA 32-032979): **A** dorsal **B** lateral **C** ventral **D** head.

openly placed long radiating setae, apparent in un-dissected specimens; genital capsule (Fig. 3AB), penis valves suddenly broadened at level of mid gonocoxite.

Coloration. Integument black, apart from antenna ventrally brown, legs orange, apart from basis of femur of front and middle legs, all coxae and trochanters brown, tegula translucent orange; marginal zone of T1-6 transparent.

Sculpture. Scutum, propodeum and tergites with close pit-reticulation, vertical part of propodeal triangle dull with fine irregular reticulation, horizontal part with transverse striae, lateral rim coarsely areolate.

Pubescence. Eyes with tiny dispersed hairs. Pubescence orange, apart from posterior margin of T6 and whole of T6-7 black, genae near eye margin whitish. Hairs on face erect, dense and long; hairs on T1 longer than on T2-4; sternites almost bare, apart from S3-4 with narrow, dense posterior fringes.

Female (SAMA 32-033291), body length circa 11 mm, head width 3.2 mm.

Relative head measurements. HW 50, HL 40, UID 28, UFW 34, LID 29, DMA 27, HVO 1, WOC 16, MOD 4, OOD 6, IAD 8, ASD 3, AOD 8, ML 21, BMW 7, MSL 2, SL 15, SW 3, FL 33.

Structure: Head: inner eye margin straight, converging below; scape reaching median ocellus, tapering basally; malar space smooth length about 0.16 width; labial palps and maxillary palps just reaching apex of extended glossa; clypeus below supraclypeal level; epistomal suture distinct, almost straight; labrum basally strongly produced, apically flat; F1,3–10 length equal to, or less than width, F2 shorter than width; antennal sockets depressed; supraclypeal area strongly raised above frons level; midtibial spur long, slender; inner hind tibial spur with 6 long, slender teeth (other examined specimens with 7 teeth); Metasoma: postgradular area with weaker sculpture than pregradular area; pygidial plate broadly rounded apically, flat, dull; T2-4 anteriorly fine reticulate striated, posteriorly open to close punctated.

Coloration: Head, integument black with dark brown to black clypeus; mandibles black, medially brown and smooth; scape almost naked, black; flagellum light brown beneath, dark brown above; Mesosomal integument black; Metasomal integument orange.

Sculpture: Scutum and scutellum posteriomedially smooth with strong open punctation; metanotum dull and rugose; propodeal triangle with several fine transverse carina, anterior area narrower than metanotum; anterior area almost smooth. Clypeus flat, shiny with open large punctures; supraclypeal area polished, flat.

Pubescence: Head: hair white, dense on lower paraocular area; mesosomal hair white; hind basitibial plate with dense, branched hairs, obscuring marginal carina; hind basitarsus with posterior open fringe of long, branched grey hairs. Metasomal hair dorsally short, sparse, white; prepygidial fimbria dark brown; T2-4 posteriorly with fringes of long white hair.

Remarks. A redescription of this species was necessary because the specimens examined under Maynard's (2013) original description of *G. comatus*, appear to belong to more than one species. The male holotype and the female are redescribed for comparative reasons.

Floral visitations. *Eucalyptus* (Myrtaceae)

Distribution. Currently known from four localities in the Flinders Ranges and northern Eyre Peninsula, South Australia.

Goniocolletes wanni **sp. n.**

http://zoobank.org/E2A89228-CB9B-46B0-A07A-98CB0A5804D2
Figs 2, 4, 6

Material examined. *Holotype*: ♂, (SAMA32-032979-AUSBS310-13), Hiltaba Station, SA, 32.11689S 135.15634E, 16 Nov 2012, R. Leijs and B. Tully, on *Eucalyptus* (SAMA).

Paratype. ♀, (SAMA 32-033292), 11 miles (17.6 km) S of Salmon Gums, WA, 11 Jan 1970, T.F. Houston, on *Eucalyptus.*

Additional material examined 5♀, 42♂. **SAMA:** 1♂, (SAMA 32-033115), Hiltaba Station, SA, 32.12293S 135.22875E, 12 Nov 2012, R. Leijs and B. Tully, on *Myoporum*; 3♂, (SAMA32-033276-AUSBS 312-13, SAMA32-033277-AUSBS 311-13, SAMA32-033277-AUSBS 311-13), Hiltaba Station, N of Progress Dam, SA, 32.23577S, 135.26811E, 12 Nov 2012, R. Leijs and B. Tully, on *Eucalyptus*; 1♂ 28 km NE Wirrulla, SA, 7 Mar 1976, C.A. & T.F. Houston, on *Eucalyptus*; 1♂ 20 miles (32km) NE Eucla, SA, 9 Jan 1970, T.F. Houston; 1♀, 3♂ Moorlands, SA, 19 Jan 1966, T.F. Houston on *Eucalyptus*. SA: 5♀, 2♂ 11 miles (17.6km) S of Salmon Gums, WA, 11 Jan 1970, T.F. Houston, on *Eucalyptus*; 15♂, 37 km W. of Balledonia, WA, 32.17556S 123.27500E, 18 Mar 1996, J. Forrest, sweeping flowering *Eucalyptus*.

QM: 20♂ Weebubbie Cave area, WNW Eucla, WA, 31.65000S 128.76667E, 23 Jan 1987, G. & A. Daniels on *Melaleuca*.

Diagnosis. This species is distinguished from other species in this genus by males with pubescence twice as long on T1 as on T2 and S8 on both sides with conspicuous bristle of dense, thick, long setae. Females with horizontal part of propodeal triangle defined by sharp transverse carina.

Description. *Holotype*. ♂ (SAMA 32-032979), body length 12 mm, head width 3.3 mm.

Relative head measurements. HW 50, HL 42, UID 31, UFW 34, LID 28, DMA 30, HVO 2, WOC 17, MOD 3, OOD 7, IAD 8, ASD 3, AOD 8, ML 21, BMW 6, MSL 1, SL 14, SW 3, FL 61,

Structure. Head: face longer than wide, clearly converging below; malar space circa 0.16× basal mandibular width; clypeus protuberant, vertex not elevated; gena little wider than 0.7 eye width viewed laterally; flagellum 1.22× head width; flagellomeres 2-5 1.6× as long as wide; flagellomere 11 2.3× as long as wide. Legs not modified: hind tarsus about as long as hind tibia; hind basitarsus 5.5× as long as wide; hind coxa with posterior ventral procession; S7 (Fig. 4C), inner lobes almost straight; S8 (Fig. 4D) bare at apex, both sides with conspicuous bristle of dense thick long setae, apparent in un-dissected specimens; genital capsule (Fig. 4A–B): penis valves progressively broadened from mid of gonocoxite to base of gonostylus.

Coloration. Integument black, apart from antenna ventrally, legs orange, apart from femur of front leg and posterior part of femur of middle leg black, tegula brown, marginal zone of T1-6 transparent.

Sculpture. Scutum, propodeum and tergites with close pit-reticulation, vertical part of propodeal triangle dull with fine irregular reticulation, horizontal part with radial striae, lateral rim coarsely areolate.

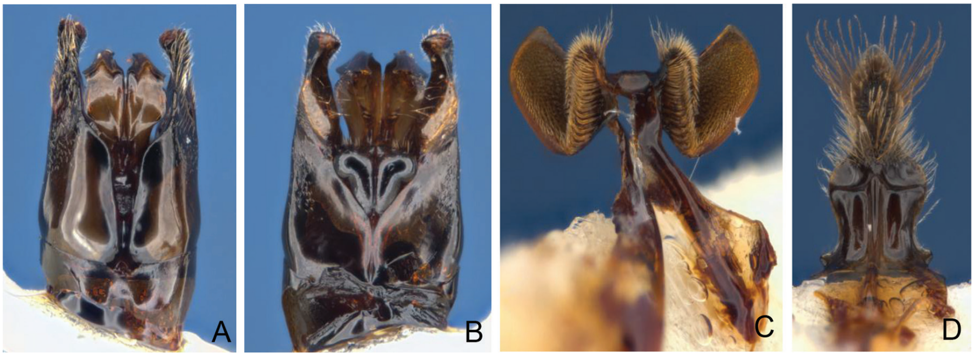

Figure 3. Male holotype *Goniocolletes comatus* (SAMA 32-032610). **A** genital capsule dorsal **B** id. ventral **C** S7 **D** S8.

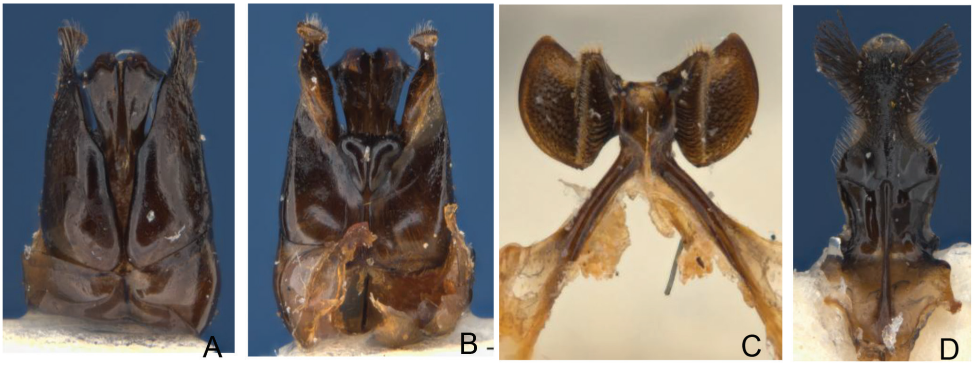

Figure 4. Male holotype *Goniocolletes wanni* sp. n. (SAMA 32-032979). **A** genital capsule dorsal **B** id. ventral **C** S7 **D** S8.

Pubescence. Eyes with tiny dispersed hairs. Pubescence orange, apart from posterior margin of T6 and whole of T6-7 black, genae near margin of eye pale orange. Hairs on face erect dense and long, hairs on T1 dense, much longer than on T2-4. Sternites almost bare, apart from S3-4 with narrow dense posterior fringes.

Paratype. ♀ (SAMA 32-033292), body length 13 mm, head width 3.8mm.

Relative head measurements. HW 50, HL 45, UID 29, UFW 35, LID 30, DMA 29, HVO 2, WOC 16, MOD 3, OOD 6, IAD 8, ASD 3, AOD 9, ML 23, BMW 8, MSL 3, SL 16, SW 3, FL 32.

Structure. Head: inner eye margin straight converging below; scape over reaching median ocellus, tapering basally; malar space smooth length about 0.16x width; labial palps reaching beyond apex of extended glossa; maxillary palps reaching beyond apex of extended glossa; clypeus below level of supraclypeus; epistomal suture distinct, almost straight; labrum basally strongly produced, apically flat and smooth; F3–10 length equal to, or less than width, F1 longer than F3; antennal sockets depressed; supraclypeal area strongly raised above frons level; midtibial spur long, robust; inner hind tibial spur with 7 long, slender teeth. Metasoma: marginal zones of tergites with weaker sculpture

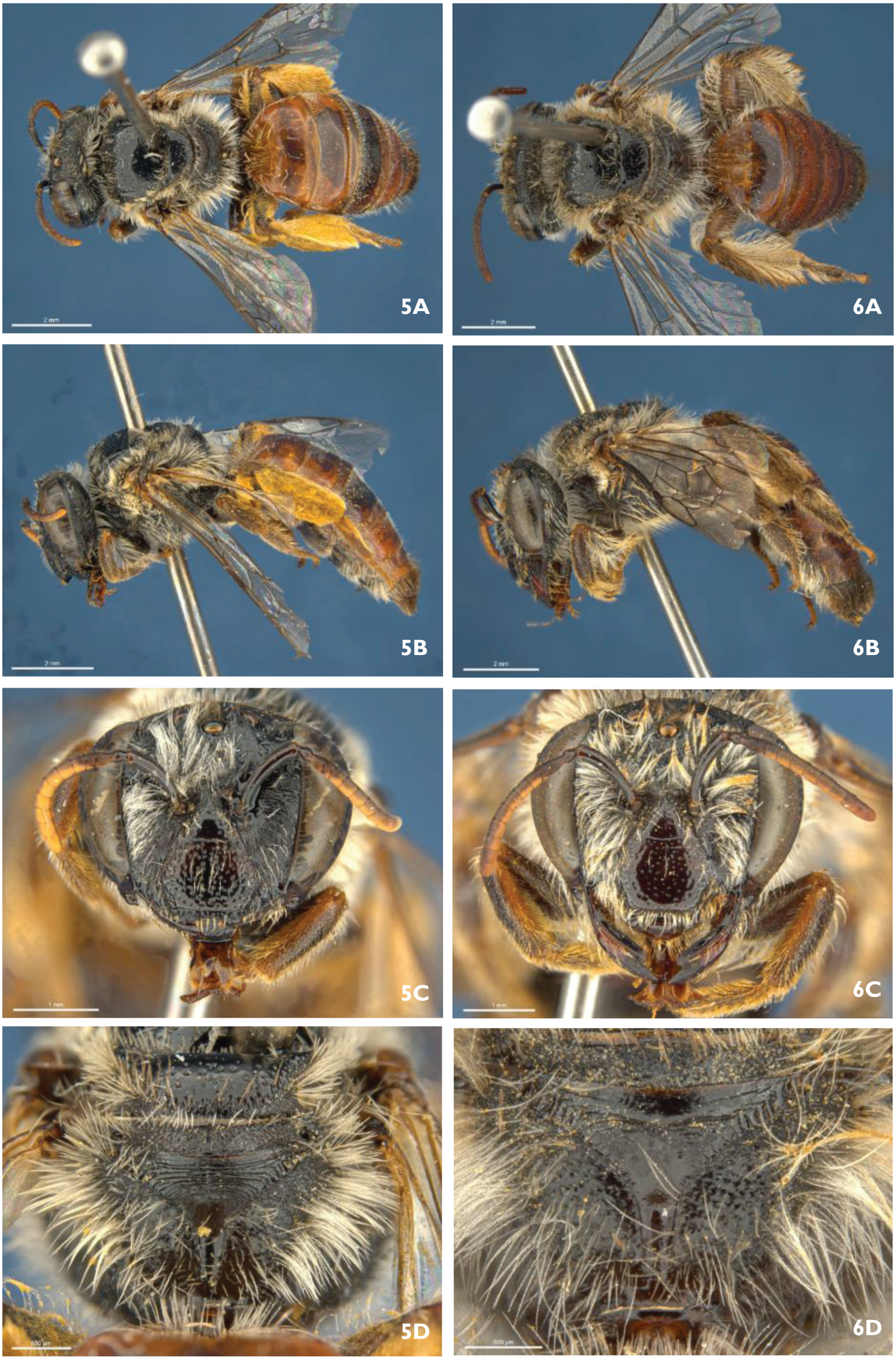

Figure 5, 6. 5 Female *G. comatus* (SAMA 32-033291): **A** dorsal **B** lateral **C** head **D** propodeum **6** Female paratype *G. wanni* sp. n. (SAMA 32-033292): **A** dorsal **B** lateral **C** head **D** propodeum.

than on disc; pygidial plate broadly rounded apically, flat, dull; T2-4 almost entirely close punctated.

Coloration. Head: integument black with dark brown to black clypeus; mandibles black, brown and smooth medially; scape almost naked, black; flagellum; light brown beneath, dark brown above. Mesosomal integument black. Metasomal integument orange.

