

Description of the third instar of *Hygrobia nigra* (Clark, 1862) (Coleoptera: Paelobiidae), with a key for the identification of mature larvae of *Hygrobia* Latreille, 1804 and phylogenetic analysis

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Abstract

The mature larva of the squeak beetle *Hygrobia nigra* (Clark, 1862) (Paelobiidae) is studied for the first time based on detailed descriptions and illustrations of selected structures, with special emphasis on morphometry and chaetotaxy. A key for the identification of mature larvae of four of the six species of *Hygrobia* Latreille, 1804 known worldwide is presented. The phylogenetic relationships of the species are analyzed based on a cladistic analysis of a combined data set including larval and adult characters. *Hygrobia nigra* shares with the other known species of the genus several larval apomorphies including the presence of paramedian lip-like lobes on the epipharynx, a well-developed gula, gills on thoracic and first three abdominal sterna, and the maxillary stipites inserted into submental pouches, and is unique in the presence of a larger number of secondary setae on the metacoxa. The presence of a compact group of minute sensilla in the place where the galea is commonly located suggests that members of *Hygrobia* lost the galea, a condition independently evolved in some dytiscid lineages. The Australian species form a well-supported clade characterized by the presence of a short nasale, fewer natatory setae on the metatibia, and a marked shortening of the antennal sensorial appendage and the last abdominal segment. However, no larval characters were discovered to resolve relationships within that clade. The Palearctic *H. hermanni* (Fabricius, 1775) lacks a distinct nasale and is resolved as sister to the clade formed by the Australian species.

Key words: Coleoptera, Paelobiidae, *Hygrobia*, mature larva, morphometry, chaetotaxy, phylogenetic relationships

Introduction

The monotypic family Paelobiidae is a small group of aquatic coleopterans commonly known as squeak beetles (Alarie *et al.* 2004; Hawlitschek *et al.* 2012). All species occur in lowland areas and live in the mud, silt, and detritus of ponds (Dettner 2005). Both adults and larvae are predators, specialized on oligochaet worms, though adults were also observed feeding on chironomid larvae (Balfour-Browne 1922). *Hygrobia* Latreille, 1804, the only genus within the family, is comprised of six species worldwide (Nilsson 2005): *H. hermanni* (Fabricius, 1775) relatively common and widespread in the Western Palearctic, *H. davidi* Bedel, 1883 only found in Southeastern China, and *H. wattsi* Hendrich, 2001, *H. australasiae* (Clark, 1862), *H. maculata* Britton, 1981 and *H. nigra* (Clark, 1862) endemic to Australia (Britton 1981; Hendrich 2001; Hawlitschek *et al.* 2012).

In terms of phylogenetic relationships, there is a general agreement that Paelobiidae is part of the adephagan clade Dytiscoidea along with Dytiscidae, Aspidytidae and Amphizoidae, and within this grouping it has been hypothesized to share a sister group relationship with Dytiscidae (Ruhnau 1986; Beutel & Haas 1996; Shull *et al.* 2001; Ribera *et al.* 2002; Alarie & Bilton 2005, Alarie *et al.* 2011) or with a clade formed by the families Dytiscidae, Aspidytidae and Amphizoidae (Balke *et al.* 2005; Hawlitschek *et al.* 2012). With respect to the relationships among the species of *Hygrobia*, a recent phylogenetic analysis based on adult morphological and molecular characters suggested a sister group relationship between *H. hermanni* and a clade formed by the Australian species, with *H. nigra* sister to *H. australasiae* (Hawlitschek *et al.* 2012).

Descriptions or treatments of larvae of Paelobiidae have been presented by several authors (Bertrand 1928, 1972; Klausnitzer 1991; Dettner 1997, 2005; Witchard *et al.* 2002; Alarie *et al.* 2004). Except for the recent paper of Alarie *et al.* (2004), however, all these contributions were deemed to be rather superficial, lacking descriptive detail, and not emphasizing chaetotaxic analysis, which has proven to be a source of useful characters to test phylogenetic relationships within Adephaga (e.g., Alarie & Bilton 2005; Michat & Torres 2009; Alarie *et al.* 2011). At the present time the larvae of three species of *Hygrobia* are described (*H. hermanni*, *H. australasiae* and *H. wattsi*). The recent discovery in Southeastern Australia of the mature larva of *H. nigra* provided the impetus for this study which aims to broaden our knowledge of the larval morphology of the family Paelobiidae. This paper therefore has the following goals: (1) to describe and illustrate in detail, for the first time, the mature larva of *H. nigra* with an emphasis on morphometry and chaetotaxy of selected structures; (2) to provide a key for the identification of the known mature larvae of this genus; and (3) to explore the phylogenetic relationships among the species based on a cladistic approach combining larval and adult characters.

Material and methods

Larvae examined, preparation and description. The description provided in this paper is based on one instar III specimen collected at the following locality: Australia: S QLD, Bundaberg reg., 2 km west of Woodgate, swamp, 33m, 27.IX.2006, 25°7'32.5"S 152°30'27.0"E, L. & E. Hendrich leg. (QLD 57). The only species of *Hygrobia* known to occur in the coastal area where the larva was collected is *H. nigra*. A second instar III larva collected only 50 km south of the place of collection of the studied larva was identified as *H. nigra* based on DNA analysis (Hawlitschek *et al.* 2012). Both larvae had the same size and a similar, almost black colour when alive, thus differing from the larvae of *H. australasiae* which are brownish or yellowish when alive. On the other hand, the DNA results from adults of *H. maculata* do not match with those of the *H. nigra* larva studied by Hawlitschek *et al.* (2012).

The larva was 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. The material is held in the larval collection of Yves Alarie (Laurentian University, Sudbury, Ontario, Canada).

The methods and terms used herein follow those employed in previous papers dealing with the larval morphology and chaetotaxy of the family Paelobiidae. The reader is referred to Alarie *et al.* (2004) for a complete list and additional explanations of the terms used in the present study. Whereas represented by instar III only, primary sensilla of *H. nigra* were identified by comparison with the *Hygrobia* groundplan wherever possible (e.g., head appendages and legs) (Alarie *et al.* 2004). In these cases, homologies were recognized using the criterion of similarity of position (Wiley 1981).

