Zootaxa 3881 (4): 362–372
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3881.4.4
http://zoobank.org/urn:lsid:zoobank.org:pub:33C50A47-49D3-4CA1-8887-EB2EE9DC611D

Description of the second and third instars of *Aspidytes wrasei* Balke, Ribera & Beutel, 2003, with comments on the identification of larvae of *Aspidytes* Ribera, Beutel, Balke & Vogler, 2002 (Coleoptera: Aspidytidae), and phylogenetic considerations

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Abstract

The second- and third instar larvae of the cliff water beetle *Aspidytes wrasei* Balke, Ribera & Beutel, 2003 are studied for the first time with special emphasis on morphometry and chaetotaxy. A review of the characters useful in the identification of larvae of *Aspidytes* Ribera, Beutel, Balke & Vogler, 2002 is presented. Confirming previous findings, larvae of this genus are unique within Hydradephaga in the dorsally oriented spiracles on the abdominal segment VIII of instars II and III. The inclusion of Aspidytidae within the superfamily Dytiscoidea is reinforced by the following putative synapomorphies: presence of pore PAp, proximal insertion of pore ANg, apical or subapical insertion of seta MX8, presence of pore LAd, and distal insertion of seta CO6. Larvae of *A. wrasei* differ from those of *A. niobe* Ribera, Beutel, Balke & Vogler, 2002 in several significant characters that may indicate that both species have a long history of independent evolution.

Key words: Coleoptera, Aspidytidae, *Aspidytes*, larva, morphometry, chaetotaxy

Introduction

Ribera *et al.* (2002) erected the water beetle family Aspidytidae based on a single genus, *Aspidytes* Ribera, Beutel, Balke & Vogler, 2002 and a single species, *A. niobe* Ribera, Beutel, Balke & Vogler, 2002 from the Western Cape Province in South Africa. Based on a phylogenetic analysis combining adult morphological and molecular data, the authors placed the new family within the adephagan superfamily Dytiscoidea, as the sister group of Paelobiidae + Dytiscidae. Balke *et al.* (2003) described a second species, *A. wrasei* Balke, Ribera & Beutel, 2003, from Shaanxi Province in central China and provided a detailed account of the adult morphology of the genus and species. Alarie & Bilton (2005) described in detail the three larval instars of *A. niobe* with emphasis on chaetotaxy, and provided a description of the habitat of adults and larvae, which are strictly madicolous, living in permanent water seepages 1–2 mm in depth, flowing over exposed vertical or nearly vertical rock faces. Jia *et al.* (2012) described and illustrated the habitat of *A. wrasei* (see also Balke 2010). Based on a phylogenetic analysis of larval characters, Alarie & Bilton (2005) supported the placement of Aspidytidae within Dytiscoidea, although the relationships with other dytiscoid families were unresolved. Finally, Balke *et al.* (2005) provided a phylogenetic analysis combining adult and larval morphological and molecular characters which supports the inclusion of Aspidytidae in Dytiscoidea as the sister group of Amphizoidae, a relationship later supported by Balke *et al.* (2008) and Alarie *et*

 Accepted by H. Fery: 14 Oct. 2014; published: 6 Nov. 2014

al. (2011b) based on molecular and larval characters respectively. Overall, the aforementioned studies provide valuable information on the morphology of adults and larvae, their way of living and phylogenetic relationships of these beetles. However, as the larvae of *A. wrasei* remained unknown, it was impossible to evaluate if the characters provided by the larvae of *A. niobe* can be applied at the genus level.

The recent discovery in central China of the larvae of *A. wrasei* (Jia *et al.* 2012) provides the opportunity to develop the larval ground plan of the genus *Aspidytes* and so to reassess the relative phylogenetic position of the family Aspidytidae within the Dytiscoidea. The present study, therefore, has the following goals: (1) to describe and illustrate in detail the second- and third instars of *A. wrasei* with an emphasis on morphometry and chaetotaxy of selected structures; (2) to recognize the characters useful in the identification of larvae of the species of *Aspidytes*; and (3) to study the phylogentic relationships of the Aspidytidae with other dytiscoid families.

Material and methods

The description provided in this paper is based on five instar II and two instar III specimens collected at the type locality of *A. wrasei*: China, Shaanxi prov., 110 km NEE Xian, Huayin vill., Hua Shan Mt., granite cliff, 1275 m, 8/9.V.2011, 34°29.5'N 110°5.1'E. The larvae were cleared in lactic acid, dissected, and mounted on glass slides in polyvinyl-lacto-glycerol. Microscopic examination at magnifications up to 1,000x and drawings were made using an Olympus CX31 compound microscope equipped with a camera lucida. Drawings were scanned and digitally inked using a Genius PenSketch tablet. SEM pictures were taken with a Philips XL 30 ESEM. The material is held in the larval collections of Y. Alarie (Laurentian University, Sudbury, Ontario, Canada) and J. Hájek (Department of Entomology, National Museum, Praha, Czech Republic). One voucher specimen is held in the Coleoptera collection of the Staatliche Naturwissenschaftliche Sammlungen Bayerns–Zoologische Staatssammlung München, Germany.

The methods and terms used herein follow those employed in previous papers dealing with the larval morphology and chaetotaxy of *Aspidytes* species. The reader is referred to Alarie & Bilton (2005) for a complete list and additional explanations of the terms used in the present study. Whereas represented by instars II and III only, primary sensilla of *A. wrasei* were tentatively identified by comparison with the *Aspidytes* ground plan wherever possible (e.g., head appendages and legs) (Alarie & Bilton 2005). In these cases, homologies were recognized using the criterion of similarity of position (Wiley 1981). Secondary sensilla were also tentatively identified by comparison with the primary sensilla of *A. niobe*.