Sculpture. Scutum and scutellum medially smooth with strong open punctation; metanotum dull and rugose; propodeal triangle with several transverse sharp carinae, basal area narrower than metanotum; Vertex closely and medium size punctated over entire width; clypeus flat, shiny with open large punctures; supraclypeal area polished, flat.

Pubescence. Head - hair white, densest on the lower paraocular area; mesosomal hair white; hind basitibial plate with dense, fine hair apparently not branched, not obscuring marginal carina; hind basitarsus with posterior fringe of dense long branched white hair widest anteriorly. Metasomal hair dorsally short, sparse; prepygidial fimbria black; T2-4 with fringes of long white hair.

Months collected. January, March, November.

Floral visitations. *Eucalyptus* (Myrtaceae), *Melaleuca* (Myrtaceae), *Myoporum* (Myoporaceae).

Distribution. Known from seven localities in southern Australia, east and west of the Nullarbor Plain.

Etymology. The species is named after Stan Wann, the grandfather of the co-collector, Beth Tully. Stan Wann grew up in the bush on the north coast of NSW, and had a profound knowledge of the birds and trees in the area.

Trichocolletes Cockerell, 1912

Remarks. Ten species of *Trichocolletes* were collected during a Bush Blitz survey at Credo Station circa 120 km NW of Kalgoorlie, Western Australia between 29 August - 9 September 2011. The majority of the *Trichocolletes* species were caught on *Mirbelia microphylla* (Fabaceae) which was profusely flowering at many sites in the reserve. The *Trichocolletes* species *avialis*, *nitens* and *rufibasis* were most common and abundant, but among these also single specimens of *aureotinctus*, *dowerinensis*, *dundanensis* and *leucogenys* were collected. A single male of *T. eremophilae* was caught in an area with flowering *Eremophila* (Myoporaceae) and *Senna* (Fabaceae). Additionally, a male (first recorded) and females of *T. luteorufus* were collected on *Senna glutinosa* spp. *chatelainiana* . Here the male of *T. luteorufus* is described.

As we only add the male of a single species, we do not produce a completely new key, but suggest modifying the key for the males presented by Batley and Houston (2013, pg. 5), by inserting couplets as follows:

32(28) Hind tibia modified: short and swollen, with plume of long orange hairs (Fig. 7a) .... ***T. luteorufus***
– Hind tibia not modified.. **32a**

32a(32 Length 15–17 mm; S2 with dense, untidy plume .... ***T. marginatus***

– Length 10–14 mm; sternal pubescence weak **33**

Trichocolletes luteorufus **Batley & Houston, 2013**

Fig. 7A–F

Material examined. 1 ♂ (RL1833), 2 ♀ (RL1834a-b), Credo Station, near Ularring Rock, WA, Australia, 31 Aug 2011, 29.92833S, 120.55209E, R. Leijs, on *Senna glutinosa* ssp. *chatelainiana.*

Diagnosis. *T. luteorufus* is the only species where the male has distinctly broadened hind tibia bearing a well developed plume of dense long orange hair (Fig. 7A).

Description. ♂ (RL1833), body length 15 mm, head width 3.8 mm.

Relative head measurements. HW 50, HL 34, UID 33, UFW 33, LID 30, DMA 32, HVO 4, WOC 13, MOD 3, OOD 8, IAD 8, ASD 3, AOD 8, ML 18, BMW 5, MSL 2, SL 12, SW 3, FL 61.

Structure. Face wider than long, sligthly converging below; malar space circa 0.4× basal mandibular width; labrum 1/2 as long as wide, convex, roughened by carinae; clypeus protuberant; vertex almost horizontal; gena little wider than 0.5 eye width viewed laterally; flagellum 1.22× headwidth; flagellomeres 4-10 1.67× as long as wide, flagellomere 11 2.1× as long as wide; legs long: hind tarsus 2× as long as hind tibia; hind tibia distinctly broadened medianly (Fig. 7A); hind basitarsus 5× as long as wide; posterior carina of basitibial plate reaches apex; genital capsule (Fig. 7F), inner apex of gonostylus dorsally with broad rectangular lobe reaching penis valve. Penis valve hooked apically; gonostylus laterally with concave vertical plate, inner margin ventrally deeply emarginated (Fig. 7F); S7 (Fig. 7E) lateral lobe, emarginated, at basis near ligulate process carrying 4 long stiff setae; posterior projection very long.

Coloration. Integument black, apart from scape, antenna ventrally and all legs from posterior part of femur onwards orange; marginal zone of tergites translucent pale orange; sternites brown; labrum and basal 2/3 of mandible transparent white, mandible apex brown.

Sculpture. Vertex and scutum with fine transverse lineo-reticulation and open to sparse punctation.

Pubescence. Eyes with tiny dispersed hairs. Face, especially clypeus, with very dense, long, orange hair, finely-branched on clypeus and inner eye margins; hair on vertex more open, fine and white. Ventral part of gena with dense long ventrally directed branched pale orange hair, outer eye margins with short white hair. Scutum with medium length pale orange hairs, remaining part of thorax with off-white hairs. Fore basitarsus with plume of orange hair; hind tibia with distinctive plume of dense long orange hair (Fig. 7A); inner area of hind tibia and basitarsi with long dispersed bended orange hairs. T1-3 openly covered with long, erect, white hair, decreasing in length; S3-5 with long bended white to orange hairs that together almost forms a corbicula like structure.

Figure 7. *Trichocolletes luteorufus*. Male (RL1833). **A** lateral **B** dorsal **C** head frontal **D** head lateral **E** S7 **F** genital capsule dorsal.

Remarks. Previously the male of *T. luteorufus* was unknown (Batley and Houston 2012). The sexes were linked based on the fact that both males and females were collected on the same flowering bush of *Senna glutinosa* subsp. *chatelainiana*, where males were chasing females of this species.

Distribution. Known from two localities, near Mt Magnet and Credo Station, North of Coolgardie, WA.

Discussion

The Australian native bee fauna is as yet not completely known, and many new species are added with each new generic revision (Batley and Hogendoorn 2009). Here we have shown that surveying remote locations in Australia can even turn up new species in genera that have only recently been revised. This emphasizes the fact that our knowledge to date remains fragmentary.

Acknowledgements

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RESEARCH ARTICLE

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Review of the *Fannia postica*-group Chillcott, 1961 of the genus *Fannia* Robineau-Desvoidy, 1830, with description of two new species from the Palearctic and Oriental regions (Diptera, Fanniidae)

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Abstract

A total of 17 species of the *Fannia postica*-group Chillcott, 1961 from the Palearctic and Oriental regions are reviewed herein, 2 of which are described from China as new: *Fannia ningxiaensis* Wang & Zhang, **sp. n.** and *Fannia subaethiops* Wang & Zhu, **sp. n.**. *Fannia labidocerca* Feng & Xue, 2006, originally placed in *F. serena*-group Chillcott, 1961, is moved to the *postica*-group and re-described. An identification key to the males of known species from these regions is provided.

Keywords

Species transference, *F. serena*-group, identification key, new Chinese species

Introduction

The *Fannia postica*-group was established in the genus *Fannia* Robineau-Desvoidy, 1830 by Chillcott (1961). Species of this group are classified under two subgroups, the *F. postica*-subgroup and the *F. spathiophora*-subgroup (Chillcott 1961). The group is currently composed of approximately 25 known species worldwide (including the species added in this paper), most of which are distributed in the Holarctic Region, with a minority of species in the Oriental Region (Wang et al. 2011). They are: *F. postica*-subgroup: *F. brevicauda* Chillcott, *F. discoculea* Xue, *F. enigmata* Chillcott, *F. flavibasis* (Stein), *F. labidocerca* Feng & Xue, *F. multisetosa* Chillcott, *F. postica* (Stein), *F. ringdahlana* Collin, *F. sequoiae* Chillcott; *F. spathiophora*-subgroup: *F. aethiops* Malloch, *F. ardua* Nishida, *F. bigelowi* Chillcott, *F. brooksi* Chillcott, *F. coculea* Nishida, *F. gotlandica* Ringdahl, *F. ningxiaensis* Wang & Zhang, sp. n., *F. nudifemorata* Wang & Zhang, *F. scyphocerca* Chillcott, *F. slovaca* Gregor & Rozkošný, *F. spathiophora* Malloch, *F. stigi* Rognes, *F. subaethiops* Wang & Zhu, sp. n., *F. tundrarum* Chillcott, *F. umbratica* Collin, *F. umbrosa* (Stein).

At the end of the nineteenth century, Stein (1895) described the Palearctic and Oriental species, *F. postica* (Stein). Since the beginning of the twentieth century, a number of papers and monographs studying the European species of *F. postica*-group have been published (Ringdahl 1926, Collin 1939, Hennig 1955, D'Assis-Fonseca 1968, Rognes 1982, Gregor and Rozkošný 2005). Pont (1986) reviewed the Palearctic fanniids, including species of the *F. postica*-group. Rozkošný et al. (1997) treated species of the Family Fanniidae from Europe and added nine species to the *F. postica*-group.

Asian species of the *F. postica*-group were mainly reported on by Nishida (1975, 1976), Pont (1977), Fan (1992), Xue and Wang (1998), Xue et al. (2001), Wang and Xue (2002) and Wu and Wang (2002). Wang et al. (2011) listed 22 known species of this group worldwide, recorded nine species of the *postica*-group from China and, based on the definition of the group by Chillcott (1961), summarized features of the male habitus and terminalia that distinguish it from other Fanniidae.

The biological characteristics of these species have never been fully studied. The study of specimens in our entomological collections has revealed that the majority of species in the group occur in wooded or shrubby habitats. According to Rozkošný et al. (1997), the immature stages of some species, such as *F. umbrosa* (Stein), live in birds' nests or in sap flowing out of rotholes in trees. The larvae of *F. postica* (Stein) and *F. umbrosa* (Stein) feed on humic substances, whereas the larvae of *F. postica* (Stein) also develop in carrion (Rozkošný et al. 1997).

In this paper, we review 17 known Palearctic and Oriental species belonging to the *F. postica*-group. Based on an extensive literature search and study of dry specimens, a key to the identification of males of known species from these regions is given, and two new species from China are described. One species, *F. labidocerca* Feng & Xue, 2006, is transferred from the *F. serena*-group to the *F. postica*-group and re-described. Illustrations of the male terminalia are included.

Material and methods

The morphological terminology used in this paper follows McAlpine (1981), except for the term "postpedicel", which follows Stuckenberg (1999). Absolute measurements in millimeters (mm) are given for body length. The specimens studied for this paper are deposited in the Institute of Entomology, Shenyang Normal University, Shenyang, China (IESNU) and the Shanghai Entomological Museum, Chinese Academy of Science, Shanghai, China (SHEM). Figure of *Fannia labidocerca* Feng & Xue, 2006 is from Feng and Xue (2006). Methods for the preparation of terminalia and illustrations follow Zhang et al. (2013).

The following abbreviations are used for characters throughout the text: *acr* = acrostichal seta(e), *ad* = anterodorsal seta(e), *av* = anteroventral seta(e), *d* = dorsal seta(e), *dc* = dorsocentral seta(e), *ia* = intra-alar seta(e), *p* = posterior seta(e), *pd* = posterodorsal seta(e), *pra* = prealar seta(e), and *pv* = posteroventral seta(e).

Taxonomic accounts

Genus *Fannia* Robineau-Desvoidy, 1830

Fannia postica-group: Chillcott 1961: 101, 222; Rozkošný et al. 1997: 48; Wang et al. 2011: 3.

For a diagnosis of the group see Wang et al. (2011).

Key to males of the known Palearctic and Oriental species in the *Fannia postica*-group

1 Hind femur with at least 2 *av* in distal half (*Fannia postica*-subgroup) **2**
– Hind femur with only 1 *av* in distal half (*Fannia spathiophora*-subgroup) ...**5**
2 *Pra* 1; hind coxa with setulae on posterior surface.... ***Fannia discoculea* Xue**
– *Pra* 2; hind coxa bare on posterior surface..**3**
3 Mid first tarsomere without a basal tooth-like spine on ventral surface; hind femur with 4 to 6 *av* in distal half; calypters yellow...***Fannia postica* (Stein)**
– Mid first tarsomere with a basal tooth-like spine on ventral surface; hind femur with only 2 *av* in distal half; calypters blackish **4**
4 Hind femur without distinct *pv*, and with 3 to 5 *av* in distal half................. ....***Fannia labidocerca* Feng & Xue**
– Hind femur with 7 or 8 *pv* in distal half, and with 2 *av* in distal half ....***Fannia ringdahlana* Collin**
5 Hind coxa with setulae on posterior surface; *pra* 2 (rarely 3); frontal setae 7 to 9; mid first tarsomere with a basal tooth-like spine on ventral surface ....***Fannia coculea* Nishida**
– Hind coxa bare on posterior surface...**6**

6 Fore tibia with 7 to 9 slender *pv*.................. ***Fannia spathiophora*** **Malloch**
– Fore tibia without slender *pv* **7**
7 Hind femur without distinct *pv*; haltere brown... ***Fannia nudifemorata*** **Wang & Zhang**
– Hind femur with *pv*..**8**
8 Hind femur with 3 to 5 *pv* in distal half...**9**
– Hind femur with 7 to 14 *pv* in distal half...**13**
9 Abdomen at least yellowish in basal part......... ***Fannia gotlandica*** **Ringdahl**
– Abdomen entirely black...**10**
10 Mid first tarsomere with a stout basal tooth-like spine on ventral surface... ***Fannia stigi*** **Rognes**
– Mid first tarsomere with a weak basal tooth-like spine on ventral surface... **11**
11 Syntergite 1+2 and tergites 3–4 each with a dark median stripe .... ***Fannia aethiops*** **Malloch**
– Syntergite 1+2 and tergites 3–4 each with an inverted T-shaped dark mark.... **12**
12 Frons, at its narrowest point, about as wide as anterior ocellus; *pra* short and weak, the anterior one about 1/2 as long as the length of posterior notopleural seta... ***Fannia ardua*** **Nishida**
– Frons, at its narrowest point, slightly wider than the distance between outer margins of posterior ocelli; *pra* slightly stout, the anterior one about 2/3 as long as the length of posterior notopleural seta .... ***Fannia subaethiops*** **Wang & Zhu, sp. n.**
13 Postocular setae in 2 rows **14**
– Postocular setae in one row..**15**
14 *Acr* mainly triserial; mid tibia strongly flattened and with a posteroventral ridge .... ***Fannia bigelowi*** **Chillcott**
– *Acr* mainly biserial; mid tibia not strongly flattened and without a posteroventral ridge......................... ***Fannia ningxiaensis*** **Wang & Zhang, sp. n.**
15 Scutum entirely black; bacilliform process long and only bent ventrally.. ***Fannia umbratica*** **Collin**
– Scutum with thin grayish pollinosity; bacilliform process long or short, twisted **16**
16 Hind femur with 10 to 15 stout *pv*; bacilliform process short .... ***Fannia umbrosa*** **(Stein)**
– Hind femur with 5 stout *pv*; bacilliform process long .... ***Fannia slovaca*** **Gregor & Rozkošný**

Catalog of known Palearctic and Oriental species in the *Fannia postica*-group, with redescription of one species and description of two new species

Fannia aethiops Malloch, 1913

Fannia aethiops Malloch, 1913: 628.
Fannia aethiops: Pont 1986: 44; Rozkošný et al. 1997: 23; Xue and Wang 1998: 813; Wang and Xue 2002: 55; Wu and Wang 2002: 563; Wang et al. 2004: 34; Wang et al. 2006: 555.