Phylogenetic analysis. The phylogenetic relationships of *H. nigra* within the genus *Hygrobia* were analyzed cladistically using the program TNT (Goloboff *et al.* 2008). The analysis was restricted to characters of instar III because instars I and II of *H. nigra* remain unknown. The analysis thus included four out of six known species, with only *H. davidi* and *H. maculata* excluded. A close relationship between the families Paelobiidae and Dytiscidae was suggested based on adult and larval characters (Ruhnau 1986; Beutel & Haas 1996; Shull *et al.* 2001; Ribera *et al.* 2002; Alarie & Bilton 2005, Alarie *et al.* 2011). For this reason, members of the genera *Matus* Aubé, 1836 and *Copelatus* Erichson, 1832, two presumably ancestral lineages within Dytiscidae according to different authors (e.g., Ruhnau & Brancucci 1984; Beutel 1994; Miller 2001), were included as outgroups, with *Matus* used to root the tree. Due to the fact that characters provided by the third-instar larval morphology are too scarce to resolve relationships within *Hygrobia*, characters provided by adult morphology were also used, with the objective of obtaining a more robust analysis. Adult characters were taken from a recent paper dealing with Paelobiidae phylogeny (Hawlitschek *et al.* 2012). Whereas the morphological characters from larvae and adults were discretized (discrete data matrix), the morphometric characters from larvae were coded as continuous (continuous data matrix). It has been suggested that continuous characters carry phylogenetic information and that inclusion of these characters is likely to improve resolution and support of the phylogeny under study (Goloboff *et al.* 2006). For the present analysis, both continuous and discrete characters were put together in a single matrix. All

characters were treated as equally weighted. Multistate discrete characters were treated as nonadditive, and continuous characters as additive. A heuristic search was implemented using ‘tree bisection reconnection’ as algorithm, with 200 replicates and saving 100 trees per replication (previously setting ‘hold 20000’). Jackknife values were calculated with 2 000 replicates and P (removal probability) = 36.

Results

Description of the third instar of *Hygrobia nigra* (Clark, 1862)

Alarie *et al.* (2004) provided a detailed study of the larvae of the genus *Hygrobia* based on the description of *H. hermanni*, *H. australasiae* and *H. wattsi*. For this reason, characters common to all described species are not included in the present paper and only diagnostic or interesting features are mentioned.

Diagnosis. The third instar of *H. nigra* can be distinguished from those of the other known species of *Hygrobia* by the following combination of characters: HW: 2.71 mm; nasale present (Fig. 1); A3' shorter than A4 (Figs. 5–6); coxa with more than 50 secondary setae (Table 1; Figs 12–15); LAS < 1.70 times HL; urogomphus longer than last abdominal segment (Fig. 17).

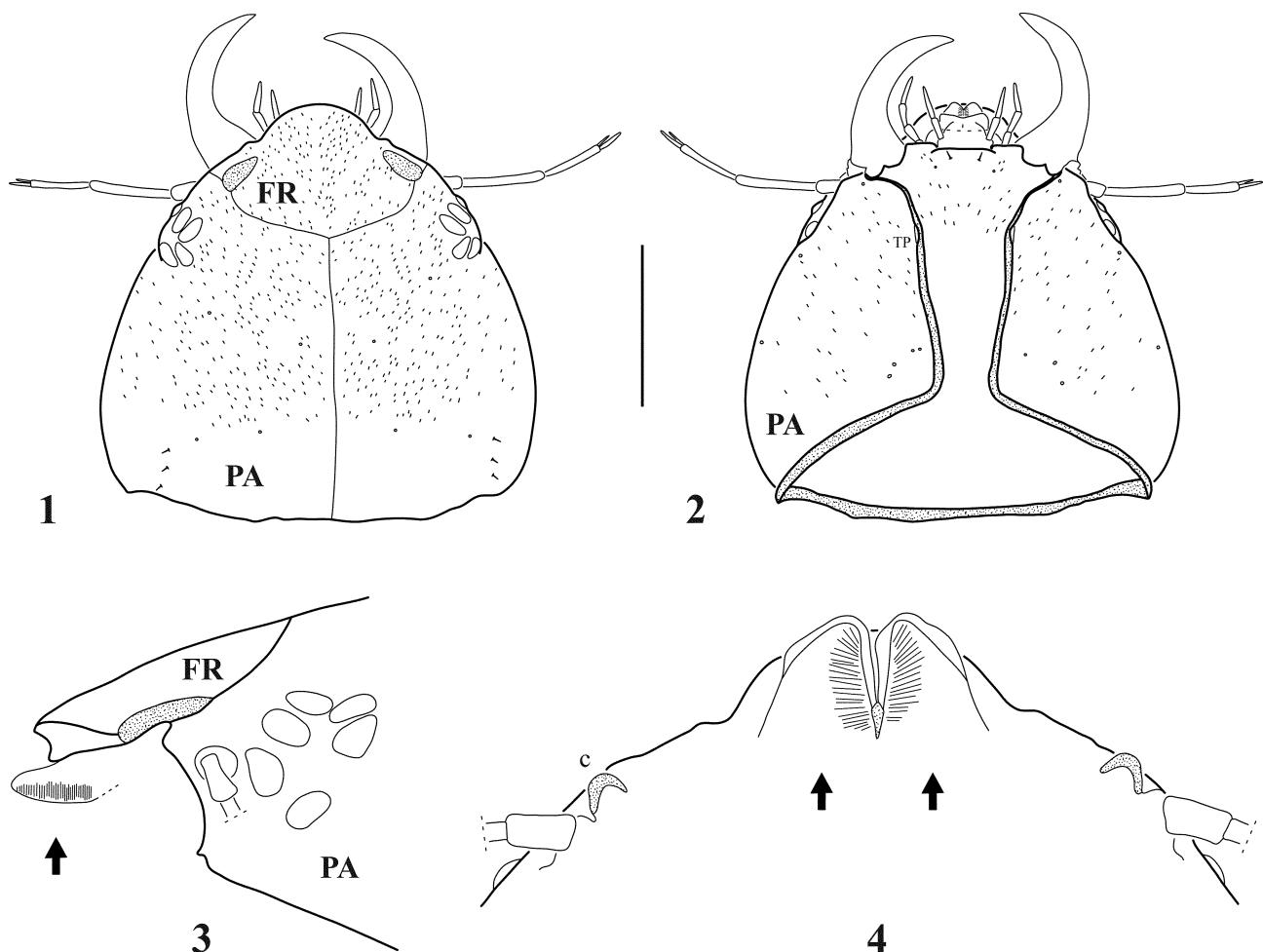
TABLE 1. Number and position of secondary setae on the legs of larvae of *Hygrobia nigra*. Numbers between slash marks refer to pro-, meso- and metathoracic leg, respectively. A = anterior, D = dorsal, NS = natatory setae, P = posterior, Pr = proximal, V = ventral, Total = total number of secondary setae on the article (including additional and natatory setae).

