Higher-level phylogeny of diving beetles (Coleoptera: Dytiscidae) based on larval characters

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Abstract. A comprehensive higher-level phylogeny of diving beetles (Dytiscidae) based on larval characters is presented. Larval morphology and chaetotaxy of a broad range of genera and species was studied, covering all currently recognized subfamilies and tribes except for the small and geographically restricted Hydrodytinae, where the larva is unknown. The results suggest several significant conclusions with respect to the systematics of Dytiscidae including the following: monophyly of all currently recognized subfamilies, although Dytiscinae when considered in a broad context is rendered paraphyletic by Cybistrinae; currently recognized tribes are monophyletic except for Agabini, Hydroporini and Laccornellini; inter-subfamily and inter-tribe relationships generally show weak support, except for a few well supported clades; three distinct clades are recognized within Dytiscinae [Dytiscini sensu lato (i.e. including the genera *Dytiscus* Linnaeus and *Hyderodes* Hope), Hydaticini sensu lato, and Cybistrini]; and recognition of Pachydrini as a distinct tribe. Other less robust results include: Methlini sister to the rest of Hydroporinae; relative basal position of Laccornini, Hydrovatini and Laccornellini within Hydroporinae; close relationship of Agabinae and Copelatinae; Matinae nested deep within Dytiscidae, as sister to a large clade including Colymbetinae, Coptotominae, Lancetinae and Dytiscinae sensu lato; the sister-group relationship of Agabetini and Laccophilini is confirmed. The results presented here are discussed and compared with previous phylogenetic hypotheses based on different datasets, and the evolution of some significant morphological features is discussed in light of the proposed phylogeny. All suprageneric taxa are diagnosed, including illustrations of all relevant synapomorphies, and a key to separate subfamilies and tribes is presented, both in traditional (paper) format and as an online Lucid interactive identification key.

Introduction

Diving beetles (Coleoptera: Dytiscidae) are a conspicuous element of the fresh water insect fauna (Fig. 1). With more than 4300 described species (Jäch & Balke, 2008; Nilsson, 2015), it is the most species-rich water beetle family on earth. Dytiscids inhabit almost every kind of continental waters (lentic, lotic, saline, hygropetric, phytotelmata, subterranean, high altitude), are distributed from the equator to both polar regions, and have

representatives among the smallest (Bidessini, Hyphydrini) as well as the largest (Cybistrini) known water beetles (Miller & Bergsten, 2016). It is therefore not surprising that such an extensive radiation has captured the attention of entomologists for a long time.

As for most holometabolous insect groups, both historical and current classifications within diving beetles have been based mainly on the study of adults. Such a pattern is also true for phylogenetic studies, which traditionally have relied more on adult morphological characters (e.g. Burmeister, 1976; Nilsson & Angus, 1992; Biström *et al.*, 1997; Miller, 2000; Miller *et al.*, 2006). In the past few years, papers addressing the matter on the