Results

Diagnosis of the larvae of *Aspidytes*

Larvae of *Aspidytes* can be distinguished from those of other dytiscoid genera by the combination of the following characters: frontoclypeolabrum extended anteriorly into a short nasale (Fig. 1); lateral lobes present (Fig. 1); lateral appendage of antennomere 3 reduced (Fig. 4); mandible with a retinaculum and mesal groove toothed along inner margin (Fig. 6); maxillary palpus subequal in length to labial palpus; trochanteral annulus lacking (Figs 11–12); primary setae FE7, FE8, FE9, and FE10 present (Fig. 11); abdomen nine-segmented, with segment IX vestigial (Fig. 14); spiracles opening dorsally on abdominal segment VIII (Fig. 13); urogomphus two-segmented (Fig. 13).

Description of the larvae of *Aspidytes wrasei* Balke, Ribera & Beutel, 2003

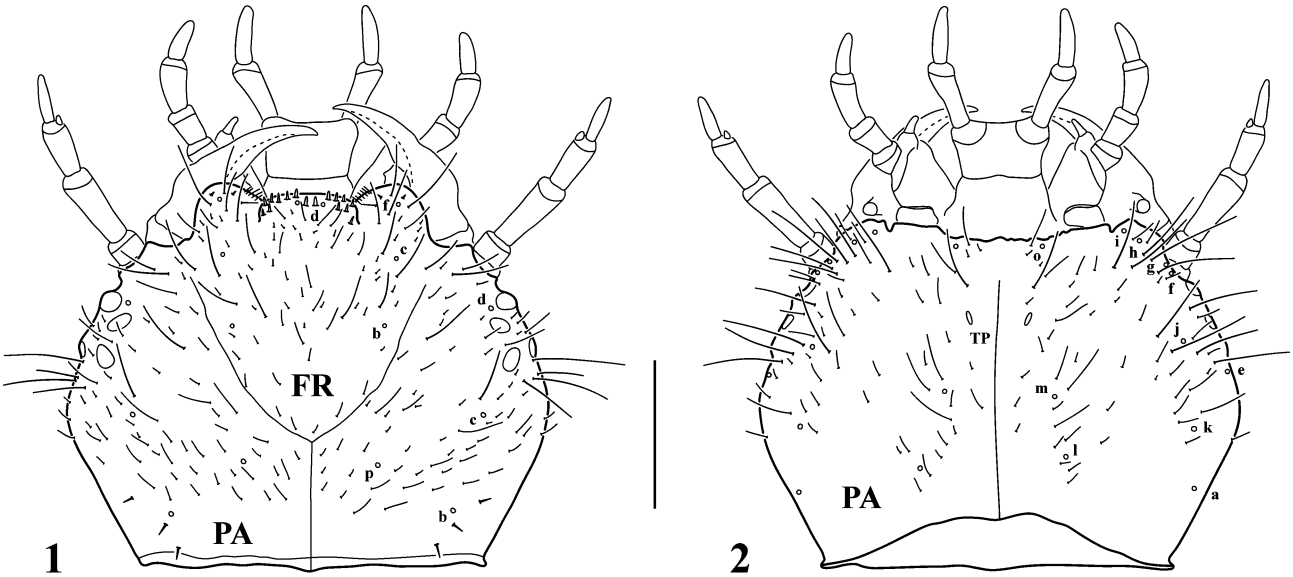
(Figs 1–17)

Diagnosis (instars II–III). Frontoclypeolabrum less than 0.7 times as long as head length, extended anteriorly into a short and subrectangular nasale (Figs 1, 15); nasale with 12 (instar II) or 16 (instar III) lamellae clypeales (Figs 1, 15); egg bursters absent (Figs 1, 15); coronal suture about 0.3 times as long as head length; antenna less than 1.7 times longer than maxillary palpus; apical lateroventral process of antennomere 3 short, bulge-like (Fig. 4); galea

two-segmented (Figs 7–8); maxillary palpomere 1 less than 0.75 times as long as maxillary palpomere 2; maxillary palpomere 3 about as long as maxillary palpomere 2; length of metathoracic leg less than 2 mm, about twice longer than HW; femur with one secondary seta on posteroventral surface (Fig. 12); tibia lacking additional pores (Figs 11–12); abdominal segments I–VII membranous ventrally; abdominal segment VIII extended posteriorly into short siphon (Fig. 13); urogomphus more than 3.3 times longer than last abdominal segment.

Description, instar III (Figs 1–14). Colour: Cephalic capsule light brown in anterior half, brown in posterior half; antennomeres 3 and 4, maxillary palpomeres 2 and 3, labial palpomere 2, and distal half of mandible brown, rest of head appendages light brown; thoracic and abdominal sclerites light brown, somewhat darker centrally, bearing few darker maculae; siphon brown; membranous parts testaceous; legs and urogomphi light brown, tibia and tarsus somewhat darker than rest of leg.