Material examined. China: Jilin: 1 male, Mt. Changbai, 42.33°N, 127.27°E, 22.VI.1980, Coll. Z.Y. Ma (IESNU). Shanxi: 1 male, Ningwu, Mt. Luya, 38.73°N, 111.93°E, 12.VI.1987, Coll. M.F. Wang (IESNU).

Distribution. Nearctic: throughout Canada, USA (Alaska, North Carolina, south to California); Palearctic: China (Jilin, Neimenggu, Shanxi), Sweden.

Fannia ardua Nishida, 1976

Fannia ardua Nishida, 1976: 135.
Fannia ardua: Pont 1986: 44; Wang and Xue 2002: 55; Wang et al. 2006: 555.

Material examined. China: Jilin: 1 male, Mt. Changbai, 42.33°N, 127.27°E, 10.VII.1998 (IESNU).

Distribution. Palearctic: China (Jilin), Japan.

Fannia bigelowi Chillcott, 1961

Fannia bigelowi Chillcott, 1961: 115.
Fannia bigelowi: Pont 1986: 45.

Distribution. Nearctic: Canada, USA (Alaska); Palearctic: Norway.

Fannia coculea Nishida, 1975

Fannia coculea Nishida, 1975: 368.
Fannia cocula: Pont 1977: 448; Xue and Wang 1998: 815; Wang and Xue 2002: 56.

Distribution. Oriental: China (Taiwan).

Fannia discoculea **Xue, 1998**

Fannia discoculea Xue, 1998: 815.
Fannia discoculea: Wang and Xue 2002: 56.

Type specimens examined. Holotype male: China, Xinjiang, Jakesi, 43.82°N, 81.12°E, 6.VIII.1957, Coll. G. Wang (IESNU).

Distribution. Palearctic: China (Xinjiang).

Fannia gotlandica **Ringdahl, 1926**

Fannia gotlandica Ringdahl, 1926: 106.
Fannia gotlandica: Pont 1986: 48; Rozkošný et al. 1997: 39.

Distribution. Palearctic: throughout Europe.

Fannia labidocerca **Feng & Xue, 2006**

Fig. 1

Fannia labidocerca Feng & Xue, 2006: 217.

Redescription. MALE. Body length 4.8 mm. Eye with short and distant hairs or bare; postocular setae in 2 rows, those of the anterior row sparse and long, curved anteriorly, those of the posterior row short; fronto-orbital plate and parafacial with silvery-white pollinosity; frons at narrowest point slightly wider than the distance between outer margins of posterior ocelli; frontal vitta black, at narrowest point about as wide as fronto-orbital plate; frontal setae 12, stout, situated on the lower 4/5 of frons, orbital setae absent; parafacial bare, at middle about 1/3 as wide as the width of postpedicel; antenna black, postpedicel about 2.0 to 2.5x as long as wide, arista ciliated, the longest individual hairs shorter than aristal base; epistoma not projecting beyond vibrissal angle, vibrissal angle behind frontal angle in profile; genal height about 1/14 of eye height; prementum shining, about 3.0x as long as wide; palpus dark brown, claviform, slightly shorter than prementum. Thorax ground-color black, notum with dark brown pollinosity; presutural *acr* biserial, long, one pair of them slightly stout, only prescutellar pairs stout, *dc* 2+3, *ia* 0+2, *pra* 2, about 2/5 of length of posterior notopleural seta; notopleuron bare; basisternum, proepisternum, anepimeron, meron and katepimeron bare; katepisternal setae 1+1, katepisternum without a ventral spine; spiracles brown; calypters mostly brown or brownish, brownish on the outer margin, the lower one small and tongue-like, about 1/2 as long as the upper one. Wing brownish; veins and wing-base yellow; basicosta brownish-yellow; costal spine inconspicuous; node of Rs bare on ventral and dorsal surfaces; vein M_{1+2} straight, parallel to vein R_{4+5} distally; crossveins not clouded; haltere

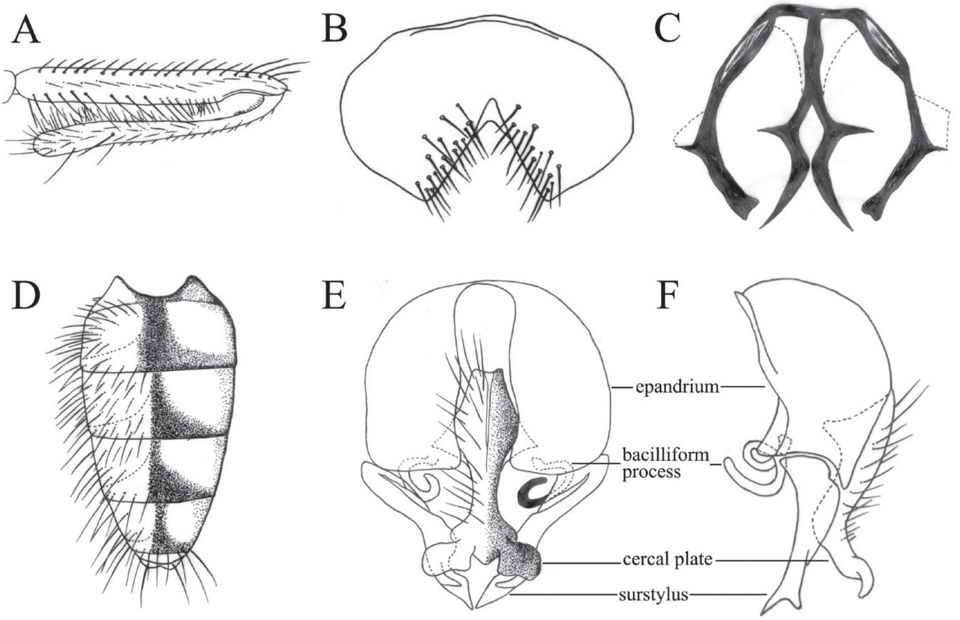

Figure 1. *Fannia labidocerca* Feng & Xue, 2006, male, holotype: (A–F in figure 1 without scale are all from Feng & Xue 2006, specimen from Sichuan, deposited in IESNU). **A** Mid leg, anterior view **B** Sternite 5, ventral view **C** Sternite 9, ventral view. **D.** Abdomen, dorsal view **E** Terminalia, ventral view **F** Terminalia, lateral view.

brown in basal part, yellowish at middle and dark brown in distal part. Legs entirely black, sometimes dark brown or brown; fore tibia without *p*; mid coxa without any hook-like spine or spine-like seta; mid femur concave on ventral surface in apical part, becoming swollen from distal 1/3 towards basal part, with a row of *av*, stout in basal part, becoming shorter and denser in distal 1/4, with a cluster of spine-like setae in distal 1/3, a complete row of *ad*, slightly short (Fig. 1A), with a complete row of slender *p*, slightly situated on the posteroventral surface, with a row of *pv* in basal 4/5, and with a row of setulae at middle towards distal 1/4; mid tibia slightly swollen towards apex, in distal half with one *ad*, one preapical *d*, one *pd*, and with numerous slender setulae on ventral surface, most of the setulae longer than mid tibial width in distal part (Fig. 1A); mid first tarsomere with a basal tooth-like spine on ventral surface; hind coxa bare on posterior surface; hind femur with 3 stout *av* in distal 1/3, without *pv*; hind tibia with one *av*, one *ad* and one median *d*. Abdomen long and flattened (Fig. 1D), ground-color black, with thin gray pollinosity; syntergite 1+2 and tergites 3–5 each with one dark median triangular vitta (Fig. 1D); sternite 1 with 4 long lateral marginal setae; for morphology of sternites 5 and 9 and terminalia, see Fig. 1B, C, E, F.

FEMALE. Unknown.

Remarks. Feng and Xue (2006) placed *F. labidocerca* into the *F. serena*-subgroup of the *F. serena*-group while recording the species from the Mt. Emei Region, Sichuan, China. However, this species should be placed into the *F. postica*-subgroup by sharing the following morphological characters with all other species of the subgroup: mid first tarsomere with a distinct basal tooth-like spine on ventral surface; lower calypter short, tongue-like (Fig. 1E, F).

Type specimens examined. Holotype male: China, Sichuan, Emeishan, Mt. Emei, 29.59°N, 103.30°E, 3099 m, 22.VI.1984, Coll. Y. Feng (IESNU). Paratypes: 1 male, China, Sichuan, Yaan, Hanyuan, Mt. Jiaoding, 3550 m, 8.VII.1987, Coll. Y. Feng (IESNU); 1 male, China, Yunnan, Lushui, Pianma, Mt. Gaoligong, 2400 m, 24.VI.2010, Coll. Y.Y. Zhou (IESNU).

Distribution. Oriental: China (Yunnan); Palearctic: China (Shaanxi, Sichuan).

Fannia ningxiaensis Wang & Zhang, sp. n.

http://zoobank.org/C3089E00-6329-40D5-B852-ECBF3D1F70BB
Fig. 2

Description. MALE. Body length 4.0 mm. Eye bare; postocular setae in one row, slender and curved anteriorly, occipital setae situated behind the postocular setae on vertex and in one row; fronto-orbital plate and parafacial with grayish-silvery pollinosity; frons at narrowest point slightly narrower than the distance between outer margins of posterior ocelli, about 2/3 as wide as postpedicel; frontal vitta black, linear at narrowest point; frontal setae 7 to 9, stout, nearly reaching ocellar triangle, the gaps between them without setulae; orbital setae absent; parafacial bare and narrow, at middle about 2/5 of width of postpedicel; antenna black, postpedicel about 1.5x as long as wide, arista black and ciliated, slightly swollen in basal part, the longest individual hairs shorter than aristal base; epistoma not projecting beyond vibrissal angle, vibrissal angle behind frontal angle in profile; subvibrissal setulae in one row, lateral of it with 2 or 3 fine setae; gena and genal dilation with black setulae, upper margin of gena without upcurved setae; prementum with thin grayish pollinosity, slightly shining, about 2.3x as long as wide; palpus black, claviform, about as long as prementum. Thorax ground-color black, notum with thin dark brown pollinosity, without a distinct vitta; presutural *acr* biserial, slightly stout, prescutellar pairs stout, the distance between the 2 rows of *acr* narrower than the distance between rows of *acr* and *dc*; *dc* 2+3, *ia* 0+2, *pra* 2, the anterior one about 3/5 as long as posterior notopleural seta; notopleuron bare; proepisternal setae 2, proepimeral seta 1, with about 10 slender setulae around it; basisternum, proepisternum, anepimeron, meron and katepimeron bare; katepisternal setae 1+1, katepisternum without a ventral spine, with only some fine and curved setae; anterior spiracle brown, posterior spiracle dark brown; calypters brownish with yellow-brownish margin, the lower calypter slightly smaller than the upper one and not projecting beyond the upper one. Wing brownish; veins brown; wing-base of similar color to other parts of wing; tegula black; basicosta brown; costal spine inconspicuous; node of Rs bare on ventral and dorsal surfaces; vein R_{4+5} straight, parallel to vein M_{1+2} distally; crossveins not distinctly clouded; haltere brownish-yellow. Legs entirely black; fore coxa without a spine on anterior ventral surface; fore femur with a complete row of *pv*; fore tibia

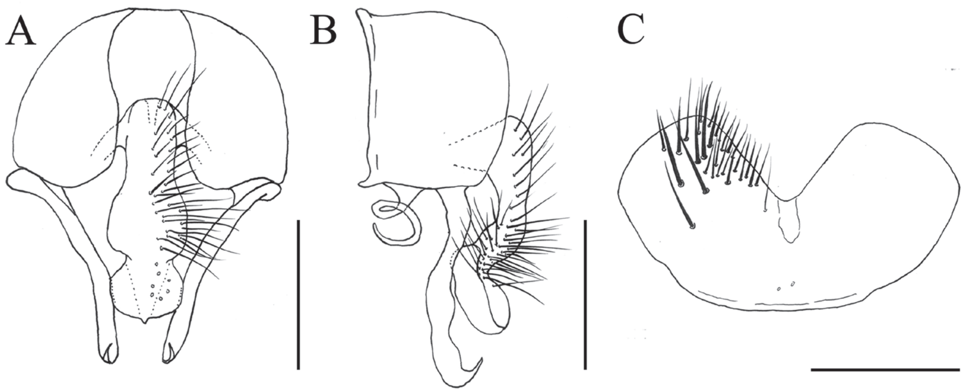

Figure 2. *Fannia ningxiaensis* Wang & Zhang, sp. n., male, holotype: (specimen from Ningxia, deposited in IESNU). **A.** Terminalia, ventral view **B** Terminalia, lateral view **C** Sternite 5, ventral view. Scale for **A–C** = 0.25 mm.

without *ad* and median *p*, and with only one stout preapical *d*; fore first tarsomere with several longish basal setae on ventral surface; mid coxa without a hook-like spine or spine-like seta; mid femur with 6 to 8 stout *av* in basal part, becoming gradually shorter and denser towards apex, with a gap in preapical part, 2 to 4 comb-like setae in distal part, and with a row of stout *pv*, slightly biserial in median part, with a gap in preapical part, with 4 or 5 comb-like setae in distal part, and with a row of slender *p*; mid tibia slightly swollen in distal half, with one *ad* and one *pd* in distal half, and with numerous slender setulae on ventral surface, the longest one about 3/4 of mid tibial width in distal part; mid first tarsomere without a basal tooth-like spine on ventral surface, and with only short basal clustered setulae; hind coxa bare on posterior surface; hind femur with only one stout *av* in preapical part, with 8 to 10 stout *pv* in distal half; hind tibia with one *av*, one *ad*, and one median *d*, and with 8 or 9 slightly erect median setae on posterior surface. Abdomen oval and flattened, ground-color black, with dense grayish-blue pollinosity; syntergite 1+2 and tergites 3–4 each with one dark broad median triangular vitta, tergite 5 with one dark median stripe in basal part; sternite 1 with setulae, sternite 5 broad (Fig. 2C); cercal plate longish, from ventral view, apex of cercal plate projecting, large and rounded, as broad as middle part of cercal plate and slightly broader than the basal part (Fig. 2A); bacilliform process twisted (Fig. 2B); surstylus slender, hook-like at apex and pointed posteriorly (Fig. 2A, B).

FEMALE. Unknown.

Remarks. The new species is attributed to the *spathiophora*-subgroup of the *postica*-group. It can be distinguished from a similar European species, *Fannia stigi* Rognes, 1982, by the following character states: mid first tarsomere with only short basal clustered setulae on ventral surface; hind femur with 8 to 10 stout *pv* in distal half; abdominal syntergite 1+2 and tergites 3–4 each with one dark broad median triangular vitta; apex of cercal plate projecting, large and rounded in ventral view (Fig. 2A); bacilliform process twisted (Fig. 2B), while *F. stigi* Rognes mid first tarsomere with a basal tooth-like spine; hind femur with 4 or 5 stout *pv* in distal half; abdominal syntergite 1+2 and tergites 3–4 each with a dark median stripe; apex of ceral plate not projecting, half round in ventral view; bacilliform process not twisted.

Etymology. The specific name is derived from name of the type locality, Ningxia.