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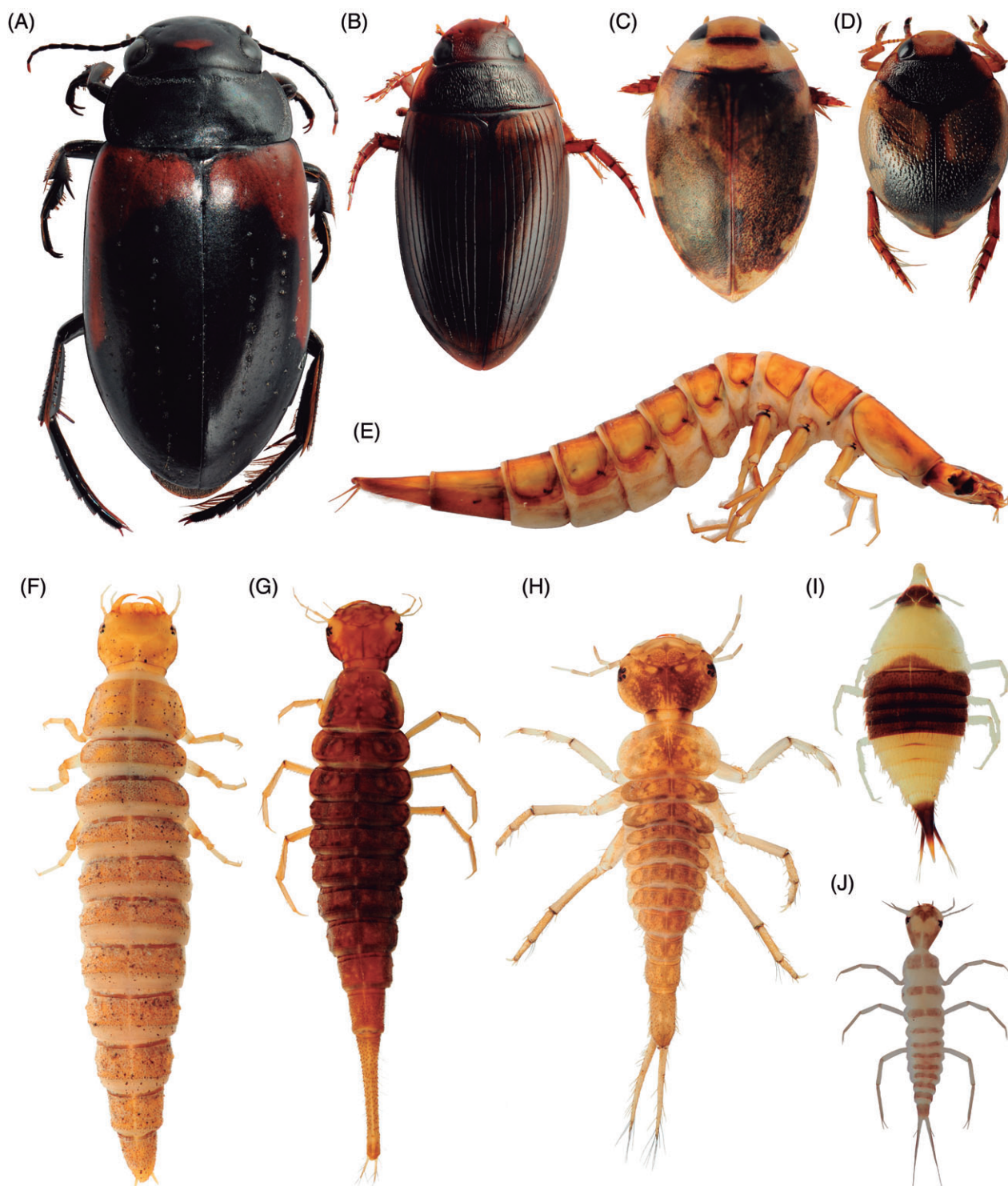
Fig. 1. Some habitus forms in Dytiscidae. (A–D) Adults, dorsal view: (A) *Bunites distigma*; (B) *Copelatus* sp.; (C) *Laccophilus* sp.; (D) *Desmopachria* sp. (E) *Thermonectus* sp., instar III, lateral view. (F–J) Larvae, dorsal view: (F) *Copelatus* sp., instar III. (G) *Meridiorhantus calidus* (Fabricius), instar III; (H) *Bunites distigma*, instar I; (I) *Desmopachria* sp., instar III; (J) *Laccophilus* sp., instar I.
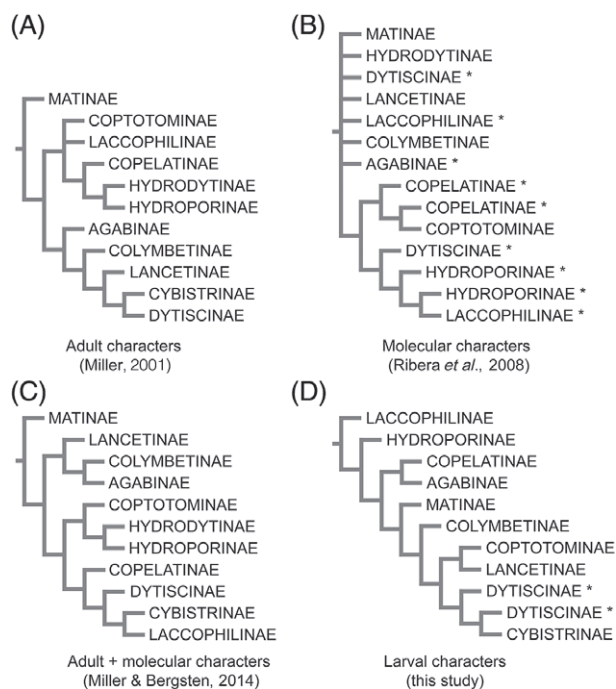
Fig. 2. Most recent hypotheses on higher-level relationships within Dytiscidae, based on different datasets (A–D). Nonmonophyletic groups (according to current classification; see Miller & Bergsten, 2014) are indicated with asterisks.