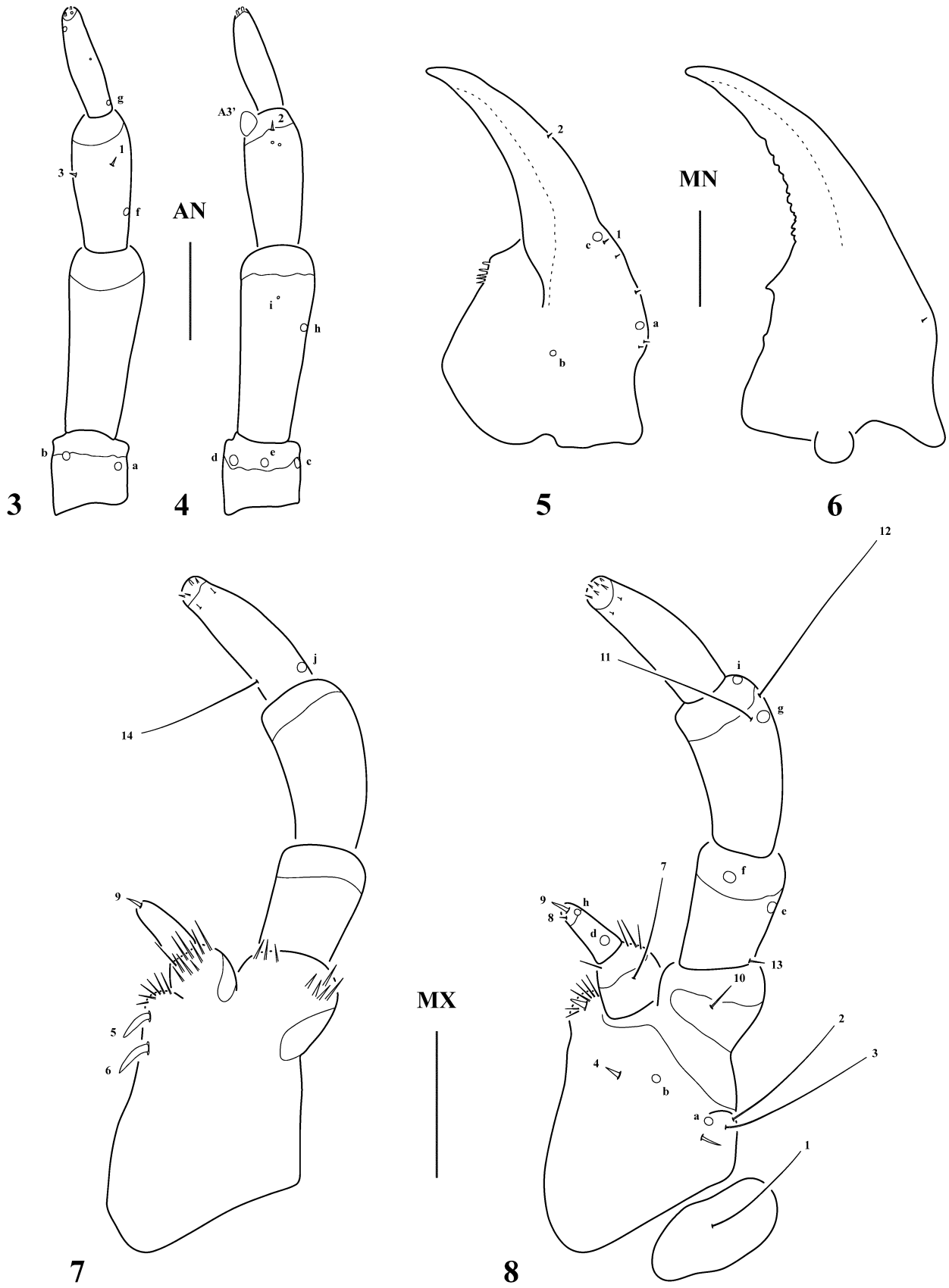
Body: Subcylindrical, narrowing towards abdominal apex. Total length (excluding urogomphi): 6.9–7.1 mm; maximum width: 1.2–1.3 mm.



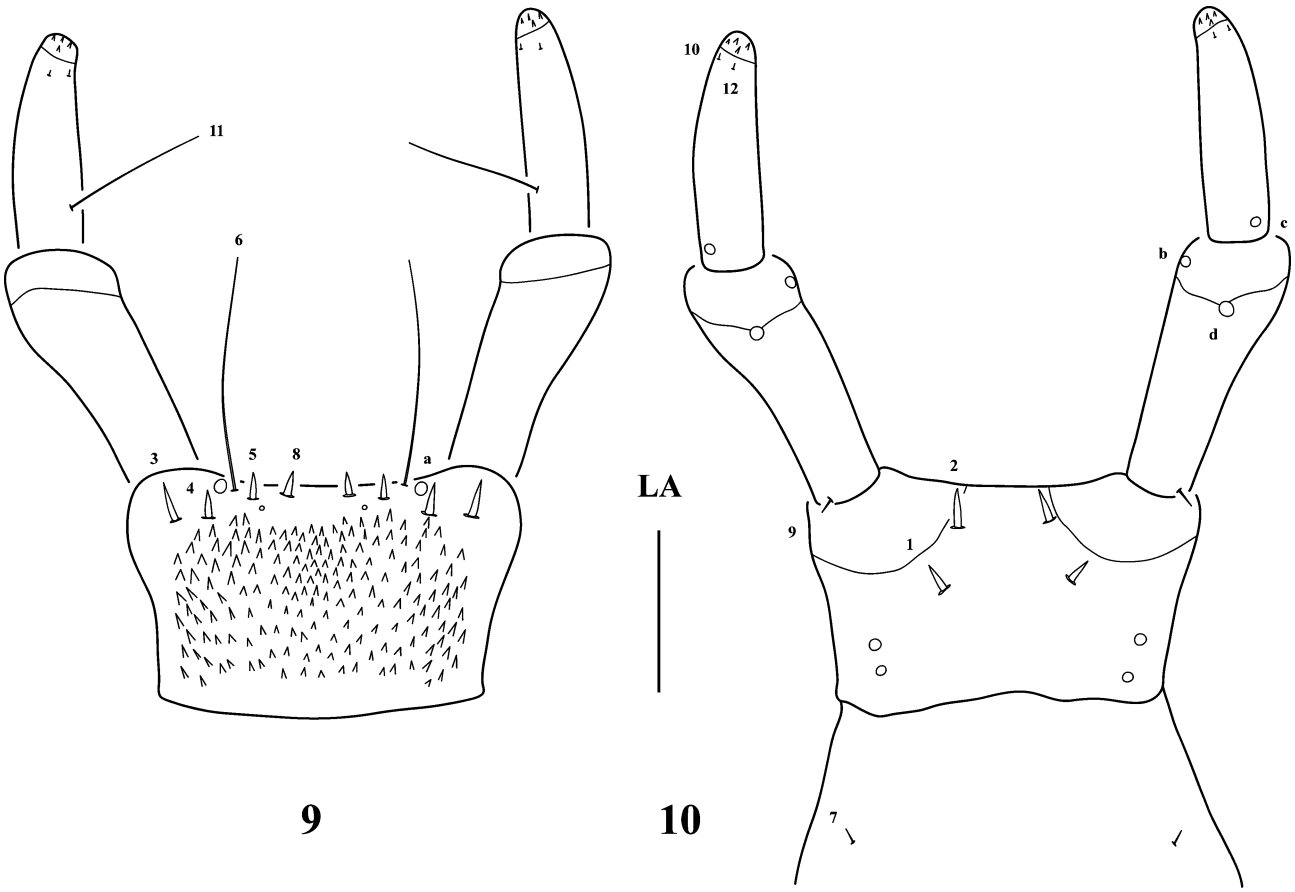
FIGURES 1–2. *Aspidytes wrasei*, third-instar larva. 1, head, dorsal aspect; 2, head, ventral aspect. Lowercase letters indicate primary pores. FR: frontoclypeolabrum; PA: parietal; TP: tentorial pit. Scale bar: 0.30 mm.