Types material. Holotype male: China, Ningxia, Guyuan, Jingyuan, Dongshanpo, 2200 m, 27.VI.2008, Coll. M.F. Wang (IESNU). Paratype: 1 male, China, Ningxia, Guyuan, Jingyuan, Dongshanpo, 2000 m, 27.VI.2008, Coll. M.F. Wang (IESNU).

Distribution. Palearctic: China (Ningxia).

Fannia nudifemorata Wang & Zhang, 2011

Fannia nudifemorata Wang & Zhang, 2011: 12.

Type specimens examined. Holotype male: China, Yunnan, Yulongxueshan, 27.09°N, 100.25°E, 3200 m, 24.V.2007, Coll. W.X. Dong (IESNU). Paratype: 1 male, same locality and time, Coll. S.C. Bai (IESNU).

Distribution. Oriental: China (Yunnan).

Fannia postica (Stein, 1895)

Fannia postica Stein, 1895: 89.

Fannia postica: Hennig 1955: 24; Chillcott 1961: 103; Pont 1986: 53; Rozkošný et al. 1997: 27; Xue and Wang 1998: 819; Wang and Xue 2002: 57; Wang et al. 2006: 555.

Material examined. China: Heilongjiang: 2 male, Xilinji, 53.48°N, 122.37°E, 19.VI.1986, Coll. C.Y. Cui (IESNU).

Distribution. Nearctic: throughout North America; Palearctic: Austria, Belgium, Bulgaria, China (Heilongjiang), Czech Republic, Denmark, Finland, former Yugoslavia, France, Germany, Iceland, Ireland, Italy, Luxembourg, Norway, Poland, Romania, Slovakia, Spain, Sweden, Switzerland, United Kingdom (England).

Fannia ringdahlana Collin, 1939

Fannia ringdahlana Collin, 1939: 143.

Fannia ringdahlana: Hennig 1955: 20; Pont 1977: 449; Pont 1986: 54; Fan 1992: 216; Wang and Wu 1996: 66; Rozkošný et al. 1997: 22; Xue and Wang 1998: 815; Wang and Xue 2002: 57; Wu and Wang 2002: 563; Wang et al. 2004: 34; Wang et al. 2006: 555.

Material examined. China: Jilin: 2 male, Mt. Changbai, Xiaotianchi, 42.58°N, 128.30°E, 25.VII.1982, Coll. L.Y. Gao (IESNU); 2 male, Mt. Changbai, 42.33°N, 127.27°E, 18.VII.1988, [collector unknown]. Shanxi: 1 male, Ningwu, Mt. Luya, 38.73°N, 111.93°E, 12.VI.1987, Coll. M.F. Wang (IESNU). Sichuan: 2 male, Jiuzaigou, 33.26°N, 103.91°E, 2800 m, 1.VI.2006, Coll. Y. Zhu (IESNU); 3 male, same locality, 2.VI.2006, Coll. D. Jing (IESNU); 9 male, same locality, 3.VI.2006, Coll. D. Wang (IESNU); 1 male, Daocheng, Kasi, 29.04°N, 100.31°E, 2750–3000 m, 12.VII.2006, Coll. C.T. Zhang (IESNU). Yunnan: 1 male, Deqin, Mt. Meili, 28.49°N, 98.93°E, 4000–4200 m, 2.VII.2006, Coll. Y. Wang (IESNU); 1 male, Xianggelila, Bitahai, 27.80°N, 99.90°E, 3700 m, 2.VII.2006, Coll. B.F. Wang (IESNU); 5 male, same locality and time, Coll. L. Chang (IESNU); 5 male, same locality and time, Coll. M.F. Wang (IESNU).

Distribution. Oriental: China (Taiwan, Yunnan); Palearctic: China (Jilin, Shanxi, Sichuan), Japan, Sweden, United Kingdom.

Fannia spathiophora Malloch, 1918

Fannia spathiophora Malloch, 1918: 294.

Fannia spathiophora: Chillcott 1961: 112; Wang and Wu 1996: 66; Rozkošný et al. 1997: 23; Xue and Wang 1998: 815; Wang and Xue 2002: 57; Wu and Wang 2002: 563; Wang et al. 2004: 34; Wang et al. 2006: 556.

Material examined. China: Heilongjiang: 1 male, Wuying, 48.11°N, 129.24°E, 16.VII.1977, Coll. C.Y. Cui (IESNU); 1 male, Guyuan, 50.58°N, 123.70°E, 26.VI.1980, Coll. C.Y. Cui (IESNU); 1 male, Bizhou, 51.94°N, 124.60°E, 13.VII.1980 [collector unknown] (IESNU). Jilin: 1 male, Baihe, 42.58°N, 128.04°E, 20.VI.1980, Coll. Z.Y. Ma (IESNU); 1 male, Mt. Changbai, 42.33°N, 127.27°E, 19.VII.1986 [collector unknown] (IESNU); 1 male, Mt. Changbai, 42.33°N, 127.27°E, 15.VII.1990 [collector unknown] (IESNU). Liaoning: 2 male, Xinbin, Gangshan, 41.72°N, 125.02°E, -.VI.1981, Coll. Z.Y. Ma (IESNU); 1 male, same locality, 08.IX.1990, [collector unknown] (IESNU); 2 male, Benxi, Yanghugou, 41.30°N, 123.73°E, 01.VII.1993, Coll. Y.S. Cui (IESNU); 1 male, same locality, 01.VII.1993, Coll. C.T. Zhang (IESNU); 1 male, Huanren, 41.27°N, 125.35°E, 09.VI.1994, Coll. D. Wei (IESNU); 3 male, Qianshan, 41.03°N, 123.13°E, 25.VI.2007, Coll. M.F. Wang (IESNU). Shanxi: 1 male, Hunyuan, 39.70°N, 113.68°E, 12.VII.1985, Coll. M.F. Wang (IESNU).

Distribution. Nearctic: Canada (Labrador, Northwest Territories, Ontario), USA (Alaska, south to Arizona & New Mexico, Minnesota); Palearctic: China (Hebei, Heilongjiang, Jilin, Liaoning, Shanxi), throughout Europe, Japan.

Fannia slovaca Gregor & Rozkošný, 2005

Fannia slovaca Gregor & Rozkošný, 2005: 519.

Distribution. Palearctic: Slovakia.

Fannia stigi Rognes, 1982

Fannia stigi Rognes, 1982: 325.
Fannia stigi: Wang, Li and Zhang 2011: 15.
Fannia tigripeda: Xue, Wang and Li 2001: 225–226; Wang and Xue 2002: 57; Su and Wang 2004: 112.

Material examined. China: Jilin: Mt. Changbai, 42.33°N, 127.27°E, 1700 m, 28.VI.1997, Coll. W.Q. Xue (IESNU). Shanxi: 1 male, Ningwu, 38.73°N, 111.93°E, 07.VI.1982, Coll. M.F. Wang (IESNU).

Distribution. Palearctic: China (Jilin, Shanxi), Norway, Sweden.

Fannia subaethiops Wang & Zhu, sp. n.

http://zoobank.org/A9405B63-59AD-4D73-829F-F0364A86A69A
Fig. 3

Description. MALE. Body length 5.0 mm. Eye bare; upper inner facets larger than the remaining facets; postocular setae in one row, short and neatly arranged, occipital setae absent; fronto-orbital plate and parafacial with grayish-silvery pollinosity; frons at narrowest point slightly wider than the distance between outer margins of posterior ocelli, about as wide as postpedicel; frontal vitta black, with grayish-silvery pollinosity, at narrowest point about as wide as fronto-orbital plate; frontal setae 5, stout, nearly reaching ocellar triangle, the gaps between them without setulae, orbital setae absent; parafacial bare, at middle about 1/2 as wide as postpedicel; antenna black, postpedicel about 1.5x as long as wide, arista ciliated, slightly swollen in basal part, the longest individual hairs shorter than aristal base; epistoma not projecting beyond vibrissal angle, vibrissal angle behind frontal angle in profile; subvibrissal setulae in one row, lateral of it with several setae; gena and genal dilation with black setulae, upper margin of gena without upcurved setae; prementum shining, without distinct pollinosity, about 2.0x as long as wide; palpus black, claviform, slightly longer than prementum. Thorax ground-color black, notum with dark brown pollinosity, without a distinct vitta; presutural *acr* biserial, slightly stout, only prescutellar pairs stout, the distance between 2 rows of *acr* narrower than the distance between rows of *acr* and *dc*; *dc* 2+3, *ia* 0+2, *pra* 2, the anterior one stout, about 2/3 as the length of posterior notopleural seta; notopleuron bare; proepisternal setae 2, proepimeral seta 1, lower part of proepimeral seta with one short setula; basisternum, proepisternum, anepimeron, meron and katepimeron bare; katepisternal setae 1+1, katepisternum without a ventral spine; spiracles brown; calypters brownish-yellow, the lower one slightly projecting beyond the upper one. Wing brownish; veins dark brown; wing-base of same color as other parts of wing; tegula dark brown; basicosta brownish-yellow; costal spine conspicuous, about 2/3 of the length of crossvein r-m; node of Rs

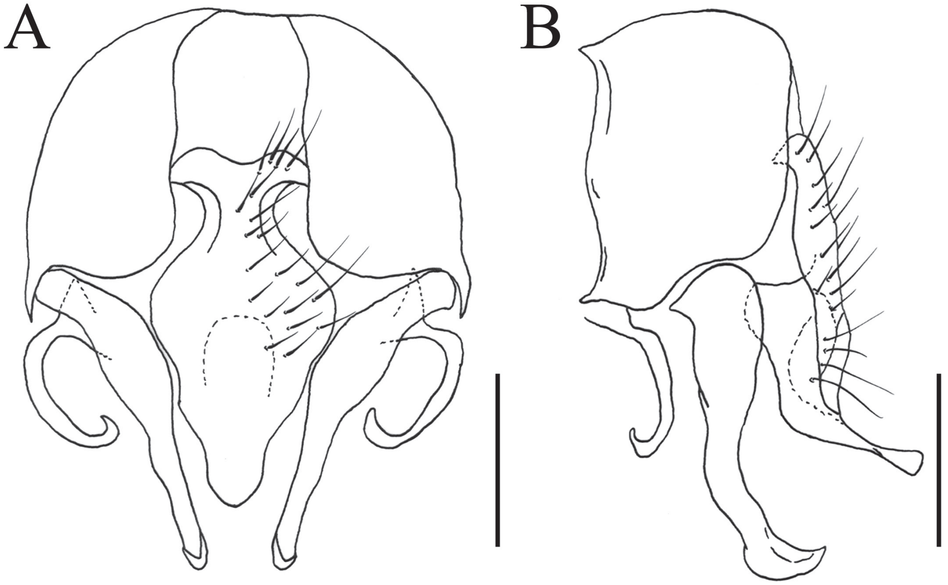

Figure 3. *Fannia subaethiops* Wang & Zhu, sp. n., male, holotype: (specimen from Heilongjiang, deposited in SHEM). **A** Terminalia, ventral view **B** Terminalia, lateral view. Scale for **A–B** = 0.25 mm.

bare on ventral and dorsal surfaces; vein R_{4+5} straight, veins M_{1+2} and R_{4+5} converging distally; crossveins not clouded; haltere brown. Legs entirely black, except knees yellow; fore coxa without a anterior spine on ventral surface; fore femur with a stout row of *pv*; fore tibia without *ad* and median *p*, with only one *d* and one *v* in apical part; fore first tarsomere with few longish basal setae on ventral surface; mid coxa without a hook-like spine or spine-like seta; mid femur with a row of stout and sparse *av* in basal half, becoming shorter and denser towards apex, with a gap in preapical part, 2 or 3 comb-like setae in distal part, a complete row of stout *pv*, slightly biserial in median part, and a row of slender *p*; mid tibia slightly narrowing in basal half, gradually swollen towards apex, about 2.0x as wide in distal part as wide in basal part, with one *ad* and one *pd* in distal half, and with numerous slender setulae on ventral surface, the longest one about 3/4 as long as mid tibial width in distal part; mid first tarsomere without a basal tooth-like spine on ventral surface, with only short basal clustered setulae; hind coxa bare on posterior surface; hind femur with only one stout *av* and 3 or 4 *pv* in preapical part; hind tibia with one *av*, one *ad* and one *d*. Abdomen long and flattened, ground-color black, with grayish-brown pollinosity; syntergite 1+2 and tergites 3–4 each with an inverted T-shaped dark mark, each tergite with stout lateral marginal setae; sternite 1 broad, with 4 long setae on each lateral margin, sternites 2 to 4 narrow, with long setulae, sternite 5 with slightly dense setae in posterior margin; cercal plate longish, from ventral view, cercal plate slightly indented in each lateral margin, middle part of cercal plate strongly broader than the apex and the basal part

(Fig. 3A); bacilliform process curved (Fig. 3A, B); surstylus slender, curved at apex and pointed posteriorly (Fig. 3A, B).

FEMALE. Unknown.

Remarks. The new taxon is similar to the holarctic species *F. aethiops* Malloch, 1913 but differs from it for the following character states: frontal setae only 5; anterior *pra* about 2/3 as long as posterior notopleural seta; sternite 1 with 4 long setae on each lateral margin; in ventral view, cercal plate broadest in median part, slightly indented in each lateral margin (Fig. 3A); bacilliform process curved (Fig. 3A & B), while *F. aethiops* Malloch frontal setae 9; anterior *pra* about 1/2 as long as posterior notopleural seta; sternite 1 with 1–2 long setae on each lateral margin; in ventral view, cercal plate not indented in each lateral margin; bacilliform process not curved.

Etymology. This specific name refers to the similarity between the new species and *F. aethiops* Malloch.

Types material. Holotype male: China, Heilongjiang, Yichun, Wuying, 3.V.1975, Coll. S.Y. Fang (SHEM).

Distribution. Palearctic: China (Heilongjiang).

Fannia umbratica Collin, 1939

Fannia umbratica Collin, 1939: 144.
Fannia umbratica: Hennig 1955: 90; Pont 1986: 57; Rozkošný et al. 1997: 47.

Distribution. Palearctic: throughout Europe.

Fannia umbrosa (Stein, 1895)

Fannia umbrosa (Stein, 1895): 75.
Fannia umbrosa: Hennig 1955: 90; Pont 1986: 57; Rozkošný et al. 1997: 47.

Distribution. Palearctic: throughout Europe.

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RESEARCH ARTICLE

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Description of a new catfish genus (Siluriformes, Loricariidae) from the Tocantins River basin in central Brazil, with comments on the historical zoogeography of the new taxon

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Abstract

This study presents the description of a new genus of the catfish subfamily Neoplecostominae from the Tocantins River basin. It can be distinguished from other neoplecostomine genera by the presence of (1) three hypertrophied bicuspid odontodes on the lateral portion of the body (character apparently present in mature males); (2) a large area without odontodes around the snout; (3) a post-dorsal ridge on the caudal peduncle; (4) a straight tooth series in the dentary and premaxillary rows; (5) the absence of abdominal plates; (6) a conspicuous series of enlarged papillae just posterior to the dentary teeth; and (7) caudal peduncle ellipsoid in cross section. We used maximum likelihood and Bayesian methods to estimate a time-calibrated tree with the published data on 116 loricariid species using one nuclear and three mitochondrial genes, and we used parametric biogeographic analyses (DEC and DECj models) to estimate ancestral geographic ranges and to infer the colonization routes of the new genus and the other neoplecostomines in the Tocantins River and the hydrographic systems of southeastern Brazil. Our phylogenetic results indicate that the new genus and species is a sister taxon of all the other members of the Neoplecostominae, originating during the Eocene at 47.5 Mya (32.7–64.5 Mya 95% HPD). The present distribution of the new genus and other neoplecostomines may be the result of a historical connection between the drainage basins of the Paraguay and Paraná rivers and the Amazon basin, mainly through headwater captures.