basis of other datasets such as larval morphology (e.g. Ruhnau & Brancucci, 1984; Nilsson & Hilsenhoff, 1991; Alarie & Michat, 2007a; Michat & Alarie, 2008, 2013; Alarie *et al.*, 2011a), DNA sequence (Ribera *et al.*, 2003a,2003b, 2004; Balke *et al.*, 2004, 2007a,2007b; Morinière *et al.*, 2015) or a combination of morphology and DNA (Miller, 2003; Miller *et al.*, 2007, 2009; Ribera & Balke, 2007; Miller & Bergsten, 2014) have increased considerably. The study of the larvae has proven to be particularly fruitful in providing good characters at various taxonomic levels. Most of these papers, however, are focused only on groups such as subfamilies, tribes or even genera. So far, studies treating dytiscid phylogeny in a broader sense are relatively scarce and have addressed the subject based on adult morphology (Miller, 2001), DNA sequence data (Ribera *et al.*, 2002, 2008), or both (Miller & Bergsten, 2014). All these contributions (and others) have precipitated a classification in which several higher taxa (i.e. subfamilies and tribes) have been erected based on well-supported, natural groups. Other taxa, however, are more problematic and appear to be either para- or polyphyletic, weakly supported statistically, not defined by unambiguous characters or contradictorily resolved by the different analyses (e.g. Agabinae, Copelatinae, Hydroporini, Hygrotini, Hyphydrini, Hydaticini). Higher-level relationships (i.e. among subfamilies and tribes) have also been the subject of much debate, and the different analyses have generally resulted in much disagreement (Fig. 2). The most recent study on Dytiscidae phylogeny (Miller & Bergsten, 2014) provided an updated classification of the family, including several changes

at higher taxonomic levels. This classification is followed in our study.

Despite considerable effort in the last decades (Figure S1), and an increased emphasis on chaetotaxy during the last 25 years (Alarie & Michat, 2014), characters from diving-beetle larvae still remain underexplored compared with other datasets. This was due in part to the large number of key taxa of which larvae remained unknown. Larval characters, however, alone or in combination with other datasets, were capable of producing the most significant phylogenetic hypotheses for beetles as a whole (Solodovnikov, 2007). As different expressions of the same genotype, larval characters help to complement adult characters that have traditionally been the primary basis for classification (Alarie & Watts, 2004).