| Article | Position | Instar III (n = 1) |
|------------|----------|-----------------------------|
| Coxa | A | 18 / 20–23 / 18–20 |
| | D | 28–32 / 42–48 / 53–58 |
| | V | 8 / 5 / 3–4 |
| | Total | 54–58 / 70–73 / 74–82 |
| Trochanter | Di | 2–3 / 3 / 2 |
| | Pr | 1–2 / 1–2 / 2–3 |
| | Total | 4 / 4–5 / 4–5 |
| Femur | AV | 19–20 / 19–20 / 21 |
| | PV | 0–1 / 0 / 0–1 |
| | Total | 20 / 19–20 / 21–22 |
| Tibia | AV | 20–24 / 23–25 / 21–24 |
| | PD (NS) | 142–144 / 118–122 / 99–103 |
| | PV | 1 / 1 / 1–2 |
| Tarsus | Total | 163–169 / 144–146 / 122–128 |
| | PD (NS) | 70–75 / 67–68 / 63–64 |
| | V | 10–12 / 9–10 / 11 |
| | Total | 80–87 / 77 / 74–75 |

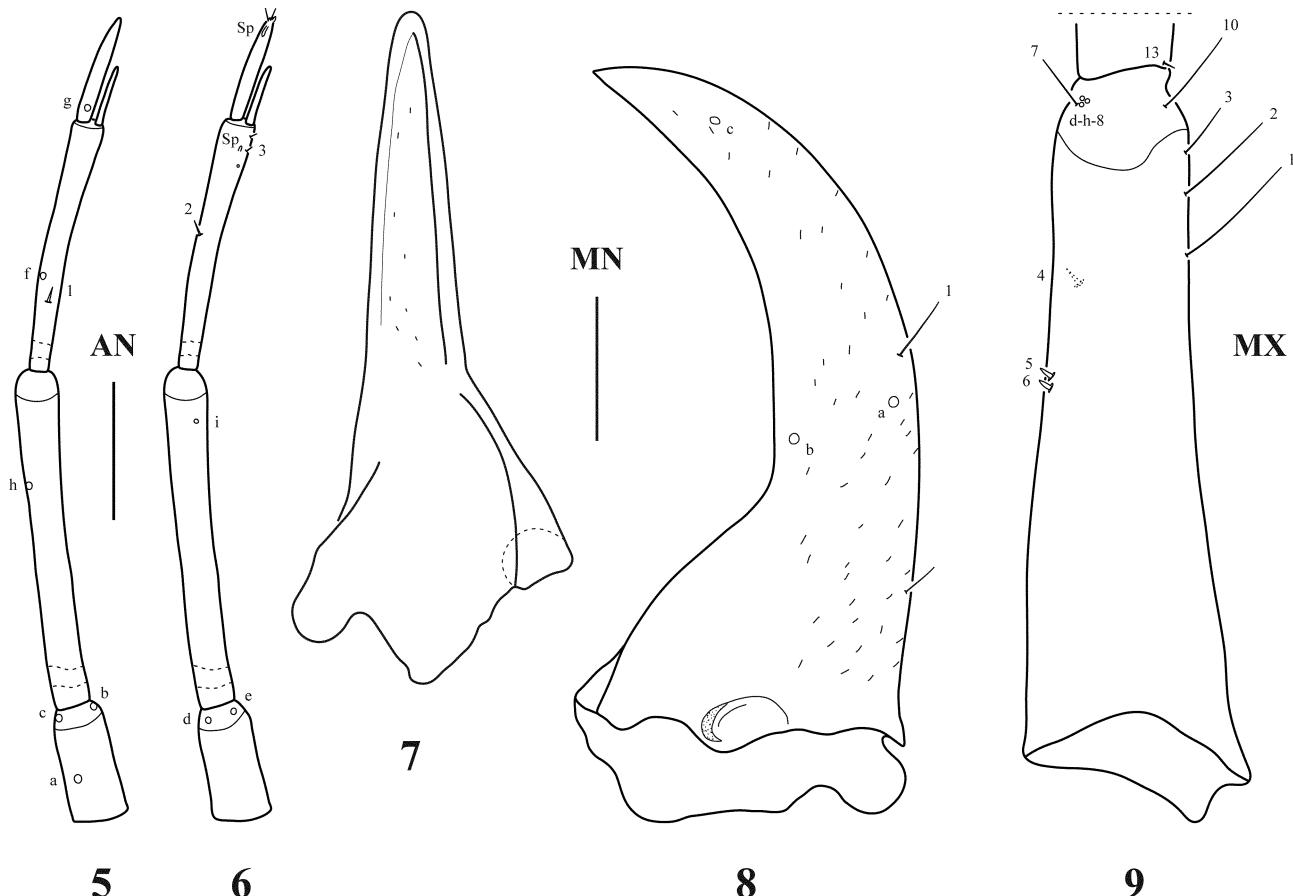
Description (Figs. 1–17). Colour. Cephalic capsule uniformly brown dorsally, somewhat lighter ventrally; head appendages testaceous except for brown mandible; thoracic and abdominal sclerites brown except for posterior part of pronotum testaceous, middle part of abdominal tergites IV–V somewhat darker, and last abdominal segment (excluding siphon) dark brown; basal third of siphon dark brown, distal two thirds light brown; membranous parts testaceous; legs light brown, coxae slightly darker than remaining articles; basal third of urogomphus light brown, distal two thirds testaceous.

Body. Bent at level of abdominal segments I–II, somewhat gibbous in lateral view. TL: 15.00 mm; MW: 3.50 mm.