Head (Figs 1–2): HL: 0.75–0.8 mm; HW: 0.95–1.0 mm; FCL: 0.5 mm; OCW: 0.7–0.75 mm. *Cephalic capsule*: Subovate, somewhat broader than long (HL/HW: 0.8); neck constriction and occipital suture absent; ecdysial suture well visible except anterior portion, coronal suture 0.35 times as long as HL; HW/OCW: 1.3–1.4; frontoclypeolabrum 0.65 times as long as HL, with anterior margin produced medially into a short and broad subrectangular nasale, lacking egg bursters; tentorial pits visible ventrally on each side of midline; occipital foramen broad, slightly indented ventrally; ocularium as in Figs 1–2. *Antenna* (Figs 3–4): Short, composed of four antennomeres; length of antenna/HL: 0.55; A2 > A3 > A4 > A1; A1/A2: 0.35–0.4; A3/A2: 0.7–0.75; A4/A2: 0.6–0.65; A3' prominent, bulge-like; ventroapical spinula of A3 absent. *Mandible* (Figs 5–6): Short, broad basally, distal half projected inwards, apex sharp; 2.15–2.25 times longer than broad, 0.5 times as long as HL; ventral edge of mesal groove slightly dentate; retinaculum present. *Maxilla* (Figs 7–8): Short, robust; cardo well developed, subovate; stipes broad, subtrapezoidal; galea two-segmented, 0.5–0.6 times as long as palpomere 1; palpifer well differentiated from the stipes, length of palpifer/length of palpomere 1: 0.65–0.75; maxillary palpus short, composed of three palpomeres, length of antenna/length of maxillary palpus: 1.55–1.6; length of palpomere 1/ length of palpomere 2: 0.65–0.75; length of palpomere 3/length of palpomere 2: 0.8–0.9. *Labium* (Figs 9–10): Well developed; prementum broader than long; labial palpus relatively elongate, composed of two palpomeres, length of maxillary palpus/length of labial palpus: 1.05; length of palpomere 2/length of palpomere 1: 0.85.

Thorax: Terga convex, pronotum somewhat shorter than meso- and metanotum combined, meso- and metanotum subequal, wider than pronotum; protergite subovate, meso- and metatergite transverse, with anterior transverse carina; sagittal line visible on the three tergites; sterna membranous; spiracles present on mesothorax. *Legs* (Figs 11–12): Short, composed of six segments; prothoracic leg shortest, metathoracic leg longest, length of metathoracic leg: 1.85 mm, 1.2 times longer than prothoracic leg, 1.1 times longer than mesothoracic leg,



FIGURES 3–8. *Aspidytes wrasei*, third-instar larva. 3, right antenna, dorsal aspect; 4, left antenna, ventral aspect; 5, right mandible, dorsal aspect; 6, left mandible, ventral aspect; 7, right maxilla, dorsal aspect; 8, left maxilla, ventral aspect. Numbers and lowercase letters indicate primary setae and pores respectively. Additional and/or secondary sensilla not labelled. A3': apical lateroventral process of antennomere 3; AN: antenna; MN: mandible; MX: maxilla. Scale bars: 0.08 mm.



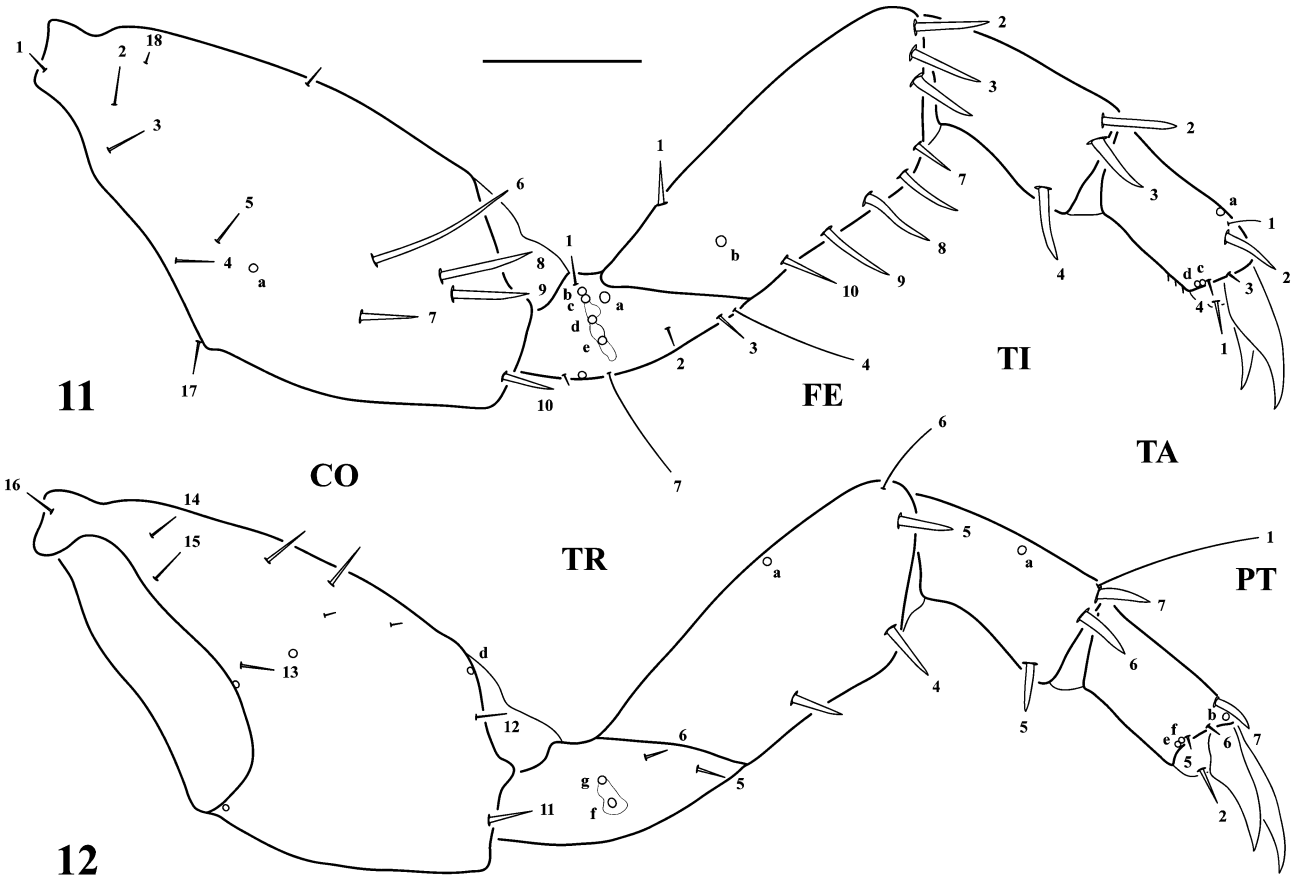
FIGURES 9–10. *Aspidytes wrasei*, third-instar larva. 9, labium, dorsal aspect; 10, labium, ventral aspect. Numbers and lowercase letters indicate primary setae and pores respectively. Additional and/or secondary sensilla not labelled. LA: labium. Scale bar: 0.08 mm.