As a functionally important part of the exoskeleton, chaetotaxy of beetle larvae is morphologically diverse and contains evolutionary information (Solodovnikov, 2007). Dytiscid larvae, in particular, are well suited for the detection of mechanical stimuli and have developed a number of specialized structures for this purpose. Recent studies have demonstrated the taxonomic and phylogenetic value of chaetotaxy in studying larval Adephaga (e.g. Bousquet & Goulet, 1984; Alarie *et al.*, 2004, 2011b; Alarie & Bilton, 2005). There is an overall pattern of primary sensilla, which is widespread among taxa, though it is modified in a variety of groups. This generalized pattern is consistent enough to be used for phylogenetic analysis and yet sufficiently variable to allow for taxonomic distinction.

The purpose of this study is to perform a comprehensive phylogenetic analysis of Dytiscidae based on larval characters. Emphasis has been placed on including all the major lineages within the family, and on discovering as many meaningful characters (both previously used and new) as possible to produce a higher-level phylogeny of the group. Suprageneric taxa (namely subfamilies and tribes) are diagnosed and discussed in the context of current classification, and relevant synapomorphies are illustrated. Evolution of some significant morphological features is discussed in light of the proposed phylogeny. A key to separate subfamilies and tribes is also included, in both traditional (paper) and digital (interactive) formats.