Head (Figs 1–4). HL: 2.60 mm; HW: 2.71 mm; FRL: 0.83 mm; OCW: 2.23 mm. *Cephalic capsule*. Slightly broader than long (HL/HW: 0.96); occipital foramen well developed (HW/OCW: 1.22); coronal suture long, 0.68 times as long as head length; frontoclypeolabrum short, with a lightly sclerotized area on each side, 0.32 times as long as head length, extended anteriorly into a short nasale; lateral lobes of frontoclypeolabrum slightly evident (Fig. 1); epipharyngeal lobes well developed, projected forward slightly beyond anterior margin of nasale (Figs. 3–4); ocularium as in Fig. 3; parietals lacking lateral horizontal keel; gula very broad and lightly sclerotized, continuous with submentum (Figs. 2, 10–11). *Antenna* (Figs. 5–6). Shorter than head width (A/HW: 0.45); A2 longest, A3 somewhat shorter than A2 (A3/A2: 0.76), A1 and A4 shortest, subequal (A1/A3: 0.44); sensorial appendage of A3 shorter than A4 (A3'/A4: 0.59); A2 and A3 fractured basally. *Mandible* (Figs. 7–8). Acute apically, very broad basally, 2.22 times longer than broad, 0.39 times as long as head length; condyle well-developed; mesal margin not toothed, with well-developed dorsal cutting edge and slightly insinuated ventral cutting edge (Fig. 7); mandibular channel absent. *Maxilla* (Figs. 9–11). Cardo fused to stipes; stipes almost entirely inserted in a membranous pouch of submentum (Figs. 10–11), palpus free; palpifer vestigial, not differentiated from stipes; palpus shorter than antenna (A/MP: 2.64); MP3 longest, MP2 slightly shorter than MP3 (MP3/MP2: 1.27), MP1 slightly shorter than MP2 (MP2/MP1: 1.15). *Labium* (Figs. 10–11). Well developed, ventrally entirely visible (Fig. 11), dorsally closely associated with (and almost entirely concealed by) hypopharynx (Fig. 10); submentum strongly expanded, covering maxillary stipites and mentum; prementum and to a lesser degree mentum completely sclerotized ventrally and laterally; ligula strongly developed (Figs. 10–11); palpifer absent; labial palpus shorter than maxillary palpus (MP/LP: 1.29); LP2 1.35 times longer than LP1. *Hypopharynx* (Fig. 10). Strongly developed, broad, sclerotized dorsally, with distinct dorsointernal sclerotized sagittal process.



FIGURES 1–4. *Hygrobia nigra*, third-instar larva. 1, head, dorsal aspect; 2, head, ventral aspect; 3, anterior portion of head with mouth parts removed, lateral aspect; 4, epipharyngeal lobes, ventral aspect. Arrows indicate epipharyngeal lobes. c: anterior (dorsal) articulation of mandible; FR: frontoclypeus; PA: parietal; TP: tentorial pit. Scale bar = 1.00 mm.

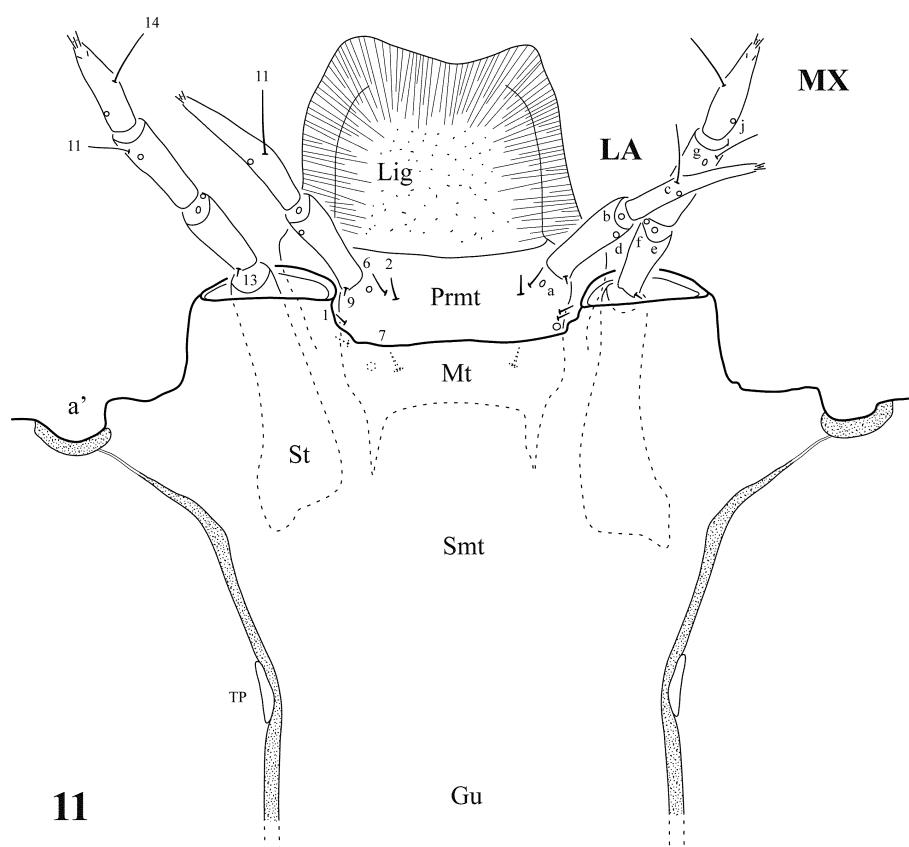
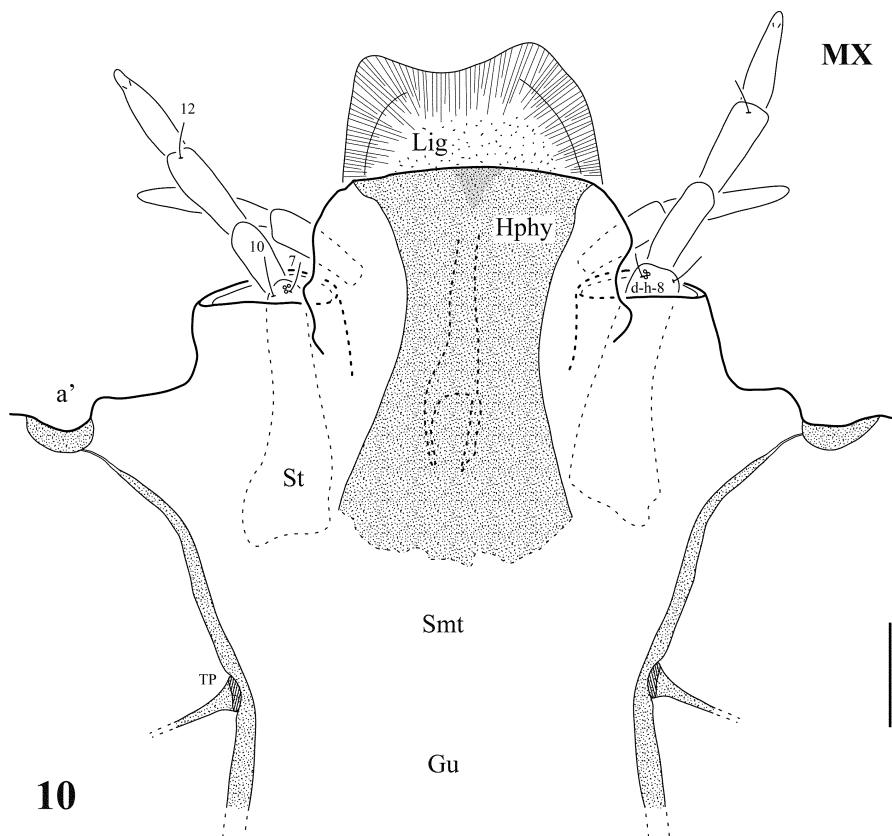


FIGURES 5–9. *Hygrobia nigra*, third-instar larva. 5, left antenna, dorsal aspect; 6, right antenna, ventral aspect; 7, right mandible, mesal aspect; 8, right mandible, dorsal aspect; 9, right stipes, dorsal aspect. Numbers and lowercase letters indicate primary setae and pores respectively. Additional and secondary setae and pores not labeled. AN: antenna; MN: mandible; MX: maxilla; Sp: spinula. Scale bars = 0.20 mm.