1.85–1.95 times longer than HW; metathoracic leg: CO/FE: 1.25, TR/FE: 0.25–0.3, TI/FE: 0.65, TA/FE: 0.55; PT with two slightly curved claws, longest (anterior) metathoracic claw 0.7 times as long as metatarsus; trochanter with membranous areas surrounding pores TRb–TRe and TRf–TRg, not forming distinct annulus.

Abdomen: Nine-segmented; segments I–VII sclerotized dorsally and laterally, membranous ventrally; segment VIII subtrapezoidal, length: 0.45–0.5 mm, completely sclerotized except for a narrow longitudinal band ventrally (Figs 13–14), 0.5 times as long as HW; sclerites I–VIII with anterior transverse carina; spiracles present on segments I–VII; siphon present, short, partly membranous and bearing spiracles dorsally; segment IX vestigial, surrounded by urogomphal bases (Fig. 14). *Urogomphus* (Figs 13–14): Long, two-segmented, total length: 1.6–1.75 mm (length of urogomphomere 1: 1.0–1.05 mm), 1.65–1.8 times longer than HW (length of urogomphomere 1/HW: 1.05), 3.35–3.55 times longer than segment VIII (length of urogomphomere 1/length of segment VIII: 2.15); length of urogomphomere1/length of urogomphomere 2: 1.5–1.75.

Chaetotaxy (Figs 1–14): Cephalic capsule with numerous secondary setae both dorsally and ventrally (Figs 1–2); anterior margin of nasale with 16 lamellae clypeales (Fig. 1); pore FRa absent (Fig. 1); antenna bearing all primary sensilla and two minute pores on ventroapical surface of A3 (Fig. 4); mandible bearing all primary sensilla and few secondary setae on external margin (Figs 5–6); maxilla bearing all primary sensilla (Figs 7–8); stipes with one short secondary seta on ventral surface, contiguous to pore MXa (Fig. 8), and three robust spine-like (secondary?) setae on internal margin, two of them probably homologous to primary setae MX5 and MX6 of other Adephaga (Fig. 7); labium bearing all primary sensilla except additional setae on prementum (Figs 9–10); prementum with two pores on ventral surface, most likely secondary structures (Fig. 10); setae LA10 and LA12 minute, inserted distally on second palpomere (Fig. 10); thoracic tergites bearing numerous minute and some hair-like setae; legs bearing all primary sensilla except additional pore on anterior surface of tibia (Fig. 11); coxa with 0–1 anterior and 2–4 dorsal secondary setae and 1–2 posterior and 0–1 ventral secondary pores; trochanter with one secondary seta and one secondary pore on ventral surface; femur with one secondary seta on posteroventral

surface; abdominal tergites I–VIII bearing numerous minute and some hair-like setae (Figs 13–14); ventral surface of abdominal tergite VIII with 5–7 secondary pores (Fig. 14); urogomphus bearing all primary sensilla (Figs 13–14); primary pores not labelled because varying slightly in number (6–8 pores recorded); urogomphomere 1 with 4–6 spine-like secondary setae on basal half and group of 4–6 secondary pores on basoventral surface (Figs 13–14).



FIGURES 11–12. *Aspidytes wrasei*, third-instar larva. 11, left metathoracic leg, anterior aspect; 12, right metathoracic leg, posterior aspect. Numbers and lowercase letters indicate primary setae and pores respectively. Additional and/or secondary sensilla not labelled. CO: coxa; FE: femur; PT: pretarsus; TA: tarsus; TI: tibia; TR: trochanter. Scale bar: 0.15 mm.