Material and methods

Taxon sampling and methods

A broad taxon sampling was examined, including 113 dytiscid and nine outgroup species in all currently recognized tribes and 10 of 11 subfamilies (Table S1). The larvae of Hydrodytinae are unknown and could not be included. The range of morphological diversity within the family was covered by including as many different genera as possible (Table S2). In total, 75 genera/subgenera were included, many of them represented by multiple species. Voucher specimens of most species are deposited in the research larval collections of MCM (Laboratory of Entomology, Buenos Aires University, Argentina) and YA (Department of Biology, Laurentian University, Sudbury, Ontario, Canada).
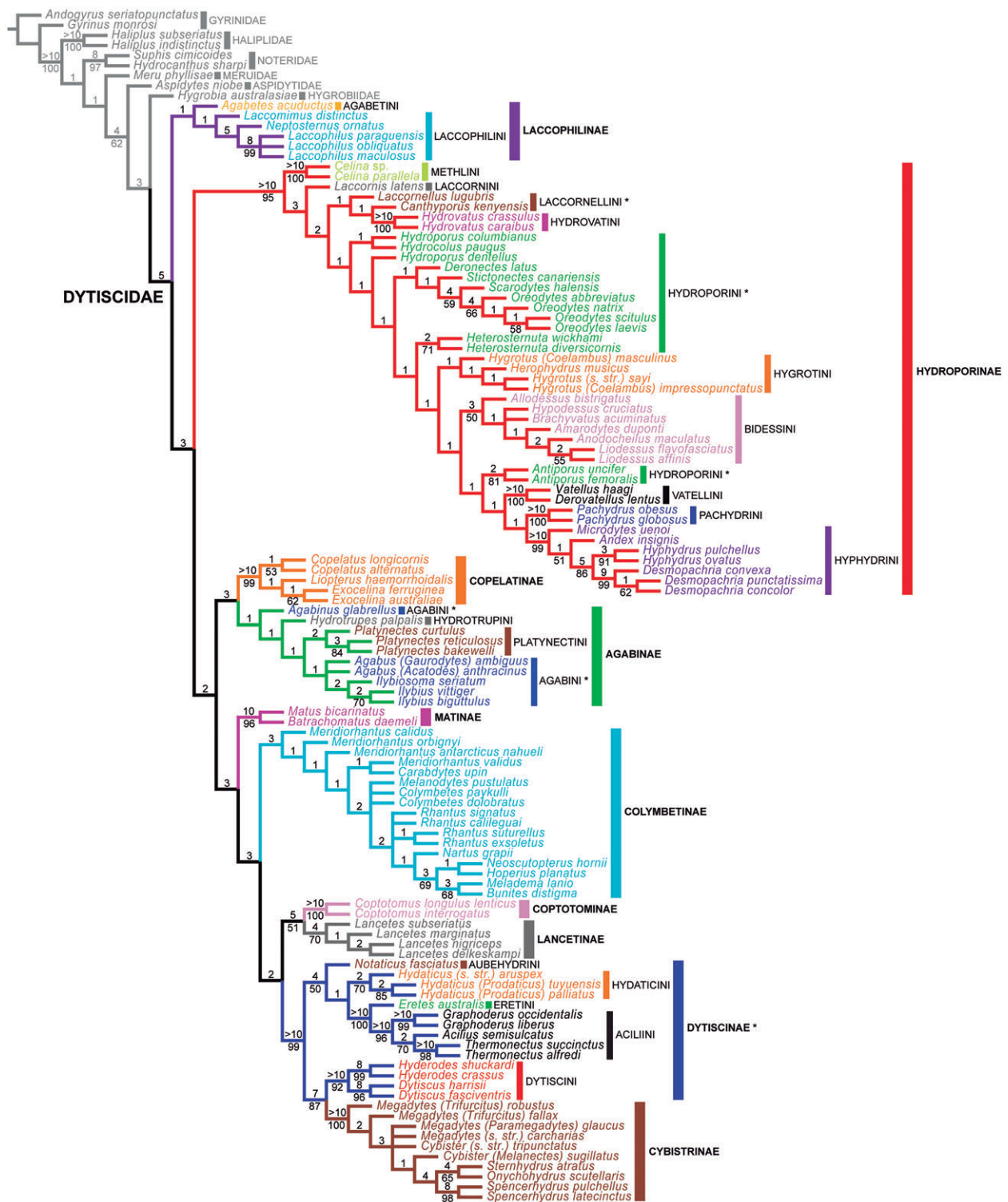
Fig. 3. Strict consensus cladogram with Bremer support values indicated above branches and bootstrap support values above 50 indicated below branches. Subfamilies and tribes are indicated with colours. Nonmonophyletic groups are indicated with asterisks.
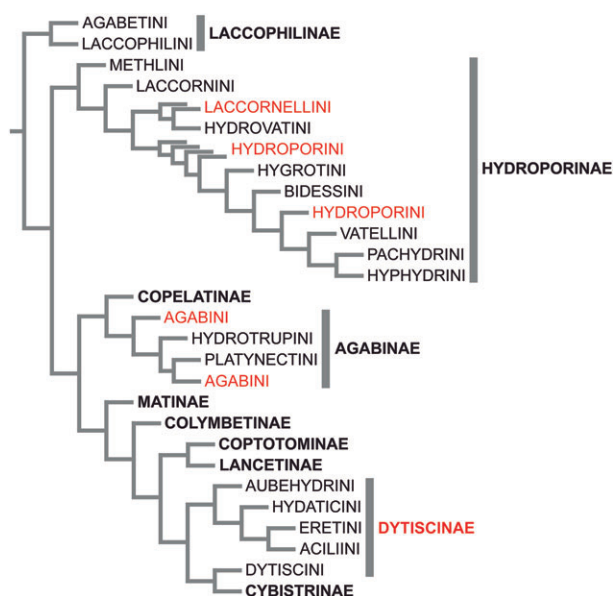
Fig. 4. Condensed tree showing higher-level relationships within Dytiscidae. Nonmonophyletic groups are indicated with colour.

Specimens were cleared in lactic acid, dissected and mounted on glass slides in polyvinyl-lacto-glycerol or Hoyer's medium. Observation (at magnifications up to 1000×) and drawings were made using an Olympus CX31 and Olympus BX50 (Olympus Corporation, Tokyo, Japan) compound microscopes equipped with a camera lucida. Drawings were scanned and digitally inked using a Genius PenSketch tablet (KYE Corporation, Taipei, Taiwan).