Keywords

Molecular phylogeny, Freshwater fishes, headwater capture, catfish, taxonomy

Introduction

The Loricariidae, an endemic Neotropical family of freshwater fish, is the largest group of catfish, with about 900 valid species (Eschmeyer and Fong 2015). Within the Loricariidae, the subfamily Neoplecostominae has a long complex taxonomic and systematic history, with a number of major morphological and molecular studies being conducted since the nineteenth century (e.g. Eigenmann and Eigenmann 1890; Regan 1904; Gosline 1947; Isbrücker 1980; Howes 1983; Schaefer 1987; Montoya-Burgos et al. 1998; Armbruster 2004; Chiachio et al. 2008; Roxo et al. 2012a, 2014).

The neoplecostomines are small-bodied catfishes which were, until now, restricted to southern and southeastern Brazil, where they are found in small- to medium-sized streams with clear and shallow water, of up to 1 m in depth (Langeani 1990). Previous studies (e.g. Chiachio et al. 2008; Roxo et al. 2012a, 2014) concluded that the considerable diversity of this subfamily can be accounted for primarily by the geomorphological processes (i.e. tectonics and erosion) that have shaped the South American continent over the past 100 Mya, influencing fish distribution and speciation patterns (Ribeiro 2006; Albert and Reis 2011). In this context, one of the principal processes is river capture (also known as stream capture or headwater capture), an important landscape-level mechanism that can isolate lineages and promote diversification (Waters et al. 2006; Winemiller et al. 2008; Albert and Crampton 2010) by changing the connectivity of adjacent river basins (Smith 1981; Hocutt and Wiley 1986; Mayden 1988; Lundberg et al. 1998). The consequences of this process for the local fauna can be profound, changing watershed boundaries and allowing previously isolated species to disperse and colonize new environments (Grant et al. 2007; Muneepeerakul et al. 2008; Bertuzzo et al. 2009).

Here, we recognize a new genus and species of neoplecostomine catfish based on specimens collected during a recent expedition to the Tocantins River basin in Goiás state, Brazil. The new taxon is described in detail below.

Material and methods

Morphological analysis

Body plate nomenclature follows Schaefer (1997) and measurements, Armbruster (2003), except for the dorsal-adipose distance, adipose-spine length, dorsal adipose-caudal distance, ventral adipose-caudal distance, adipose-anal distance and mouth width. Measurements and counts were taken on the left side of the specimens and were taken point to point, to the nearest 0.1 mm with digital calipers. Specimens were cleared and stained (c&s) according to the method of Taylor and Van Dyke (1985). Dorsal fin ray counts include the spinelet as the first unbranched ray. Counts of vertebrae include the five vertebrae that comprise the Weberian apparatus, while the compound caudal centrum (PU1 + U1) was counted as a single element. Zoological nomenclature follows the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999).

Molecular analysis

Taxon sampling

The molecular analysis included 157 specimens representing 116 loricariid species (115 species from the study of Roxo et al. [2014], and one sample of the new genus, see Suppl. material 1 for all taxa). *Diplomystes mesembrinus* (Ringuelet, 1982) was used as the outgroup to root all phylogenies (Arratia 1987; de Pinna 1993, 1998; Grande 1987; Grande and de Pinna 1998; Mo 1991; Sullivan et al. 2006). Samples of *Corydoras imitator* Nijssen & Isbrücker, 1983, *Corydoras oiapoquensis* Nijssen, 1972, *Hoplosternum littorale* (Hancock, 1828), *Callichthys callichthys* (Linnaeus, 1758), *Astroblepus* spp. 1 and 2, *Hemipsilichthys gobio* (Lütken, 1874), *H. papillatus* Pereira, Oliveira & Oyakawa, 2000, *Delturus parahybae* Eigenmann & Eigenmann, 1889b, *Rineloricaria lanceolata* (Günther, 1868), *Spatuloricaria* sp. 1, *Hypostomus ancistroides* (Ihering, 1911), *H. nigromaculatus* (Schubart 1964) and *H. microstomus* Weber, 1987 were also included in the analysis as outgroups.

Vouchers of the samples were those catalogued by Roxo et al. (2014), except for the samples of the new genus, which was deposited in the collection of Auburn University Natural History Museum (AUM), Auburn; Laboratório de Biologia e Genética de Peixes (LBP), Botucatu; and Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo.

DNa extraction and sequencing

Total DNA was extracted from muscle samples collected from two specimens of the new genus preserved in ethanol using the protocol described by Aljanabi and Martinez (1997). Partial sequences for two genes, Cytochrome B (CytB), forward 5’-CCA TCC AAC ATC TCA GCA TGA TGA AA 3’, reverse 5’-AAC CTC CGA TCT TCG GAT TAC AAG AC 3` (Oliveira et al. 2011), and 16S rRNA, forward 5’-ACG CCT GTT TAT CAA AAA CAT-3’, reverse 5’-CCG GTC TGA ACT CAG ATC ACG T-3’ (Kocher et al. 1989) were amplified by polymerase chain reaction (PCR). The amplification was conducted in a total volume of 12.5 µl with 1.25 µl of 10 X buffer (10 mM Tris-HCl+15 mM MgCl2), 0.5 µl of the dNTPs (200 nM of each), 0.5 µl of each 5 mM primer, 0.05 µl of platinum Taq polymerase (Invitrogen), 1 µl of template

DNA (12 ng), and 8.7 µl of dd H_2O. The PCR reactions consisted of 30–40 cycles, 30 s at 95 °C, 15–30 s at 48–58 °C, and 45–90 s at 72 °C. All the PCR products were first identified visually on a 1% agarose gel and then purified using ExoSap-IT (USB Corporation) following the manufacturer's instructions. The purified PCR products were sequenced using the Big DyeTM Terminator v 3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems), purified by ethanol precipitation and loaded into a 3130-Genetic Analyzer automatic sequencer (Applied Biosystems).

Sequencing and phylogenetic analysis

The individual sequences of each species were initially analyzed in the BioEdit 5.0.9 software (Hall 1999), and a consensus sequence was obtained for each DNA segment. The sequences were then aligned in MUSCLE (Edgar 2004) using the default parameters, and inspected visually. To evaluate the saturation of the matrix by substitution, we calculated the index of substitution saturation (Iss), as described by Xia et al. (2003) and Xia and Lemey (2009), and the transition/transversion rate, in DAMBE 5.2.31 (Xia and Xie 2001). The Iss was calculated without taking gaps into account.

Maximum likelihood analyses were run in RAxML Web-Servers (Stamatakis et al. 2008). Bootstrap (BS) resampling (Felsenstein 1985) was used to evaluate the support for each node, based on 1000 replicates. Random starting trees were used for each independent ML tree search, while all other parameters were set at the default values. The ML analyses were based on the GTR model.

Time calibration and estimates of ancestral ranges

The uncorrelated relaxed (lognormal) molecular clock was calibrated using BEAST v.1.7.5. All clade-age estimates are presented as the mean and 95% highest posterior density (HPD) values. We included two calibration points to constrain the divergence dates for the 157 clades identified in our phylogenetic tree. The first calibration point was implemented as a normally-distributed prior, with an offset of 125 million years ago (Mya), and a standard deviation of 15 million years. Data from the stratigraphic record and the geographic distribution of living taxa indicate that the Siluriformes originated during the Lower Cretaceous (145–100 Mya; Lundberg 1993; Sullivan et al. 2006; Lundberg et al. 2007).

The second calibration point was implemented using a log-normal prior set at 55 Mya, with a mean and standard deviation of 1 for the origin of the family Callichthyidae. The oldest known callichthyid fossil, *Corydoras revelatus* Cockerell (1925) was dated to the Paleocene by Marshall et al. (1997), assuming 55 Mya as a minimum age. We used a macroevolutionary Birth–Death model to estimate diversification likelihood values, with a starting tree obtained from the RAxML analysis. These analyses were conducted under the GTR model. The ML tree obtained in this analysis was used as a starting tree for the MCMC searches. This analysis was run for 100 million generations and sampled every

10,000th generation. Stationarity and the sufficient mixing of parameters (ESS>200) were verified using Tracer v1.5 (Rambaut and Drummond 2007a). A consensus tree was built in TreeAnnotator v1.7.5 (Rambaut and Drummond 2007b).

Data on the geographic distribution of the species in each of the three subfamilies analyzed here (Hypoptopomatinae, Neoplecostominae and Otothyrinae) were obtained from the original species descriptions and the catalog of Eschmeyer (2015), with the species classification following Roxo et al. (2014). Species ranges are located within five biogeographic regions: A, Drainage basins of the Atlantic coast of southeastern Brazil; B, Upper Paraná basin; C, Paraguay, Lower Paraná and Uruguay basins; D, Amazon and Orinoco basins; E, São Francisco basin and the coastal drainage basins of northeastern Brazil.

We estimated the likelihood of ancestral range evolution using the Dispersal-Extinction-Cladogenesis (DEC: Ree and Smith 2008) and jumping (DECj: Matzke 2013a) models of species range evolution. These models are composed of two (DEC) or three (DECj) parameters including: 1) dispersal (D), where ancestral ranges expand by adding new geographic units, 2) extinction (E), where ancestral ranges are reduced by extirpating geographic units, and 3) jumping events (j), where j specifies the weight of the jumping events beyond an ancestral range (Matzke 2014). The two models of range evolution (i.e. DEC and DECj) were implemented in the R package BioGeoBEARS (Matzke 2013b). The global likelihood of the six biogeographic scenarios found using the two models (i.e. DEC and DEC+J models) were compared using the Akaike Information Criterion (AIC) (Akaike 1973) (Suppl. material 2). The model that obtained the lowest AIC values was model 2 with the DEC+J model (M2 – DEC + J), which constrained the dispersal rates between adjacent areas at 1.0 and areas separated by one or more intercalated areas at 0.5.

Results

Microplecostomus gen. n.

http://zoobank.org/077BD513-6BF2-47D9-AB4C-4A496FE33115
Figs 1, 6

Type species. *Microplecostomus forestii* sp. n.

Diagnosis. The new genus and species differs from all members of the Loricariidae by having (1) three hypertrophied bicuspid odontodes on the lateral portion of the body (character apparently present only in mature males – observed in the holotype, but not present in the paratypes) (Fig. 2a, b); and differs from all members of the Neoplecostominae by having (2) a large area without odontodes around the snout, observed in all specimens, Fig. 3 (*vs.* margin of snout bearing odontodes); and from all members of the Neoplecostominae, except *Hirtella carinata* Pereira, Zanata, Cetra & Reis, 2014, *Pareiorhina carrancas* Bockmann & Ribeiro, 2003 and *Pareiorhina hyptiorhachis* Silva, Roxo & Oliveira, 2013 by (3) the presence of a post-dorsal ridge on

Figure 1. *Microplecostomus forestii* sp. n., MZUSP 118673, holotype, male, 38.3 mm SL, Goiás state, Brazil, Tocantins River basin.

the caudal peduncle, see dorsal view of holotype in Figs 1, 4 (*vs.* the absence of a post-dorsal ridge). *Microplecostomus forestii* sp. n. differs from species of the genera *Isbrueckerichthys*, *Neoplecostomus* and *Pseudotocinclus* by (4) the absence of abdominal plates, Fig. 1 (*vs.* abdomen covered by pentagonal or hexagonal platelets); from *Kronichthys* by having (5) the tooth series in dentary and premaxillary rows straight (*vs.* tooth series strongly curved medially); from *Neoplecostomus* by (6) the absence of a conspicuous

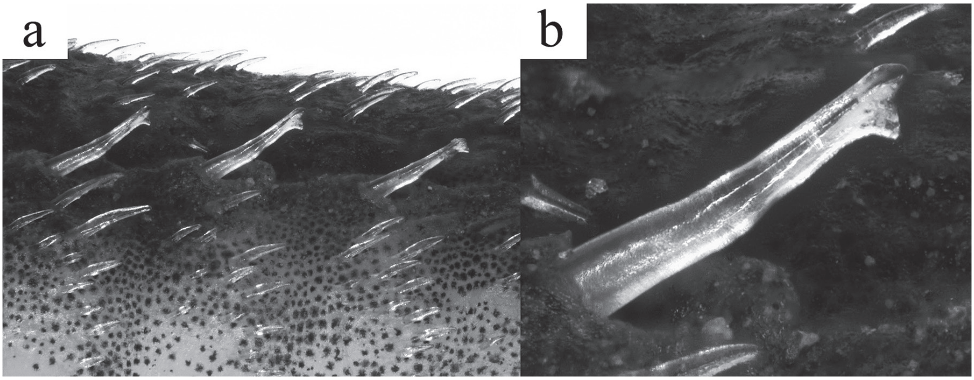

Figure 2. Photographs showing **a** the three hypertrophied bicuspid odontodes on the lateral portion of the body of the holotype of *Microplecostomus forestii* sp. n., MZUSP 118673; **b** Detail of the hypertrophied bicuspid odontodes.

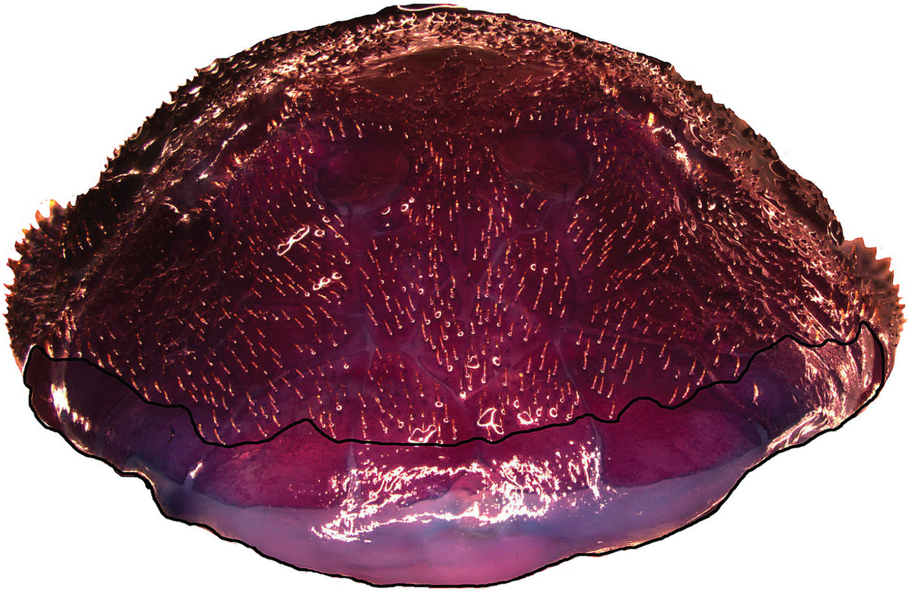

Figure 3. *Microplecostomus forestii* sp. n. showing a large area without odontodes around the snout, LBP 19017, 29.0 mm SL.

series of enlarged papillae just posterior to the dentary teeth (*vs.* presence of enlarged papillae); and from *Pseudotocinclus* by having (7) the caudal peduncle ellipsoid in cross section (*vs.* caudal peduncle square in cross-section).