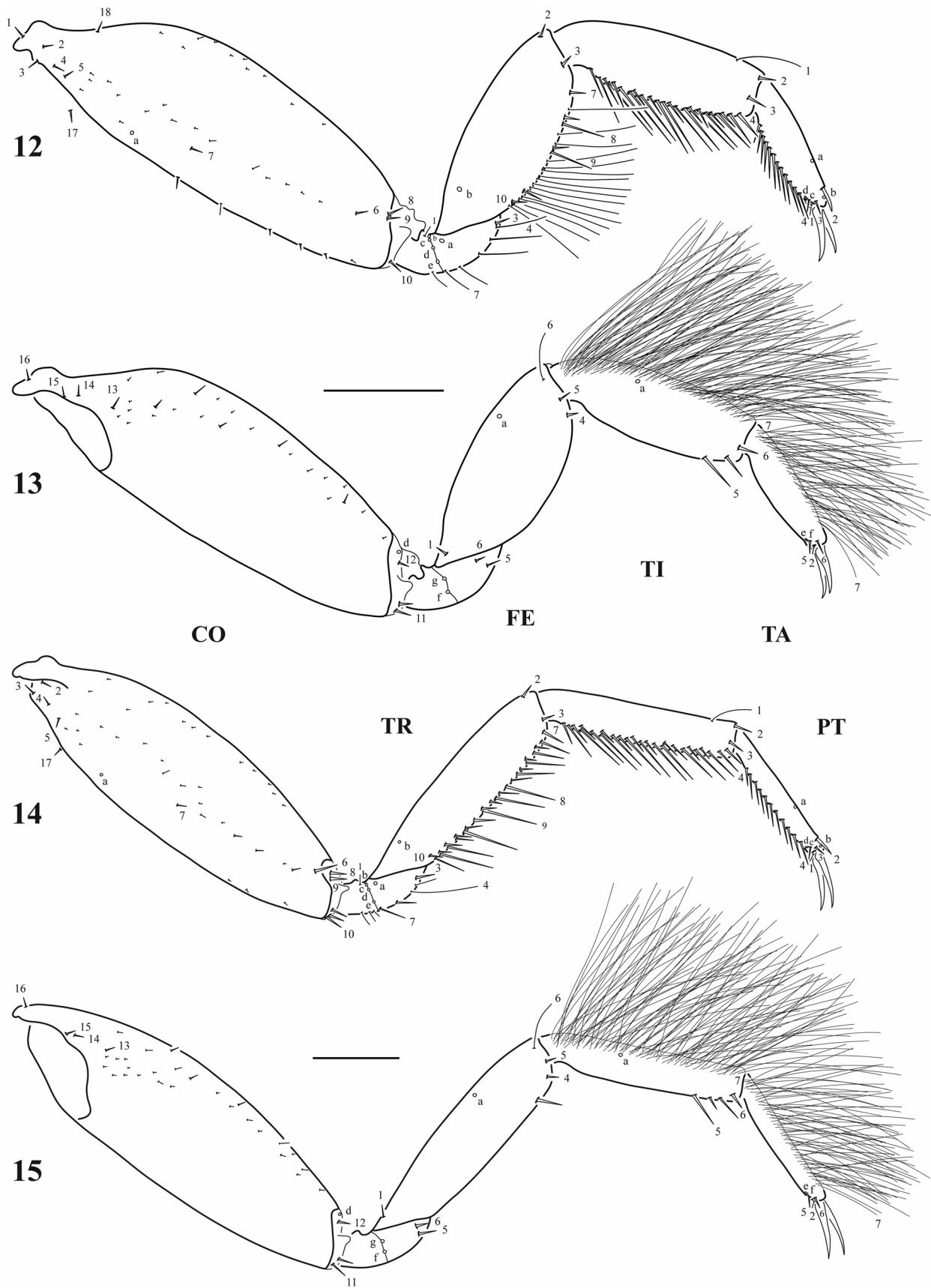
Thorax. Meso- and metathoracic tergites with anterior transverse carina; paired gills present ventrally on three segments, near coxal insertions; spiracles present on mesothorax. Legs (Figs. 12–15). Metathoracic leg longest (L3/HW: 1.89) (Figs. 14–15); mesothoracic leg somewhat shorter than metathoracic leg (L3/L2: 1.11); prothoracic leg shortest (L3/L1: 1.34) (Figs. 12–13); total length of L1–L2–L3: 3.82 mm, 4.63 mm, 5.13 mm, respectively. Coxa longest, relative length of remaining segments on metathoracic leg: TR/CO: 0.11, FE/CO: 0.63, TI/CO: 0.51, TA/CO: 0.36; longest metathoracic claw 0.43 times as long as metatarsus; relative length of remaining segments on prothoracic leg: TR/CO: 0.11, FE/CO: 0.52, TI/CO: 0.49, TA/CO: 0.31; longest prothoracic claw 0.41 times as long as protarsus.

Abdomen (Figs. 16–17). Segments I–VII sclerotized dorsally (Fig. 16); segments I–III membranous and bearing paired gills ventrally; segments IV–V each with a single large ventral sclerite independent from dorsal sclerite; segments VI–VII almost completely sclerotized ventrally, with ventral and dorsal sclerites separated by a thin membranous band; spiracles present on segments I–VII; segment VIII completely sclerotized, ring-like (LAS: 3.75 mm); sclerites I–VIII with anterior transverse carina; siphon very long, urogomphomere-like (LAS/HW: 1.44) (Fig. 17). *Urogomphus* (Fig. 17). Very long (total length: 5.40 mm), 1.44 times longer than last abdominal segment, 1.99 times longer than head width.