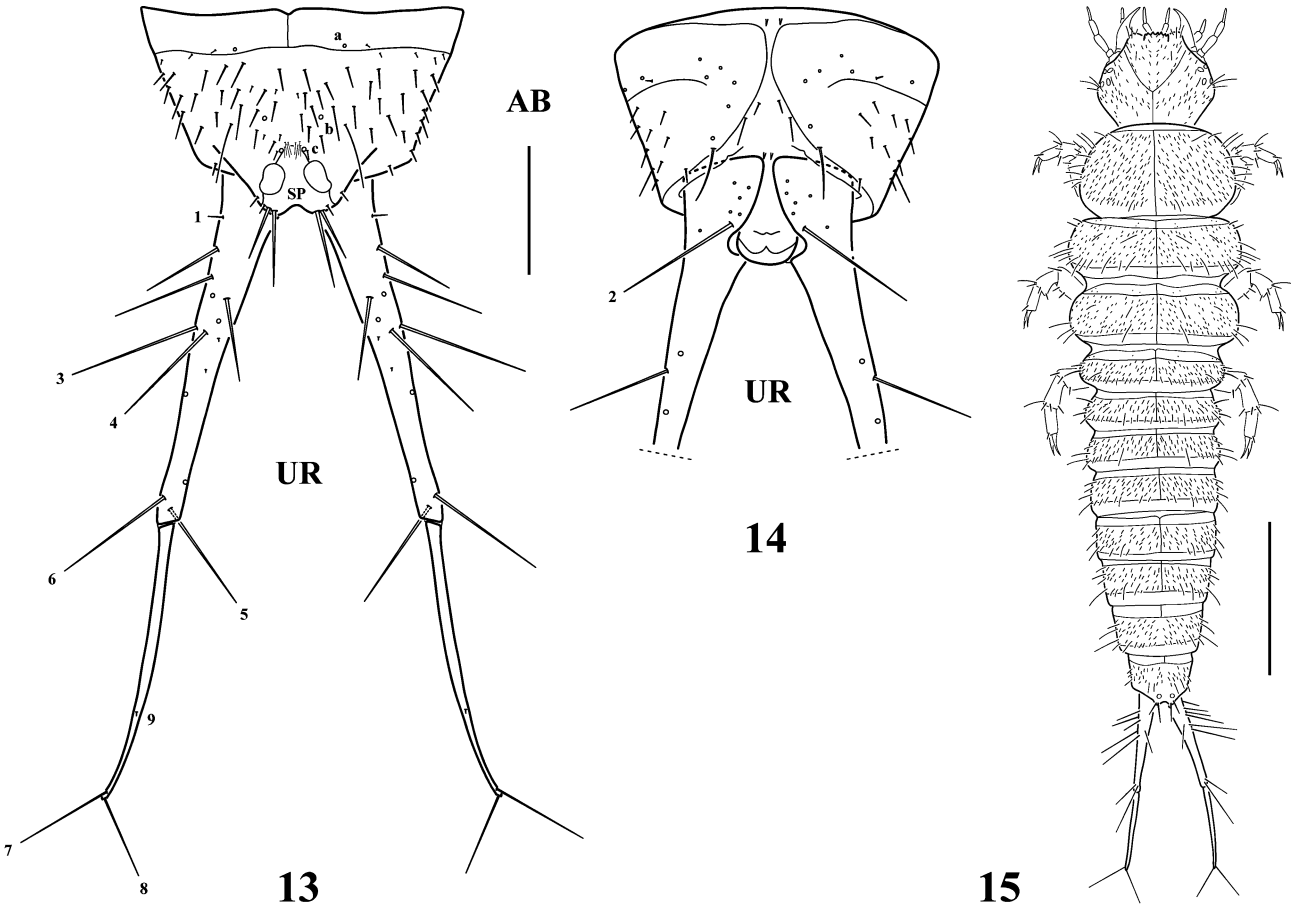
Description, instar II (Figs 15–17). As instar III except as follows:

Body: Total length (excluding urogomphi): 4.5–5.0 mm; maximum width: 0.9–1.0 mm.

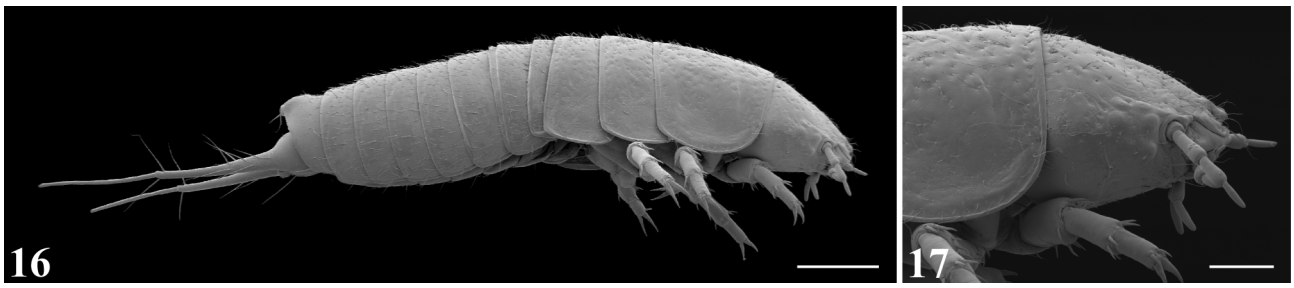
Head: HL: 0.6–0.65 mm; HW: 0.75–0.8 mm; FCL: 0.45 mm; OCW: 0.55 mm. *Cephalic capsule*: HL/HW: 0.8–0.85, coronal suture 0.3–0.35 times as long as HL; HW/OCW: 1.4; frontoclypeolabrum 0.65–0.7 times as long as HL. *Antenna*: Length of antenna/HL: 0.55–0.6; A1/A2: 0.35; A3/A2: 0.85–0.9; A4/A2: 0.7–0.85. *Mandible*: 1.95–2.05 times longer than broad, 0.5 times as long as HL. *Maxilla*: Galea 0.6 times as long as palpomere 1; length of antenna/length of maxillary palpus: 1.5; length of palpifer/length of palpomere 1: 0.75; length of palpomere 1/length of palpomere 2: 0.55–0.6; length of palpomere 3/length of palpomere 2: 0.9–0.95. *Labium*: Length of maxillary palpus/length of labial palpus: 1.05–1.1; length of palpomere 2/length of palpomere 1: 1.05–1.1.

Thorax: *Legs*: Length of metathoracic leg: 1.45–1.55 mm, 1.2–1.25 times longer than prothoracic leg, 1.1 times longer than mesothoracic leg, 1.9–1.95 times longer than HW; metathoracic leg: CO/FE: 1.3, TR/FE: 0.3, TI/FE: 0.65, TA/FE: 0.6; longest metathoracic claw 0.7–0.75 times as long as metatarsus.