Etymology. The generic name is a combination of Greek, *micro* (mikrós) = small, related to the small size of the adult type-specimen, and *plecostomus* = a former generic name of species currently included in Loricariidae, also in reference to the small adult size of the type-species. A masculine name.

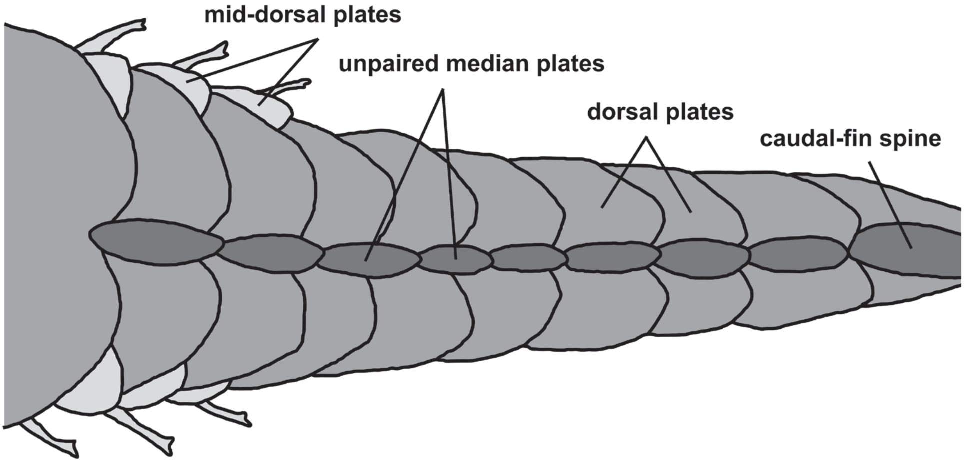

Figure 4. Dorsal view of the caudal peduncle in *Microplecostomus forestii* sp. n., LBP 19017, 29.0 mm SL, showing the presence of a series of unpaired median plates that form a post-dorsal ridge.

Microplecostomus forestii sp. n.

http://zoobank.org/2A1A0D93-ED90-4C5F-9562-D3209D951630
Figs 1, 6; Table 1

Holotype. MZUSP 118673 (adult male, 38.3 mm SL), Brazil, Goiás state, municipality of São João D'Aliança, Roncador Stream, a tributary of das Brancas Stream, tributary of the Tocantizinho River, Tocantins River basin, 14°53'47.2"S, 47°34'58.4"W, 9 November 2014, FF Roxo, GSC Silva, LEO Ochoa, LH Roxo.

Paratypes. All from Brazil, Goiás state, Tocantins River basin (15 specimens). AUM 67015, 1, 29.4 mm SL, municipality of Água Fria de Goiás, córrego das Brancas, tributary of rio Tocantizinho, 14°53'47.2"S, 47°34'58.4"W, 9 November 2014, FF Roxo, GSC Silva, LEO Ochoa, LH Roxo. LBP 17318, 2, 24.2–30.3 mm SL, municipality of São João D'Aliança, Roncador Stream, a tributary of das Brancas Stream, 14°43'51.3"S, 47°32'34.0"W, 21 November 2012, BF Melo, GSC Silva, JHM Martinez, R Devidé. LBP 19000, 2, 29.8–32.2 mm SL, collected with the holotype. LBP 19017, 1, 24.8 mm SL, 1 c&s 29.0 mm SL, municipality of Água Fria de Goiás, das Brancas Stream, a tributary of the Tocantizinho River, 14°53'47.2"S, 47°34'58.4"W, 30 June 2014, FF Roxo, GSC Silva, LE Ochoa. LBP 19319, 3, 24.4–28.4 mm SL, municipality of Água Fria de Goiás, das Brancas Stream, tributary of the Tocantizinho River, 14°53'47.2"S, 47°34'58.4"W, 16 August 2014, BF Melo, C Oliveira, GSC Silva, MI Taylor. LBP 19467, 2, 27.6–28.4 mm SL, municipality of Água Fria de Goiás, das Brancas Stream, a tributary of the Tocantizinho River, 14°53'47.2"S, 47°34'58.4"W, 9 November 2014, FF Roxo, GSC Silva, LEO Ochoa, LH Roxo. LBP 19468, 1, 27.7 mm SL, municipality of São João D'Aliança, Roncador Stream, a tributary of das Brancas Stream, 14°43'51.3"S, 47°32'34.0"W, 9 November 2014, FF Roxo, GSC Silva, LE Ochoa, LH Roxo. MZUSP 113919, 2, 21.7–25.0 mm SL, municipality of Água Fria de Goiás, das Brancas Stream, a tributary of the Tocantizinho River, 14°53'47.2"S, 47°34'58.4"W, 27 November 2012, AM Zanata, P Camelier, M Melo, OT Oyakawa.

Diagnosis. Same as for the genus.

Description. Morphometric and meristic data in Table 1. In lateral view, dorsal profile of head strongly convex from snout tip to distal margin of supraoccipital; straight from supraoccipital to dorsal-fin origin; concave and slightly decreasing to end of caudal peduncle. Ventral surface of body, slightly concave at head, straight to convex from posterior end of head to pelvic-fin insertion, and straight but angled to posterior caudal peduncle. Snout tip rounded in dorsal view. Nostril small. Trunk and caudal peduncle rectangular in cross-section. Greatest body depth at dorsal-fin origin. Body progressively narrowing posteriorly from cleithrum to caudal peduncle. Head flat to slightly convex between orbits; superior margin of orbits elevated. Head lacking crests. Head and body plates covered with minute, uniformly sized and evenly distributed odontodes. Head with large area without odontodes around snout. Eye small, situated dorsolaterally just posterior of midpoint.

Tip of snout formed by two triangle rostral plates, without odontodes. Nasal plates almost rectangular forming medial nostril margin and contacting pre-nasals anteriorly. Nasal plates posteriorly contacting frontal bones. Lateral margin of head formed by four or five postrostral plates. Complete infraorbital plate series composed of five plates; all infraorbital plates containing latero-sensory canals; first and second infraorbitals largest and third, fourth and fifth smallest. Preopercle elongate, bearing a branch of laterosensory canal. Subocular cheek plates present ventral to preopercle plate. Top of head composed of compound pterotic, supraoccipital, prefrontal, frontal, and sphenotic (Fig. 5); compound pterotic as with fenestrae irregularly distributed and with different sizes and shapes. Anterior margin of mesethmoid pointed and projected anteriorly to condyle.

Lateral ethmoid exposed without odontodes in dorsal view. Lateral ethmoid strut short and broad, nasal capsule partially closed, lateral ethmoid surrounding more than 50% of nasal capsule. Compound pterotic roughly quadrangular, without posterior process, with several fenestrae non-uniform in shape and size. Parieto-supraoccipital not contributing to dorsal portion of swimbladder capsule. Metapterygoid channel present. Hyomandibular square and not sutured to compound pterotic, hyomandibular adductor palatine crest present. Quadrate triangle. Lips large; oral disk rounded and papillose. Premaxillary teeth 44–65 (mode 46). Dentary teeth 45–69 (mode 48). Teeth bicuspid. Maxillar barbel short. Upper pharyngeal tooth-plate small and triangular. Five ceratobranchials with accessory process present and long. Five teeth in ceratobranchial. Four branchiostegal rays.

Dorsal-fin rays II,7; dorsal-fin originating at vertical through posterior end of pelvic-fin base; distal margin slightly convex; dorsal-fin spinelet short and oval in shape. Pectoral-fin rays I,6; distal margin slightly convex; unbranched pectoral-fin ray reaching pelvic-fin origin; unbranched pectoral-fin ray covered with large and pointed odontodes. Pectoral girdle not exposed ventrally. Arrector fossae, partially enclosed by ventral lamina of coracoids, opening relatively large, extending laterally towards base

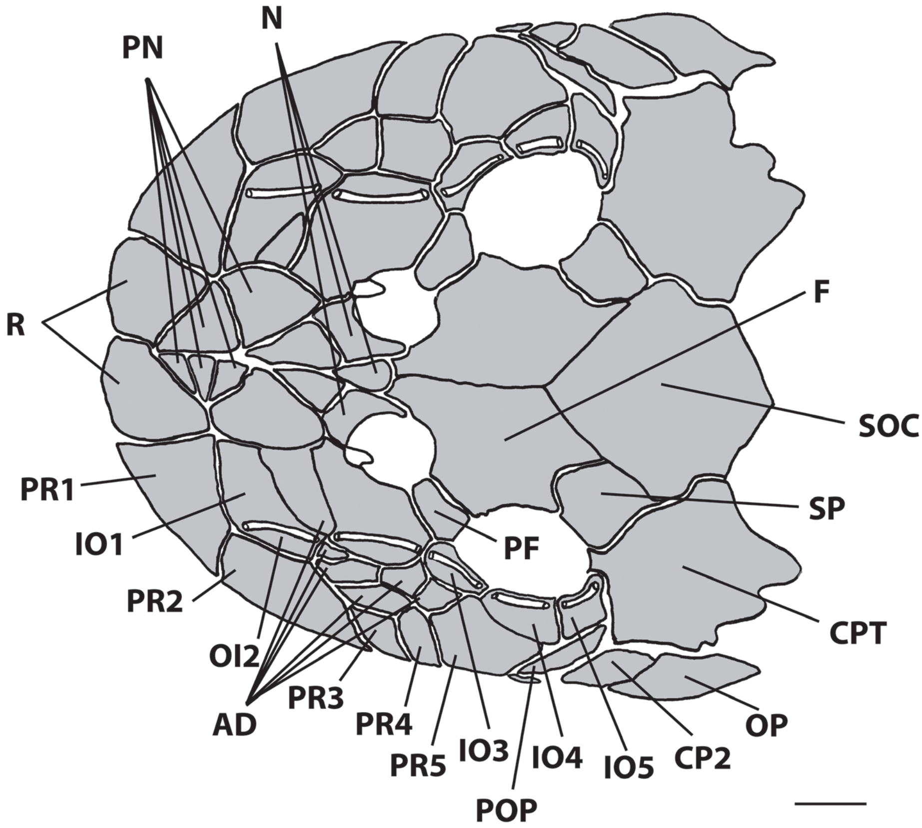

Figure 5. Dorsal view of the head plates in *Microplecostomus forestii* sp. n., LBP 19017, 29.0 mm SL. CPT = compound pterotic; F = frontal; IO1-5 = infraorbitals; N = nasal; OP = opercle; PR1-4 postrostral plates; PF = prefrontal; PN = prenasal; POP = preopercle; R = rostral plate; SP = sphenotic; CP2 = subocular cheek plate; SOC = parieto-supraoccipital; AD = additional plates.

of pectoral fin. Pelvic-fin rays I,5; distal margin of fin slightly convex; tip of adpressed pelvic-fin almost reaching anal-fin origin; unbranched pelvic-fin ray covered with conspicuously pointed, and uniformly distributed odontodes, larger at ventral portion. Pelvic girdle with slender lateropterigium. Basipterygium lacking anterior fenestrae. Anal-fin rays I,5; distal margin slightly convex. Adipose-fin absent. Caudal-fin rays I,7–7,I, truncated with ventral unbranched principal ray longer than dorsal ray.

Compound hypurals 1 and 2 almost completely fused to compound hypurals 3–5, and lower and upper halves fused to last vertebra. Upper and lower lobes of hypural plates of same length. Epural present and separated from hypural plate. Body entirely covered by bony plates, except for ventral surface of head, abdomen and region between compound pterotic and first medial plate. Dorsal series of plates 22–23, mid-dorsal 4–7, median perforated plates 22–23, mid-ventral 11, and ventral 18–20. Trunk with conspicuous, elongated, post-dorsal ridge formed by 14–15 raised, unpaired, median

Figure 6. *Microplecostomus forestii* sp. n., live specimen, LBP 19319, paratype, 28.4 mm SL, Tocantins River, Goiás state, Brazil. Photograph: MI Taylor.

Table 1. Morphometric data for *Microplecostomus forestii* sp. n. SD = standard deviation.

	Microplecostomus forestii **sp. n., 15 paratypes and the holotype**			
	Holotype	**Range**	**Mean**	**SD**
SL	38.3	21.7–38.3	27.9	–
Percentage of SL				
Predorsal length	45.5	44.5–50.8	47.9	1.8
Head length	34.9	34.5–39.9	37.4	1.5
Head-dorsal length	12.5	10.0–13.5	11.4	1.1
Cleithral width	32.5	31.2–35.2	33.3	1.0
Head-pectoral length	29.7	22.4–32.7	30.1	2.4
Thorax length	19.1	17.1–20.5	19.1	1.0
Pectoral-spine length	19.9	19.2–25.3	21.4	1.7
Abdominal length	21.8	19.4–24.3	21.8	1.2
Pelvic-spine length	20.8	17.0–22.3	20.3	1.6
Post-anal length	34.2	31.8–34.9	33.2	0.9
Anal-fin spine length	12.2	10.6–13.6	12.1	0.8
Dorsal-pectoral distance	25.7	25.3–34.5	28.1	2.2
Dorsal spine length	19.4	18.2–23.0	20.9	1.4
Dorsal-pelvic distance	20.2	16.8–22.3	20.1	1.6
Dorsal-fin base length	18.5	15.1–19.4	17.6	1.2
Caudal peduncle depth	9.5	8.1–10.5	9.6	0.6
Dorsal-anal distance	13.6	13.6–16.8	15.0	1.0
Pelvic-dorsal distance	22.5	20.3–25.7	23.2	1.6
Percentage of HL				
Head-eye length	32.3	30.9–36.7	33.9	1.9
Orbital diameter	15.8	13.2–17.2	15.1	1.2
Snout length	61.8	52.9–61.8	57.8	2.8
Internares width	17.0	14.8–19.2	16.7	1.2
Interorbital width	31.5	28.8–34.3	32.1	1.5
Head depth	58.8	55.8–66.6	61.1	2.6
Mouth length	55.8	45.6–66.9	58.7	5.8
Barbel length	4.8	1.2–5.5	3.2	1.2
Dentary tooth cup length	24.2	20.1–27.0	23.3	1.6
Premaxillary tooth cup length	21.7	18.3–25.2	23.3	1.8