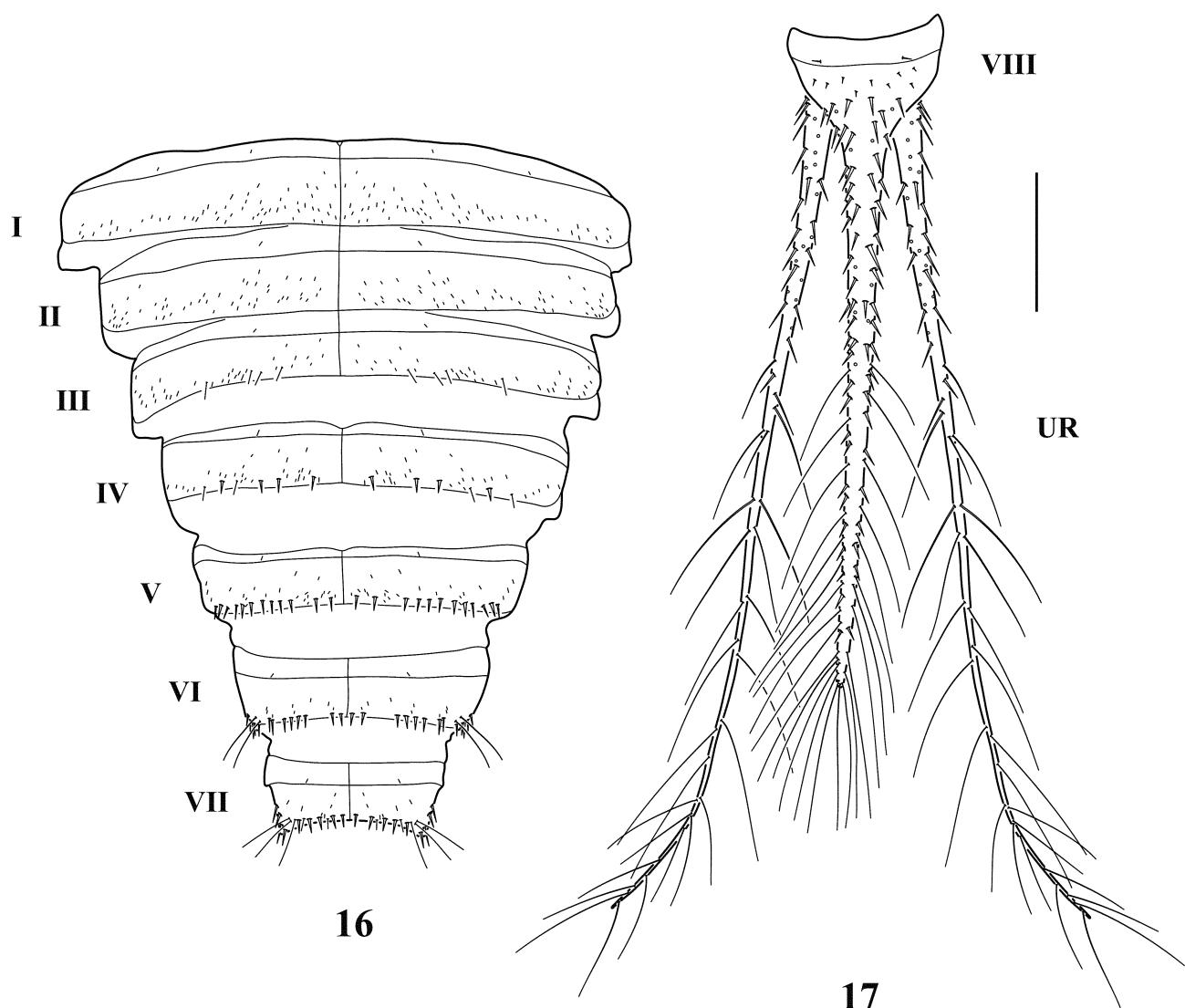
Chaetotaxy (Figs. 1–2, 5–17). Cephalic capsule with numerous secondary setae both dorsally and ventrally (Figs. 1–2); antenna bearing all primary sensilla and two structures (one minute seta and probably one minute pore) on ventroapical surface of A3 (Fig. 6); mandible with numerous secondary setae both dorsally and ventrally (Figs. 7–8); we were unable to find primary pore MNd; maxilla bearing all primary sensilla except pore MXi (we were unable to find it) (Figs. 9–11); galeal sensilla present on stipes in position where galea commonly located (Figs. 9–10); labium bearing all primary sensilla plus one seta and one pore on lateral margin of prementum being probably secondary structures (Fig. 11); thoracic tergites bearing numerous minute and some hair-like setae; legs



FIGURES 10–11. *Hygrobia nigra*, third-instar larva. 10, maxillae, labium and hypopharynx, dorsal aspect; 11, maxillae and labium, ventral aspect. Numbers and lowercase letters indicate primary setae and pores respectively. Additional and secondary setae and pores not labeled. a': posterior (ventral) articulation of mandible; Gu: gula; Hphy: hypopharynx; LA: labium; Lig: ligula; Mt: mentum; MX: maxilla; Prmt: prementum; Smt: submentum; St: stipes; TP, tentorial pit. Scale bar = 0.20 mm.



FIGURES 12–15. *Hygrobia nigra*, third-instar larva. 12, left prothoracic leg, anterior aspect; 13, right prothoracic leg, posterior aspect; 14, left metathoracic leg, anterior aspect; 15, right metathoracic leg, posterior aspect. Numbers and lowercase letters indicate primary setae and pores respectively. Additional and secondary setae and pores not labeled. CO: coxa; FE: femur; PT: pretarsus; TA: tarsus; TI: tibia; TR: trochanter. Scale bars = 0.40 mm.



FIGURES 16–17. *Hygrobia nigra*, third-instar larva. 16, abdominal segments I–VII, dorsal aspect; 17, abdominal segment VIII and urogomphi, dorsal aspect. Scale bar = 0.80 mm.

bearing all primary sensilla (we were unable to find seta CO1 on metathoracic leg) (Figs. 12–15); coxa with numerous secondary setae; trochanter with some secondary setae on ventral surface; femur, tibia and tarsus with a dense row of secondary setae on ventral surface (Table 1; Figs. 12, 14); tibia and tarsus bearing a dense and compound row of natatory setae on posterodorsal surface (Figs. 13, 15); abdominal tergites I–III bearing numerous minute and some hair-like setae on disc and posterior margin; abdominal tergites IV–VII bearing a row of spine-like setae on posterior margin and several minute and hair-like setae (Fig. 16); abdominal segment VIII with numerous spine-like and hair-like setae (Fig. 17); urogomphus with several spine-like setae on basal third and numerous hair-like setae on distal two thirds (Fig. 17).

Habitat. The single larva was collected in a half-shaded and peaty roadside swamp, rich in floating *Nymphaea* plants, among rotten leaves of *Baumea* sp. at a depth of 50 cm (Fig. 18). It was associated with adults of *Antiporus wilsoni* Watts, 1978, *Hyphydrus decemmaculatus* Wehncke, 1877, *Limbodessus compactus* (Clark, 1862), *Sternopriscus clavatus* Sharp, 1882, *S. tarsalis* Sharp, 1882 (all Dytiscidae) and *Hydrocanthus australasiae* Wehncke, 1876 (Noteridae).