Photographs were taken using a Nikon D90 digital camera equipped with a Nikon AFS VR Micro-NIKKOR 105 mm f/2.8G IF-ED lens. For smaller specimens, Raynox DCR-250 and MSN-202 lenses (Tokyo, Japan) were coupled to the camera lens. The images were stacked using HELICON FOCUS 6.7.1 PRO software (Kharkov, Ukraine) and digitally edited.

Chaetotaxic analysis

The nomenclature used in this paper follows that employed in previous papers dealing with the larval chaetotaxy of the family Dytiscidae (Nilsson, 1988; Alarie & Harper, 1990; Alarie *et al.*, 1990, 2000, 2001, 2002a, 2011a; Alarie, 1991, 1995, 1998; Alarie & Michat, 2007b; Michat & Torres, 2009; Michat & Alarie, 2013). In the following, we briefly define the terms applied in the present paper. The reader is referred to Schneider (1964), McIver (1975), Zacharuk (1985), Zacharuk & Shields (1991) and Keil (1997) for in depth descriptions of the morphology and function of insect mechanoreceptors.

Setae. Sensory receptors articulated in the base. Depending on its shape, a seta can be either spiniform (= spine-like), i.e. robust, broad at base, with the shape of a spine and the apex generally more or less pointed, or setiform (= hair-like), i.e. thin, narrow at base, similar in width along most of its length, with the shape of a hair. Both types can vary in length, although a long seta is most likely hair-like and a short one spine-like. Natatory setae are typically long and hair-like, and are generally arranged in more or less dense rows. They are present more frequently on the legs, although they can also be found on the abdominal segments VII and VIII. These setae have a clearly natatory function. The spatulate setae are robust sensilla in which the base is broad, but contrary to the spiniform setae, the apex is broader than the base and widely rounded. In general, they are short and can be typically found on the anterior margin of the frontoclypeus where they are called lamellae clypeales after Bertrand (1972). Finally, the hair-like setae that are split into two or more branches at a certain distance from the base (common for example within the tribe Cybistrini) are referred to as multifid (see Alarie *et al.*, 2011a).

Pores. Circular or elliptical structures that do not rise above (or barely above) the cuticle surface. They look like small rings with shining margins when observed with the light microscope, and at low magnifications they may resemble setal bases. The term pore (in the broad sense used here) is rather unspecific and comprises several morphologically different structures, the most common being the sensilla placodea, campaniformia or ampullacea (Schneider, 1964). Setae and pores as a whole are here referred to as sensilla.

Spinulae. Unsocketed cuticular expansions that lack innervation. Generally they have the shape of a spine, and range from extremely short and inconspicuous to relatively elongate. Sometimes they divide into two or more branches at a variable distance from the base. In the drawings, spinulae can be distinguished from setae in the absence of a socket.

Primary sensilla. The term primary applies to those sensilla present in the first-instar larva, and to their homologous structures in subsequent instars. Primary sensilla are divided into ancestral and additional. Ancestral sensilla are those recognized and homologized in most or all taxa within the family. Additional sensilla are those evolved secondarily in the first instar, and generally restricted to a small group (genus or tribe) within the family.

Secondary sensilla. Those present in the second and third instars, excluding the primary sensilla.