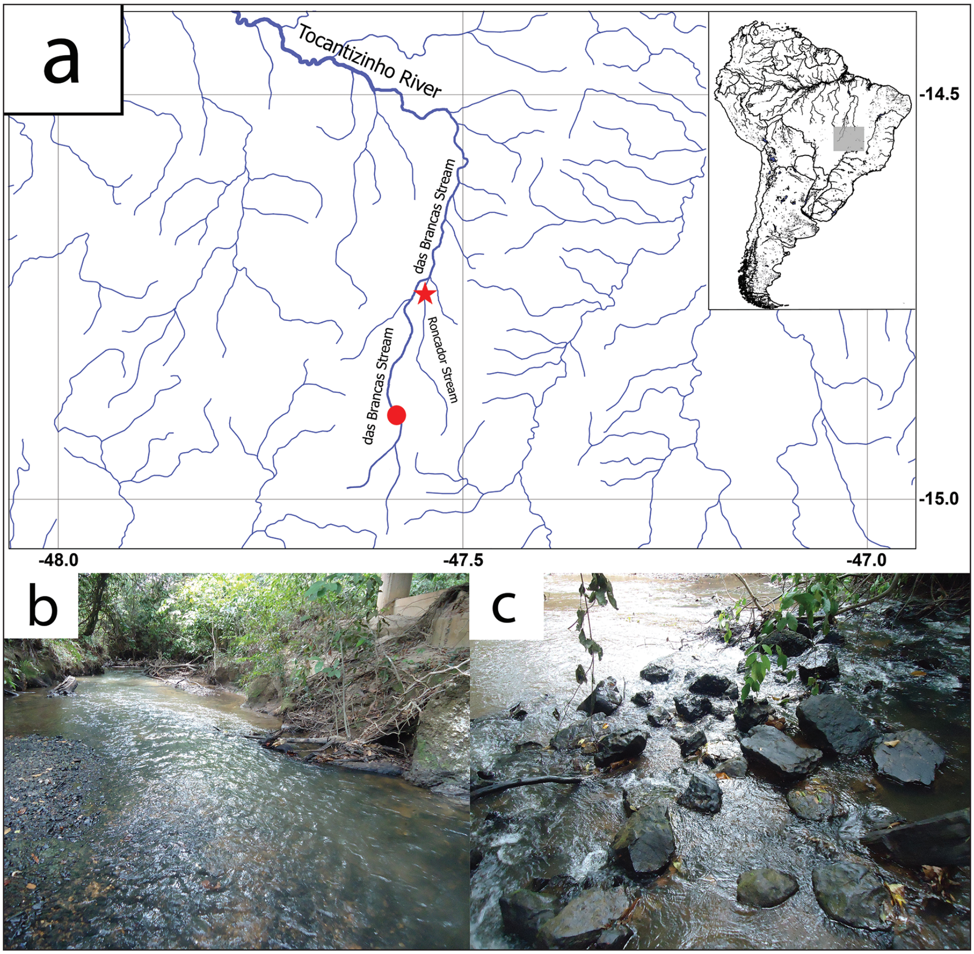

Figure 7. a Map showing the distribution of *Microplecostomus forestii* sp. n. Type locality at Roncador Stream, red star – 14°43'51.3"S, 47°32'34.0"W. Paratype locality at das Brancas Stream, red circle – 14°53'47.2"S, 47°34'58.4"W. Habitat where *Microplecostomus forestii* sp. n. is found at **b** Roncador Stream and **c** das Brancas Stream. These are small size streams with a depth of less than 1 m, clear water, the bottom covered with loose stones and shaded margins. Photographs: LH Roxo.

plates; ridge continuous posteriorly with procurrent caudal-fin rays. Six pairs of ribs associated with vertebrae 7–13. Ribs slender and poorly ossified. Total vertebrae 27.

Color in life. Background color of dorsal and ventral surfaces of body yellowish tan. Dorsal surface of head dark brown. Four dark brown saddles on dorsal surface of trunk, most anterior inconspicuous and below dorsal-fin origin, second below end of dorsal-fin, third typically in adipose-fin region, and fourth at end of caudal peduncle. Lateral portion of body with inconspicuous dark stripe from head to caudal fin. Pectoral, pelvic and dorsal fins with three irregular, poorly defined bands. Caudal fin with variegated blotches (Fig. 6).

Color in alcohol. Similar to pattern described for living individuals, but with darker brown color, and darker saddles and stripes (Fig. 1).

Sexual dimorphism. Specimens lacking main sexual dimorphic characters usually present in loricariid species, particularly in Neoplecostominae members, such as (1) a papilla present posteriorly to urogenital opening; (2) an expanded flap skin on dorsal surface of first pelvic-fin ray; and (3) a larger pelvic-fin and body size (all characters present in males), but absent in females. Three hypertrophied bicuspid odontodes are present on lateral portion of body (a characteristic that may be related to mature males), however it is only present in holotype.

Etymology. The specific name, *forestii*, is given in honor of Fausto Foresti, Professor of the university of São Paulo state "Júlio de Mesquita Filho" (Unesp) in Brazil, for his contributions to fish genetics, with more than 250 papers published in this field.

Distribution. *Microplecostomus forestii* sp. n. is known from two localities, the Roncador Stream and the das Brancas Stream, both tributaries of the Tocantizinho River, in the Tocantins basin (Fig. 7a).

Habitat. *Microplecostomus forestii* sp. n. was collected in shallow, clear waters of about 0.5 m in depth and fast-flowing currents, with an underlying substrate of rock, in areas of flat terrain. The fishes captured were associated with pebbles (Fig. 7b, c). This species is relatively hard to collect and is not abundant. In seven expeditions to the Roncador and das Brancas streams in different periods of the year, we were able to collect only 16 specimens. *Microplecostomus forestii* sp. n. is sympatric with species such as *Creagrutus* sp., *Rhinolekos capetinga* Roxo, Ochoa, Silva & Oliveira, 2015, *Hypostomus* sp., *Phenacorhamdia* sp., *Ancistrus* sp., and *Ituglanis* sp.

Sequencing and phylogenetic analysis

The sequences of all 157 specimens are shown in Suppl. material 1 (the same list of species presented by Roxo et al. 2014, but with the inclusion of the voucher and GenBank accession numbers for the specimens of the newly described genus). The concatenated dataset resulted in a matrix of 4,102 base pairs (bps), used in all the phylogenetic and biogeographic analyses, of which 1,361 bps were conserved and 2,657 bps were variable. There was no evidence of saturation in these data, considering that the Iss.c value is higher than the Iss, and the R^2 value is higher than 0.8 for transitions and transversions, for the concatenated matrix.

Our results are very similar to those of of Roxo et al. (2014), in particular, that the Hypoptopomatinae, Neoplecostominae and Otothyrinae clades are monophyletic (Figs 8–9) with strong statistical support (BS = 99 for Hypoptopomatinae; BS = 80 for Neoplecostominae; BS = 84 for Otothyrinae), that the Neoplecostominae is more closely related to the Otothyrinae than to the Hypoptopomatinae (BS = 95), and that these two clades together form the sister group of the Hypoptopomatinae (BS = 96). The new genus *Microplecostomus forestii* sp. n. was placed in the subfamily Neoplecostominae (Fig. 8), forming a sister group with all its members, with strong statistical support (BS = 80).

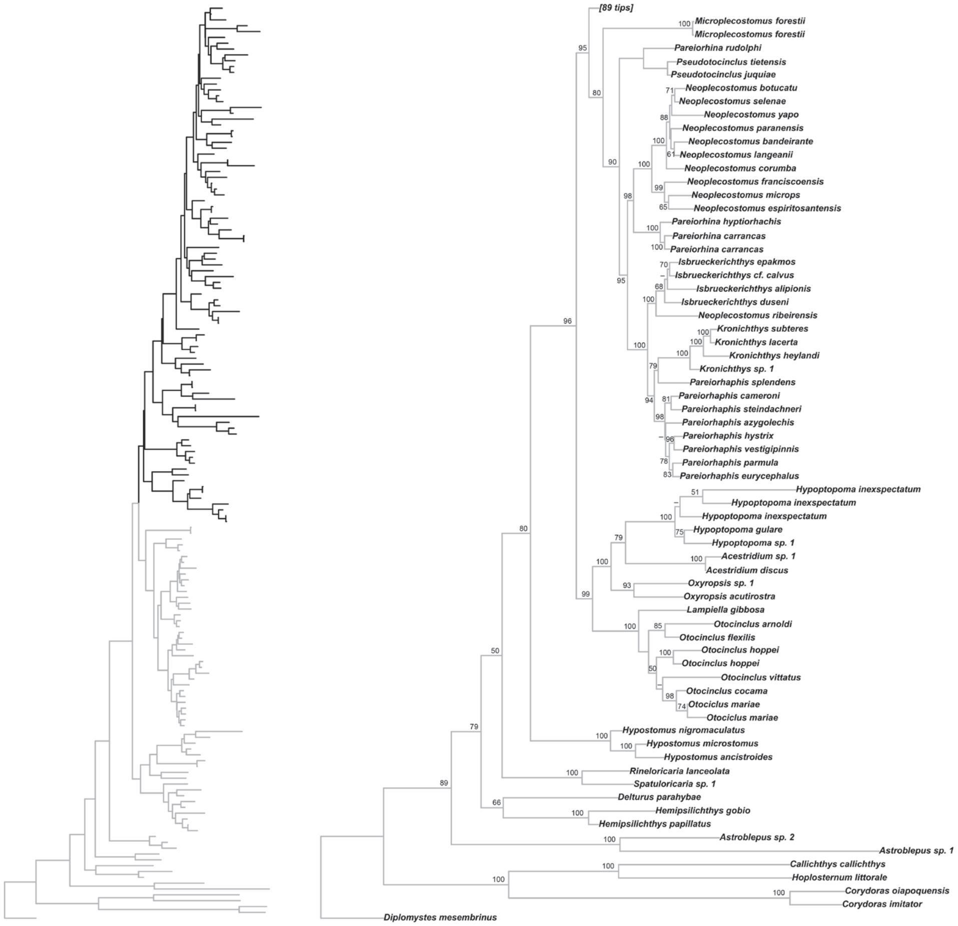

Figure 8. Partial ML tree showing the relationship among the species of the subfamilies Hypoptopomatinae and Neoplecostominae. Numbers above the branches are bootstrap values from 1000 bootstrap pseudoreplicates obtained from the ML analysis. Bootstrap values below 50% (-) are not shown.

Time calibrated tree and historical biogeography

Our time-calibrated tree estimated that the origin of the hypoptopomatine lineage was in the Paleocene, about 63.1 Mya (44.5–83.8 Mya 95% HPD), and is inferred by the DEC+J model to have been located in areas A (Atlantic Coast drainage basins) + D (Amazon and Orinoco basins) (Fig. 10 Region AD). The clade composed of the Neoplecostominae (Fig. 10 Region AD) + Otothyrinae (Fig. 11 Region AD) is estimated by BEAST to have also originated during the Paleocene, about 59.1 Mya (41.4–77.6 Mya 95% HPD), and once again, according to the DEC+J model, in areas A and D. *Microplecostomus forestii* sp. n. is found in the headwaters of the Tocantins River, one of the principal rivers of the Amazon basin (Area D). Our time-calibrated phylogeny estimated that the lineage of the new genus and species arose during the Eocene, 47.5 Mya (32.7–64.5 Mya 95% HPD).

Figure 9. Partial ML tree showing the relationship among the species of the subfamily Otothyrinae. Numbers above the branches are bootstrap values from 1000 bootstrap pseudoreplicates obtained from the ML analysis. Bootstrap values below 50% (-) are not shown.

Discussion

Taxonomy and phylogenetic relationship

The results of our molecular analyses indicated that *Microplecostomus forestii* sp. n. is the sister-group of all the other members of the Neoplecostominae (Fig. 8), with strong statistical support. *Microplecostomus forestii* sp. n. is distinguished from all other

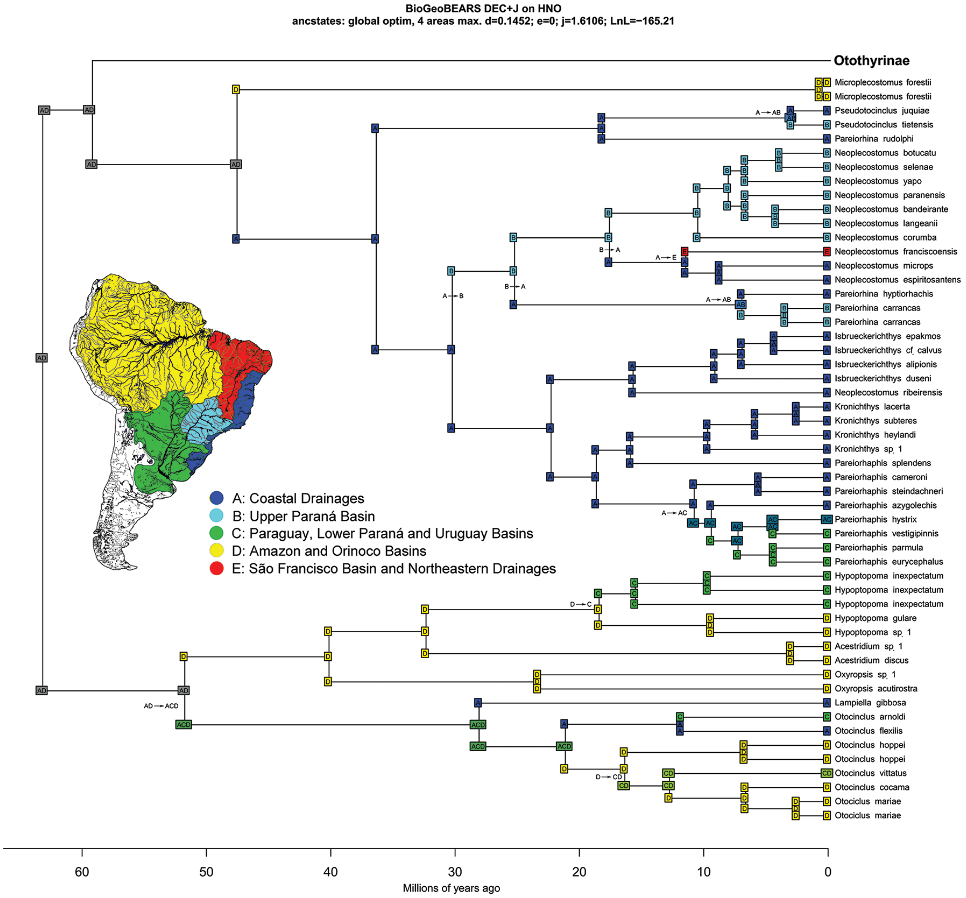

Figure 10. Time-calibrated phylogeny and ancestral range estimates for the Hypoptopomatinae and Neoplecostominae. Divergence ages were calibrated by the origins of the Siluriformes (120 Mya) and the Callichthyidae (55 Mya).

neoplecostomine species by the presence of two autapomorphic characters: (1) three hypertrophied bicuspid odontodes on the lateral portion of the body and (2) a large area without odontodes around the snout, observed in all specimens. Only one (the holotype) of the 16 specimens analyzed presented the three hypertrophied bicuspid odontodes on the lateral portion of the body. We believe that this is a sexually dimorphic character found only in large mature males, although this remains uncertain because the new taxon does not have any other obvious sexual dimorphism, and the trait is only present in the holotype, which cannot be dissected. Sexual dimorphism is very common in other neoplecostomines, in particular species of the genera *Neoplecostomus* (Langeani 1990; Zawadzki et al. 2008; Roxo et al. 2012b) and *Pareiorhaphis* (Pereira and Reis 2002; Pereira 2005). As in the present study, these species have been diagnosed primarily on the basis of the characteristics of the mature males.