FIGURE 18. Habitat of *Hygrobia nigra*, roadside swamp near Woodgate, Bundaberg Region, South Queensland, Australia.

Key to the known third-instar larvae of *Hygrobia*

The reader is referred to Alarie *et al.* (2004) for figures regarding *H. australasiae*, *H. hermanni* and *H. wattsi*.

1. Frontoclypeolabrum broadly rounded, not distinctly produced into a nasale; ratio A3'/A4 > 0.80; ratio LAS/LH > 1.90; ratio U/LAS < 0.80; meso- and metatibial natatory setae more abundant (> 135 and > 130 respectively) *H. hermanni*
- Frontoclypeolabrum produced into a short nasale; ratio A3'/A4 < 0.80; ratio LAS/LH < 1.70; ratio U/LAS > 0.80; meso- and metatibial natatory setae less abundant (< 125 and < 105 respectively) 2
2. Meso- and metacoxa with 1 ventral secondary seta; protibia with more than 35 anteroventral secondary setae *H. wattsi*
- Meso- and metacoxa with more than 3 ventral secondary setae; protibia with less than 25 anteroventral secondary setae 3
3. Urogomphi longer (total length = 5.40 mm), more than 1.40 times LAS (Fig. 17); coxae with a larger number of dorsal secondary setae (> 25, > 40 and > 50 respectively) (Figs. 12–15); ratio A3'/A4 > 0.50 *H. nigra*
- Urogomphi shorter (total length = 5.08 mm), less than 1.30 times LAS; coxae with a lesser number of dorsal secondary setae (< 20, < 20 and < 25 respectively); ratio A3'/A4 < 0.50 *H. australasiae*

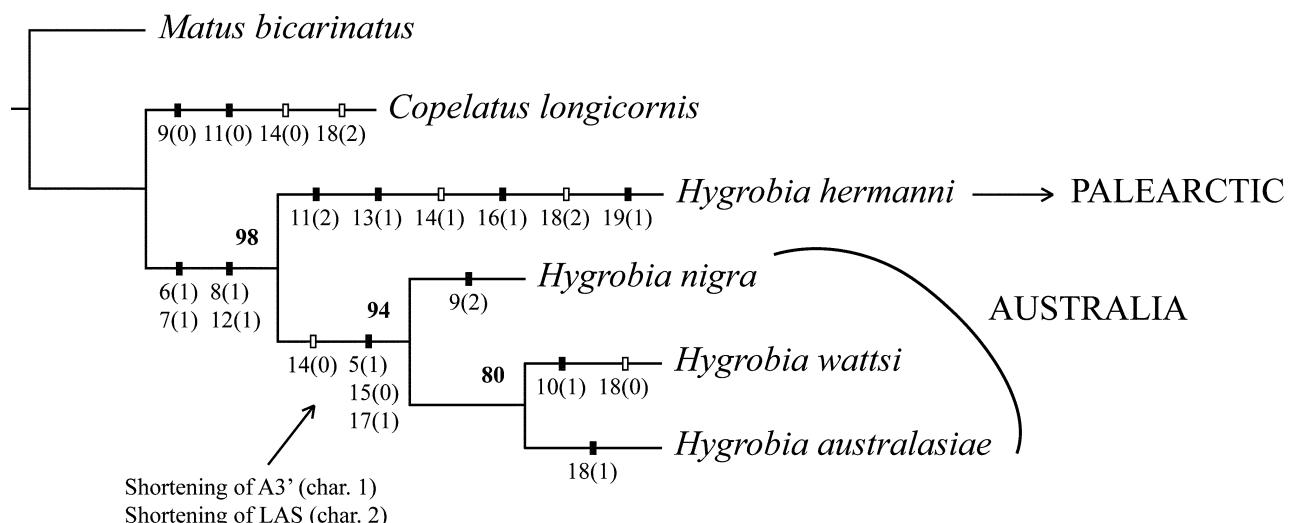


FIGURE 19. Single most parsimonious cladogram with discrete character changes mapped for each clade. Solid rectangles indicate unique character state transformations; open rectangles indicate homoplasious character state transformations. Jackknife values are indicated above branches.

Character analysis

In total, 20 characters (13 from larvae and seven from adults, Table 2) were included in our analysis, of which five were coded as continuous (characters 0–4, Table 3) and 15 as discrete (characters 5–19, Table 4). Within the discrete characters, 10 were coded as binary and five as multistate. The analysis of the combined dataset using TNT resulted in a single most parsimonious tree of 31.74 steps (CI = 0.89; RI = 0.80) (Fig. 19). The support obtained for the *Hygrobia* clade and its internal nodes was robust.

TABLE 2. Continuous and discrete characters from larvae and adults used in the cladistic analysis.

| No. | Character | Cont./disc. | Stage |
|-----|--|-------------|-------|
| 0 | Head length (mm) | Continuous | Larva |
| 1 | Ratio A3'/A4 | Continuous | Larva |
| 2 | Length of LAS (mm) | Continuous | Larva |
| 3 | Ratio LAS/HL | Continuous | Larva |
| 4 | Ratio U/LAS | Continuous | Larva |
| 5 | Nasale: (0) absent; (1) present | Discrete | Larva |
| 6 | Paramedian lip-like lobes on the epipharynx: (0) absent; (1) present | Discrete | Larva |
| 7 | Maxillary stipites: (0) not inserted into submental pouches; (1) inserted into submental pouches | Discrete | Larva |
| 8 | Gula: (0) absent; (1) present | Discrete | Larva |
| 9 | Number of secondary setae on the metacoxa: (0) less than 10; (1) 10–60; (2) more than 70 | Discrete | Larva |
| 10 | Secondary setae on the posteroventral surface of the femur: (0) present; (1) absent | Discrete | Larva |
| 11 | Natatory setae on the metatibia: (0) absent; (1) less than 110; (2) more than 130 | Discrete | Larva |
| 12 | Paired gills on thoracic and first three abdominal sterna: (0) absent; (1) present | Discrete | Larva |
| 13 | Clypeal suture: (0) faint to almost invisible; (1) well visible, strongly impressed | Discrete | Adult |
| 14 | Clypeus, shape: (0) anterior angles not significantly produced forward; (1) anterior angles produced forward into a triangular extension | Discrete | Adult |
| 15 | Labrum, shape: (0) foremargin straight; (1) foremargin concave | Discrete | Adult |
| 16 | Labrum, surface: (0) smooth; (1) with large, coarse punctures; (2) with dense, but not coarse puncturation | Discrete | Adult |
| 17 | Hind margin of pronotum in dorsal view: (0) rather straight; (1) curved on both sides; (2) triangular in the middle | Discrete | Adult |
| 18 | Protarsomeres 1–3 of male: (0) not dilated, ventrally only with short setae; (1) slightly dilated, ventrally with long adhesive setae; (2) more strongly dilated, ventrally with long adhesive setae | Discrete | Adult |
| 19 | Claws: (0) longish, curved; (1) shorter, strongly curved | Discrete | Adult |