Abdomen: Length of last abdominal segment: 0.3–0.35 mm, 0.4–0.45 times as long as HW. *Urogomphus*: Total length: 1.3–1.35 mm (length of urogomphomere 1: 0.8–0.85 mm), 1.7–1.75 times longer than HW (length of urogomphomere 1/HW: 1.05), 3.85–4.25 times longer than segment VIII (length of urogomphomere 1/length of segment VIII: 2.35–2.6); length of urogomphomere 1/length of urogomphomere 2: 1.6.



FIGURES 13–15. *Aspidytes wrasei*. 13–14, third-instar larva: 13, abdominal segment VIII and urogomphi, dorsal aspect; 14, abdominal segment VIII and urogomphi, ventral aspect. 15, second-instar larva, habitus, dorsal aspect. Only labelled: primary pores on abdominal segment VIII, primary setae on urogomphus. AB: abdominal segment VIII; SP: spiracles; UR: urogomphus. Scale bars: 0.30 mm (Figs 13–14) and 0.10 mm (Fig. 15).



FIGURES 16–17. SEMs of *Aspidytes wrasei*, second-instar larva. 16, habitus, lateral aspect; 17, head and prothorax, lateral aspect. Scale bars: 500 µm (Fig. 16) and 200 µm (Fig. 17).

Chaetotaxy: Anterior margin of nasale with 12 lamellae clypeales; mandible bearing only one secondary seta (located proximally to pore MNa); prementum bearing only one pore on ventral surface; coxa with 0–2 anterior and 2–4 dorsal secondary setae and 0–1 posterior secondary pores, ventral secondary pores absent; secondary setae on ventral surface of trochanter absent; ventral surface of abdominal tergite VIII with 2–3 secondary pores; urogomphomere 1 with 2–5 spine-like secondary setae on basal half and 1–2 secondary pores on basoventral surface.

Habitat. Larvae and adults were collected around the type locality of the species (Balke 2010; Jia *et al.* 2012), in thin (ca. 1–5 mm) water film on vertical granite rocks at altitude ca. 1.275 m a.s.l. (Fig. 18). The beetles were more abundant under small stones or leaves laying on rock. *Aspidytes wrasei* was associated with adults of *Platynectes dissimilis* (Sharp, 1873), *Platambus schillhammeri* Wewalka & Brancucci, 1995 (both Dytiscidae),

Laccobius qinlingensis Jia, Gentili & Fikáček, 2013 (Hydrophilidae) (Jia *et al.* 2013), and many Trichoptera larvae which might be prey. Despite intensive collecting efforts, we could not find *A. wrasei* in lower altitude parts of the Hua Shan mountain or at any other place in the Qinling Shan mountain range.

FIGURE 18. Habitat of *Aspidytes wrasei*, wet rocks at Hua Shan cliff, Shaanxi Province, China.

Discussion

The second- and third instar larvae of *A. wrasei* are described in detail in this contribution, which summed to the previous description of *A. niobe* by Alarie & Bilton (2005) provide a detailed account of the larval morphology of the genus *Aspidytes*. These authors postulated that the larvae of *Aspidytes* were unique within the Adephaga in retaining the egg bursters to instar II and in having dorsally orientated spiracles on the abdominal segment VIII in instars II and III. Whereas the spiracles on the abdominal segment VIII were also found to open dorsally in *A. wrasei*, the absence of egg bursters in instar II of that species suggests that this character state is autapomorphic to *A. niobe*. The presence of dorsally orientated spiracles on the abdominal segment VIII (probably an adaptation for gas exchange in the particular habitat exploited by these beetles), therefore, would represent the only unambiguous larval synapormorphy of Aspidytidae. It is worth stressing, however, another distinctive feature of larvae of this family, which is the presence of two-segmented urogomphi. Whereas this character state is not found in other hydradephagan families, its presence within some dytiscid lineages such as Agabinae, Hydroporinae, and Laccophilini suggests that it has evolved several times independently within the Dytiscoidea.

Following this study, the placement of Aspidytidae within the Dytiscoidea is reinforced. Indeed, larvae of both *A. wrasei* and *A. niobe* share with other members of this superfamily the presence of the primary pore PAe on the parietal (secondarily lost in Paelobiidae and some Hydroporinae), a small size of the cardo compared to stipital base, the presence of the primary setae FE7–FE10 on the femur, the presence of enlarged spiracles on the abdominal segment VIII (reduced in Paelobiidae), and the absence of the abdominal segment X (Alarie & Bilton 2005; Balke *et al.* 2005). Other larval characters give support to a monophyletic origin of the Dytiscoidea including Aspidytidae (Bousquet & Goulet 1984; Alarie *et al.* 2004; Alarie & Bilton 2005; Archangelsky & Michat 2007;

Michat *et al.* 2010). The primary pore PAp, located on the dorsal surface of the parietal, is absent in Carabidae, Trachypachidae, Gyrinidae, and Haliplidae but present in all dytiscoid families except members of the dytiscid subfamily Hydroporinae (Alarie 1991). The pore ANg is inserted distally in Carabidae, Trachypachidae, and Gyrinidae. It is absent in Haliplidae and inserted proximally in Dytiscoidea except the dytiscid taxa Colymbetini, Eretini, and some Matinae (not studied for Meruidae) (Alarie 1998; Alarie *et al.* 2001, 2011a). The seta MX8 is inserted proximally on the second galeomere in Carabidae, Trachypachidae, Gyrinidae, and Haliplidae. It is inserted apically or subapically in Dytiscoidea, except in those taxa that lack a galea (Paelobiidae, Cybistrini, most Hydroporinae) in which MX8 is either absent or inserted on the stipes (Alarie 1991; Alarie *et al.* 2004, 2011a). The pore LAd, located on the first labial palpomere, is absent in Carabidae, Trachypachidae, Gyrinidae, and Haliplidae but present all along Dytiscoidea (not studied for Meruidae) except in members of the dytiscid tribe Vatellini (Michat & Torres 2005, 2011). Finally, the seta CO6, which is inserted submedially to proximally on the procoxa of Carabidae, Trachypachidae, Gyrinidae, and Haliplidae, articulates distally within the Dytiscoidea (not studied for Meruidae) except in members of the dytiscid subfamily Matinae (Alarie *et al.* 2001). Given the derived position of Dytiscidae within Dytiscoidea (e.g., Balke *et al.* 2005, 2008; Alarie *et al.* 2011b), the presumably plesiomorphic character states observed in different dytiscid groups can be considered as reversals.