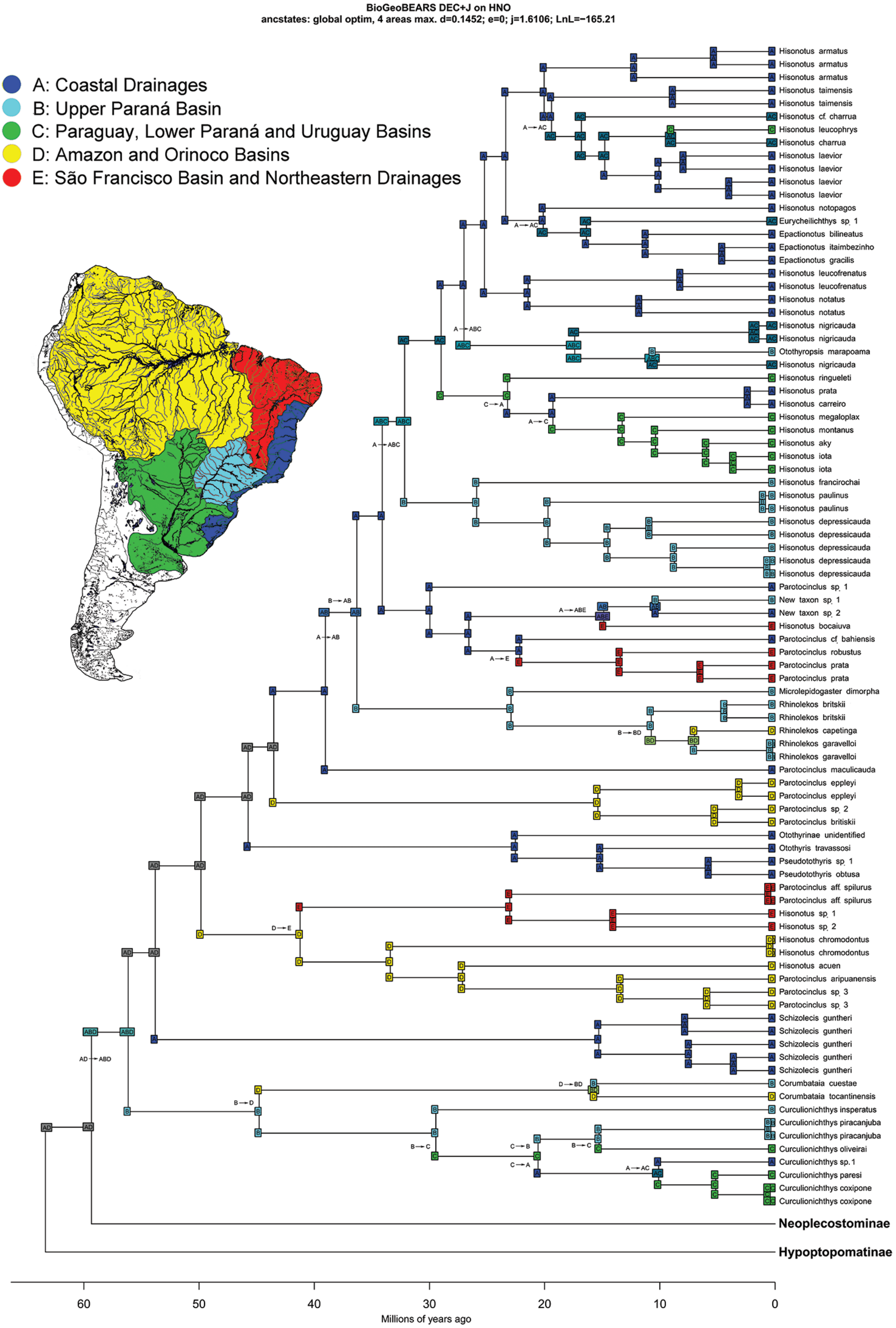

Figure 11. Time-calibrated phylogeny and ancestral range estimates for the Otothyrinae. Divergence ages were calibrated by the origins of the Siluriformes (120 Mya) and the Callichthyidae (55 Mya).

Another character used to distinguish *Microplecostomus forestii* sp. n. from other neoplecostomines is the presence of a post-dorsal ridge on the caudal peduncle. Bockmann and Ribeiro (2003) were the first authors to report this character in a neoplecostomine species (*Pareiorhina carrancas*), and Silva et al. (2013) also reported the structure in a second new species of the same genus, *P. hyptiorhachis*. This character is also present in species of *Corymbophanes* (Armbruster et al. 2000), a genus assigned to the subfamily Hypostominae, which is found throughout the Potaro River in Guyana, northern South America. Considering that these species are not closely related (Armbruster 2004; Roxo et al. 2014), the presence of a post-dorsal ridge appears to have arisen more than once during the evolution of these species.

The subfamily Neoplecostominae, as defined by Roxo et al. (2014), appears to be monophyletic and forms a sister group to the Otothyrinae, which together form a sister group to the Hypoptopomatinae (this relationship was first reported by Chiachio et al. 2008). The main deviation that we found from the arrangement proposed by Roxo et al. (2014) is in the relationship among the members of the genus *Hisonotus*. In the present study, the species *H. depressicauda* (Miranda Ribeiro, 1918b), *H. francirochai* (Ihering, 1928) and *H. paulinus* (Regan, 1908) appeared as a monophyletic group which includes most of the *Hisonotus* species found in southern Brazil, the type species of *Hisonotus* (i.e. *H. notatus* Eigenmann & Eigenmann, 1889), *Otothyropsis marapoama* Ribeiro, Carvalho & Melo, 2005, *Eurycheilichthys* and *Epactionotus*. In Roxo et al. (2014) *Hisonotus depressicauda*, *H. francirochai* and *H. paulinus* were not closely related to the former species, but formed a clade with *Parotocinclus* and *Hisonotus* species from the São Francisco River basin, albeit with reduced statistical support (see Fig. 4 in Roxo et al. 2014).

Historical biogeography

Using the DEC model to estimate ancestral species ranges, Roxo et al. (2014) suggested that the ancestral lineages of the Hypoptopomatinae, Neoplecostominae and Otothyrinae subfamilies (the HNO-Clade) originated on the Atlantic Coast of southeastern Brazil (area A, see Fig. 5 in Roxo et al. 2014). However, in a geographic area dominated by headwater capture events, ancestral lineages would be expected to be more widely distributed in adjacent hydrographic systems (see e.g. Albert et al. 2011). Given this, Roxo et al. (2014) did not reject the hypothesis that the ancestral lineages of the HNO-clade were more widely distributed in South America during the early Cenozoic, including much of the modern Atlantic Coast (area A), upper Paraná (area B), Paraguay, lower Paraná and Uruguay (area C), and Amazon and Orinoco basins (area D).

Our ancestral range estimates found, using the DECj model and including *Microplecostomus forestii* sp. n. in the HNO phylogeny, that the ancestral lineages of these three subfamilies were widely distributed on the Atlantic Coast (area A) and in the Amazon and Orinoco basins (area D). While these two areas are not adjacent (i.e. they do not share an endpoint or border), a number of studies have found evidence of the historical mixing of the faunas of the headwaters of the Amazon and Paraná rivers, and the drainage basins of the Atlantic Coast. The historical connection between the Paraguay and Amazon basins has been known for more than a century (e.g. Eigenmann and Eigenmann 1891; Jordan 1896; Eigenmann 1906; Pearson 1937; Carvalho and Albert 2011; Ribeiro et al. 2013), and may account for the geodispersal (*sensu* Albert and Reis 2011) from the Amazon drainage basins (in particular the Madeira, Tocantins and Xingu) located on the Brazilian Shield. Even so, geodispersal events in the reverse direction, i.e., from south to north should also be expected (Roxo et al. 2014), and the dispersal of the hypoptopomatine lineages (*sensu* Chiachio et al. 2008) is considered to be the result of historical connections among the Amazon, Orinoco and Paraguay basins (Albert et al. 2011; Roxo et al. 2014). A number of authors have proposed headwater capture as the likely mechanism determining the distribution of ancestral fish lineages in the Tietê, Paraíba do Sul, São Francisco and Ribeira de Iguape basins on the Brazilian Shield (Ab'Saber 1957; Ab'Saber 1998; Ribeiro 2006; Roxo et al. 2012c; 2014). The historical dispersal of ancestral fish lineages between areas A and D is thus quite conceivable.

All neoplecostomine lineages are found in southern and southeastern of South America, except *Microplecostomus forestii* sp. n. and *Pareiorhaphis regani* (Giltay, 1936), which are from the Amazon basin (area D). In a paper on the evolution of plants, Stebbins (1974) discussed the concepts of evolutionary museum and evolutionary cradle, which are used to define species occurrence patterns within an area. An evolutionary cradle is defined as an area of high speciation rates, while an evolutionary museum is an area with low extinction rates, where environmental conditions combine to preserve lineages over long periods of time. Roxo et al. (2014) suggested that in the Hypoptopomatinae, the *Lampiella gibbosa* (Miranda Ribeiro, 1908) and *Otocinclus affinis* (Steindachner, 1877b) lineages are relicts of the Atlantic Coast drainage basins, considering that other *Otocinclus* species are widely distributed in the lowland Amazon and Paraná-Paraguay basins. The new genus and species described here, *Microplecostomus forestii* sp. n., also appears to be a relict lineage of the Tocantins River basin (Amazon basin), given that all other neoplecostomine species, except *Pareiorhaphis regani,* are present in the upper Paraná, lower Paraná-Paraguay, and coastal drainage basins of the Brazilian Shield.

Comparative material

Chauliocheilos saxatilis Martins, Andrade, Rosa & Langeani, 2014: paratype, MZUSP 114758, 2, 38.9–40.2 mm SL, municipality of Itamarandiba, Minas Gerais state, Brazil, tributary of the Itamarandiba River.

Curculionichthys insperatus Britski & Garavello, 2003: LBP 4945, 5, 27.3–28.5 mm SL, 2 c&s, 28.2–29.9 mm SL, municipality of Botucatu, São Paulo state, Brazil, Tietê River basin.

Gymnotocinclus anosteos Carvalho, Lehmann A. & Reis, 2008: LBP 17125, 3, 18.8–33.0 mm SL, municipality of Alto Paraíso de Goiás, Goiás state, Brazil, Tocantins River basin.

Hisonotus acuen Silva, Roxo & Oliveria, 2014: holotype, MZUSP 115350, 25.9 mm SL, municipality of Querência, Mato Grosso state, Brazil, Xingu River basin; paratype, LBP 15755, 16, 19.5–26.0 mm SL, municipality of Ribeirão Cascalheira, Mato Grosso, Xingu basin.

Hisonotus bocaiuva Roxo, Silva, Oliveira & Zawadzki, 2013: holotype, MZUSP 112204, 24.2 mm SL, municipality of Bocaiúva, Minas Gerais state, Brazil, São Francisco River basin; paratype, LBP 9817, 9, 3 c&s, 18.3–23.2 mm SL, municipality of Bocaiúva, Minas Gerais state, Brazil São Francisco River basin.

Hisonotus notatus Eigenmann & Eigenmann, 1889a: LBP 18472, 7, 30.1–38.3 mm SL, municipality of Silva Jardim, Rio de Janeiro state, Brazil, coastal drainage basin.

Isbrueckerichthys alipionis (Gosline, 1947): LBP 7373, 17, 31.7–81.6 mm SL, municipality of Iporanga, São Paulo state, Brazil, Ribeira de Iguape River basin;

Kronichthys subteres Miranda Ribeiro 1908: LBP 515, 31, 28.4–61.9 mm SL, municipality of Iporanga, São Paulo state, Brazil, Ribeira de Iguape River basin.

Lampiella gibbosa (Miranda Ribeiro, 1908): LBP 7430, 5, 25.6–26.1 mm SL, municipality of Jacupiranga, São Paulo state, Brazil, Ribeira de Iguape River basin.

Microlepidogaster arachas Martins, Calegari & Langeani, 2013: LBP 10882, 3, 22.8–35.3 mm SL, municipality of Araxás, Minas Gerais state, Brazil, Paraná River basin.

Nannoplecostomus eleonorae Ribeiro, Lima & Pereira, 2012: LBP 19016, 51, 19.9–25.4 mm SL, municipality of Guarani de Goiás, Goiás state, Brazil, Tocantins River basin.

Neoplecostomus microps (Steindachner, 1877a): LBP 8036, 38, 41.3–65.0 mm SL, municipality of Piquete, São Paulo state, Brazil, Paraíba do Sul River basin.

Neoplecostomus franciscoensis Langeani, 1990: LBP 6489, 50, 42.8–55.9 mm SL, municipality of São Bartolomeu, Minas Gerais state, Brazil, São Francisco River basin.

Neoplecostomus paranensis Langeani, 1990: holotype, MZUSP 38572, 71.4 mm SL, municipality of Cajuru, Minas Gerais state, Brazil, Grande River basin.

Otocinclus affinis (Steindachner, 1877b): 19, 19.5–28.9 mm SL, municipality of Poconé, Mato Grosso state, Brazil, Paraguay River basin.

Otocinclus vittatus Regan, 1904: 27, 18.2–21.7 mm SL, municipality of Cáceres, Mato Grosso state, Brazil, Paraguay River basin.

Otothyropsis marapoama Ribeiro, Carvalho & Melo, 2005: LBP 4698, 6, 23.9–36.3 mm SL, municipality of Marapoama, São Paulo state, Brazil, Tietê River basin.

Pareiorhaphis splendens (Bizerril, 1995): LBP 1117, 20, 32.0–100.0 mm SL, municipality of Morretes, Paraná state, Brazil, Atlantic Coast drainage basins.

Pareiorhaphis steindachneri (Miranda Ribeiro, 1918a): LBP 739, 6, 33.8–49.0 mm SL, municipality of Jaraguá do Sul, Santa Catarina state, Brazil, Atlantic Coast drainage basins.

Pareiorhina brachyrhyncha Chamon, Aranda & Buckup, 2005: LBP 12240, 50, 26.4–36.9 mm SL, municipality of Pindamonhangaba, São Paulo state, Brazil, Paraíba do Sul River basin.

Pareiorhina cepta Roxo, Silva, Mehanna & Oliveira, 2012d: holotype, MZUSP 111095, 41.5 mm SL, municipality of São Roque de Minas, Minas Gerais state, Brazil, São Francisco basin, paratypes, LBP 10287, 13, 21.5–43.6 mm SL, municipality of São Roque de Minas, Minas Gerais, Brazil, Paraíba do Sul River basin.

Pareiorhina rudolphi (Miranda Ribeiro, 1911): LBP 8044, 18, 31.7–48.9 mm SL, municipality of Piquete, São Paulo state, Brazil, Paraíba do Sul River basin.

Parotocinclus maculicauda (Steindachner, 1877b): LBP 2869, 15, 20.2–44.7 mm SL, municipality of Miracatu, São Paulo state, Brazil, Ribeira de Iguape River basin.

Plesioptopoma curvidens Reis, Pereira & Lehmann A, 2012: LBP 17394, 39, 26.1–81.7 mm SL, municipality of Cristiano Otoni, Minas Gerais state, Brazil, São Francisco River basin.

Pseudotocinclus juquiae Takako, Oliveira & Oyakawa, 2005: LBP1081, 2, 29.0–31.9 mm SL, municipality of Juquitiba, São Paulo state, Brazil, Atlantic Coast drainage basins.

Pseudotocinclus tietensis (Ihering, 1907): LBP 2931, 3, 38.6–62.3 mm SL, municipality of Salesópolis, São Paulo state, Brazil, Tietê River basin.

Schizolecis guntheri (Miranda Ribeiro, 1918b): LBP 14335, 18, 18.3–35.3 mm SL, municipality of São Sebastião, São Paulo state, Brazil, Atlantic Coast drainage basins.

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Supplementary material 1

Table S1

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Data type: Microsoft Word document

Explanation note: Species included in the present study with voulchers and GenBank accession numbers.

Supplementary material 2

Table S2

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Data type: Microsoft Word document

Explanation note: Models tested to estimate distribution ranges inherited by the descending lineages at each node of the tree.