TABLE 3. Data matrix of continuous characters from larvae used in the cladistic analysis. Missing data coded ‘?’

| Species | Character | | | | |
|------------------------------|-----------|-----------|-----------|-----------|-----------|
| | 0 | 1 | 2 | 3 | 4 |
| <i>Matus bicarinatus</i> | 1.38–1.39 | 0.45–0.51 | 2.13–2.20 | 1.57 | 0.26–0.28 |
| <i>Copelatus alternatus</i> | 0.90–0.93 | 0.33–0.42 | 0.62–0.64 | 0.69 | 0.43–0.44 |
| <i>Hygrobia australasiae</i> | 2.80 | 0.39 | 3.88 | 1.38 | 1.31 |
| <i>Hygrobia hermanni</i> | 2.60–2.68 | 0.75–0.82 | 6.05–6.13 | 2.26–2.36 | 0.74–0.75 |
| <i>Hygrobia nigra</i> | 2.60 | 0.59 | 3.75 | 1.44 | 1.44 |
| <i>Hygrobia wattsi</i> | 2.70 | ? | 3.10 | 1.15 | ? |

TABLE 4. Data matrix of discrete characters from larvae (char. 5–12) and adults (char. 13–19) used in the cladistic analysis. Missing data coded ‘?’

| Species | Character | | | | | | | | | | | | | | |
|------------------------------|-----------|---|---|---|-----|-----|----|----|----|----|----|----|----|----|----|
| | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| <i>Matus bicarinatus</i> | 0 | 0 | 0 | 0 | 1–2 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| <i>Copelatus alternatus</i> | 0 | 0 | 0 | 0 | 0 | 0–1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 |
| <i>Hygrobia australasiae</i> | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Hygrobia hermanni</i> | 0 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 1 |
| <i>Hygrobia nigra</i> | 1 | 1 | 1 | 1 | 2 | 0–1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | ? | ? |
| <i>Hygrobia wattsi</i> | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |

Discussion

All previous studies have supported the monophyly of Paelobiidae (e.g., Beutel 1986; Beutel & Haas 1996; Ribera *et al.* 2002; Alarie *et al.* 2004). Indeed, larvae of *H. nigra* share with the other known species of the genus a remarkably high number of apomorphies including the presence of a well-developed gula (character 8.1), the maxillary stipites inserted into submental pouches (character 7.1), the absence of a galea, the presence of a strongly developed ligula, the presence of paramedian lip-like lobes on the epipharynx (character 6.1), and the presence of paired gills on the three thoracic and the first three abdominal sterna (character 12.1) (see also characters 10–29 in Alarie *et al.* 2004, musculature characters not analyzed). Several of these characters are likely adaptations to the particular feeding and breathing habits of the larvae, which are specialized on oligochaet worms that live in the muddy bottom of ponds (Balfour-Browne 1922).

Mature larvae of *Hygrobia* appear to be morphologically quite homogeneous (Alarie *et al.* 2004; this study). As this study focuses on third instar only, it is therefore not surprising that only few characters of phylogenetic importance were discovered to help in resolving interspecific relationships within the genus. Also, the unavailability of first instars of *H. nigra* prevented the comparison of several morphological and primary chaetotaxic characters that are commonly helpful in the separation of species. As a consequence, the results of this study would benefit from being tested in future, more comprehensive analyses including more characters and all larval instars.

According to our results, the Palearctic *H. hermanni* is resolved as the sister group of a clade formed by the Australian species. In fact, the clade formed by *H. nigra*, *H. wattsi* and *H. australasiae* is characterized by the presence of a short but distinct nasale (character 5.1) and the presence of a lower number of natatory setae on the metatibia (character 11.1). Also, this clade is distinguished by a marked shortening of both the sensorial appendage of third antennomere (character 1) and the last abdominal segment (character 2) (Fig. 19). The peculiar shape of the mandible in the first instar (bluntly rounded apically; see Alarie *et al.* 2004) is another putative synapomorphy for this clade, but the character could not be evaluated in *H. nigra* due to lack of first instars. This result is in agreement with a previous hypothesis based mainly on DNA sequence data (Hawlitschek *et al.* 2012) that postulated a monophyletic origin of the Australian species. Britton (1981) had already suggested, based on adult morphology, that the Australian species may be closely related and may have diverged more recently. *Hygrobia hermanni* bears a higher number of natatory setae on the metatibia (character 11.2) and lacks a distinct nasale (character 5.0).

A second branching separates *H. nigra* from a clade formed by *H. wattsi* and *H. australasiae*. A closer relationship between *H. nigra* and *H. australasiae* would have been expected because both species were recovered as sister in a recent molecular phylogeny of the group (Hawlitschek *et al.* 2012). It is worth mentioning that the lack of characters in support of this clade in our analysis does not allow making robust conclusions. *Hygrobia nigra* is unique among the species studied in the presence of a large number of secondary setae on the metacoxa (character 9.2).

Larvae of Adephaga are generally characterised by the presence of a galea, with two primary setae (MX8, MX9) and two primary pores (MXd, MXh), which are deemed to be part of the ground-plan condition of the

suborder (Alarie *et al.* 2004; Alarie & Bilton 2005). The galea is lacking within *Hygrobiidae* (Alarie *et al.* 2004). However, the presence of a compact group of minute sensilla in the place where the galea is commonly located (Figs. 9–10) is suggestive that the galea is lost. Such a loss is a likely explanation given the derived condition of this family within Adephaga (Ruhnau 1986; Beutel & Haas 1996; Shull *et al.* 2001; Ribera *et al.* 2002; Balke *et al.* 2005; Alarie & Bilton 2005, Alarie *et al.* 2011; Hawlitschek *et al.* 2012). Other adephagan groups in which the larvae lack a galea are the diving beetle subfamily Hydroporinae (most of the species) (Alarie & Michat 2007; Michat *et al.* 2007) and the tribe Cybistrini (Michat 2006, 2010). The absence of a galea in these three groups is most likely the result of independent evolution.

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