The description of the larvae of *A. wrasei* provided in this paper reinforces the idea that *Aspidytes* possess a mixture of primitive character states. Indeed, larvae of both *A. niobe* and *A. wrasei* lack a trochanteral annulus, and have retained a reduced abdominal segment IX and a small retinaculum on the mandible. The presence of a retinaculum was postulated to be part of the ground plan condition of the adephagan larval mandible (Beutel 1993). On the other hand, a trochanteral annulus is lacking within Adephaga with the exception of Paelobiidae and Dytiscidae (Alarie *et al.* 2004). Alarie & Bilton (2005) also identified the presence of the primary seta UR9 and the primary pores URd–URg on the urogomphus as other plesiomorphic character states of *Aspidytes*, which presence in *A. wrasei* could not be confirmed owing to the lack of first instars.

As mentioned above, larvae of both *A. niobe* and *A. wrasei* have retained the abdominal segment IX, albeit in a reduced form (not seen in dorsal view in *A. wrasei* due to the development of a short siphon), which reinforces the argument that Aspidytidae could be sister to other Dytiscoidea. As suggested previously (Alarie & Bilton 2005; Alarie *et al.* 2011b), this condition would indicate a transitional stage between the fully developed abdominal segment IX found in Carabidae, Trachypachidae, Gyrinidae, Meruidae, and Haliplidae (the reduced abdominal segment IX in larvae of *Peltodytes* Régimbart, 1879 is considered as independently evolved) and its absence amongst other dytiscoid families. The absence of a complete and well delineated trochanteral annulus in *A. wrasei* reinforces also the hypothesis that the Aspidytidae represent a transitional stage from a fully terrestrial to a fully aquatic condition within the Dytiscoidea. Indeed, the absence of a trochanteral annulus separates Meruidae, Noteridae, Aspidytidae and Amphizoidae from Dytiscidae and Paelobiidae. It has been suggested that the presence of a trochanteral annulus represents an evolutionary novelty within Adephaga, likely to have a direct functional relationship to swimming ability in providing greater leg flexibility to an aquatic larva (Alarie *et al.* 2011b).

Whereas the characters of the larvae of *A. wrasei* described in this paper leave little doubt about a close phylogenetic relationship to *A. niobe*, several striking and unique larval features might suggest that *A. wrasei* represents a distinct lineage within the family Aspidytidae. Among these are the presence of a subrectangular nasale (subconical in *A. niobe*), the presence of 16 lamellae clypeales along the anterior margin of the nasale in instar III (six in *A. niobe*), the presence of a two-segmented galea (the first galeomere of *A. niobe* is not clearly differentiated from the stipes), the abdominal segments III-VII sclerotized dorsally and membranous ventrally (completely sclerotized in *A. niobe*), an abdominal segment VIII partly membranous ventrally and extending posteriorly into a short siphon (fully sclerotized and lacking a siphon in *A. niobe*), and the absence of an additional anterodistal pore on the tibia (present in *A. niobe*). These features allied to the different shapes of the cephalic capsule and last abdominal segment, the absence of egg bursters in instar II and some additional chaetotaxic characters may indicate that both species have a long history of independent evolution, a reasonable hypothesis given the marked disjunction between these two taxa. In absence of a more comprehensive analysis including adult and molecular characters, however, we prefer at this point not to make any changes, keeping both species within *Aspidytes*. A summary of the characters useful to distinguish *Aspidytes* species is presented in Table 1.

TABLE 1. Characters useful to distinguish larvae of the species of *Aspidytes*. The reader is referred to Alarie & Bilton (2005) and Balke *et al.* (2005) for figures regarding *A. niobe*.

Character	*A. niobe*	*A. wrasei*
Frontoclypeolabrum	Extended anteriorly into a short and narrow subconical nasale, more than 0.8 times as long as head length	Extended anteriorly into a short and broad subrectangular nasale (Fig. 1), less than 0.7 times as long as head length
Coronal suture	About 0.2 times as long as head length	About 0.3 times as long as head length (Fig. 1)
Antenna	More than 2.5 times longer than maxillary palpus	Less than 1.7 times longer than maxillary palpus
Apical lateroventral process of antennomere 3	Not prominent	Prominent, short, bulge-like (Fig. 4)
Maxillary palpomere 1	Subequal or longer than maxillary palpomere 2	Less than 0.75 times as long as maxillary palpomere 2
Maxillary palpomere 3	More than 1.5 times longer than maxillary palpomere 2	About as long as maxillary palpomere 2
Metathoracic leg	More than 2 mm long, more than 2.7 times longer than head width	Less than 2 mm long, about twice as long as head width
Abdominal segments III–VII	Completely sclerotized, ring-like	Membranous ventrally
Abdominal segment VIII	Subquadrate, not extended posteriorly into a short siphon	Subtrapezoidal, extended posteriorly into a short siphon (Fig. 13)
Urogomphus	Less than 2.8 times longer than last abdominal segment	More than 3.3 times longer than last abdominal segment
Anterior margin of nasale	With at most six lamellae clypeales	With 12–16 lamellae clypeales (Fig. 1)
Femur	Lacking secondary setae on posteroventral surface	With one secondary seta on posteroventral surface (Fig. 12)
Tibia	With one additional anterodistal pore	Lacking additional pores

Acknowledgements

We are grateful to R.G. Beutel (Institut für Spezielle Zoologie und Evolutionsbiologie, FSN Jena, Germany) for the SEM pictures of *A. wrasei*, and to two anonymous referees for their valuable comments on the manuscript. M.C. Michat was supported by project PIP 112–201101–01087 from CONICET. Financial support was also provided by the Natural Sciences and Engineering Research Council of Canada in the form of a discovery research grant to Y. Alarie. The work of J. Hájek was partly supported by the Ministry of Culture of the Czech Republic (DKRVO 2014/13, National Museum, 0002327201).

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