Primary setae and pores were distinguished in the cephalic capsule, head appendages, legs, abdominal segment VIII and urogomphus, and coded by two capital letters, in most cases corresponding to the first two letters of the name of the structure on which they are located, and a number (setae) or a lower-case letter (pores). The following abbreviations were used: AB, abdominal segment VIII; AN, antenna; CO, coxa; FE, femur; FR, frontoclypeus; LA, labium; MN, mandible; MX, maxilla; PA, parietal; PT, pretarsus; TA, tarsus; TI, tibia; TR, trochanter; UR, urogomphus. Setae and pores present in the first-instar
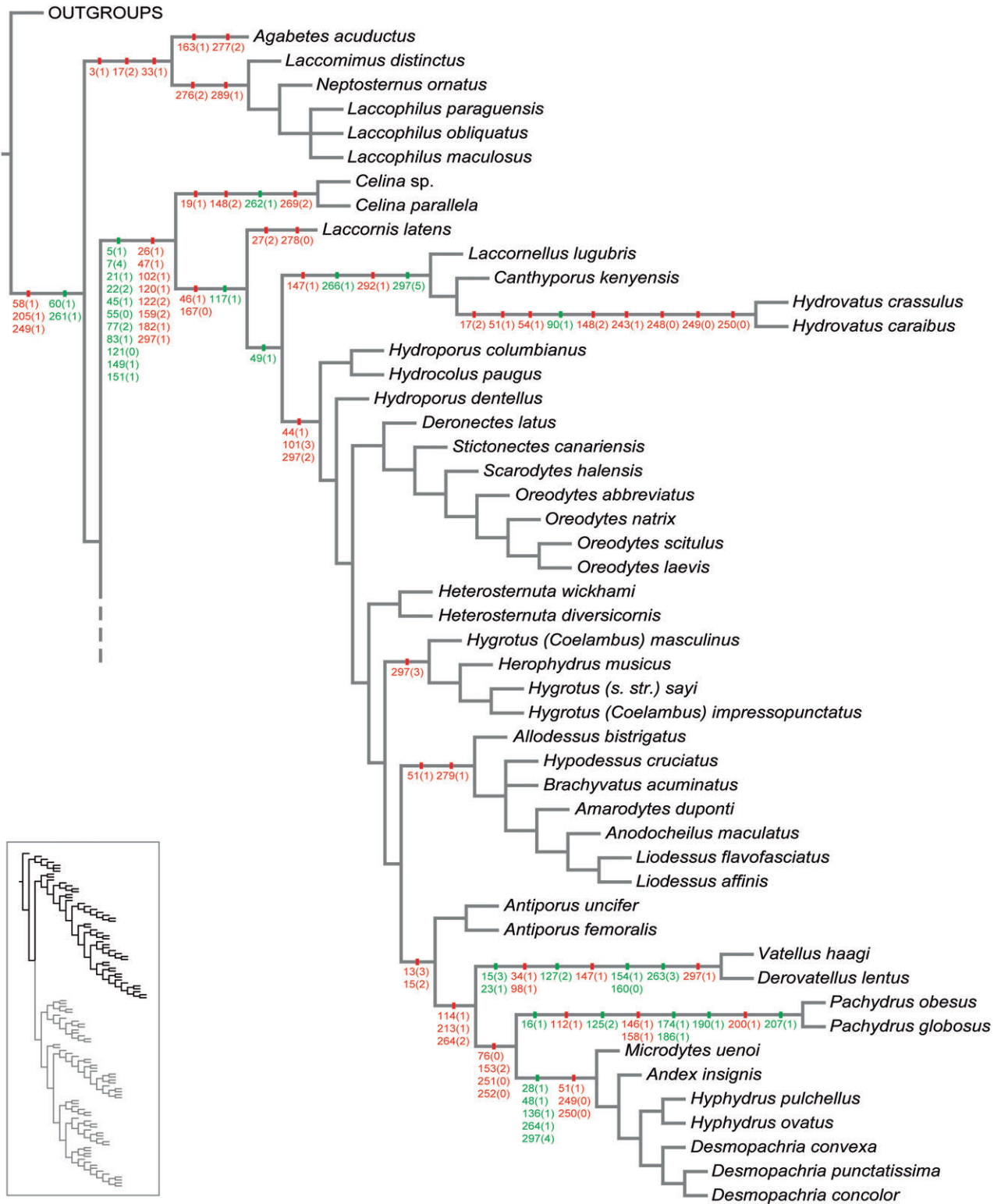
Fig. 5. Portion of one of the most parsimonious trees with selected character changes mapped for each clade. Characters in green indicate unique transformations; characters in red indicate homoplasious transformations.
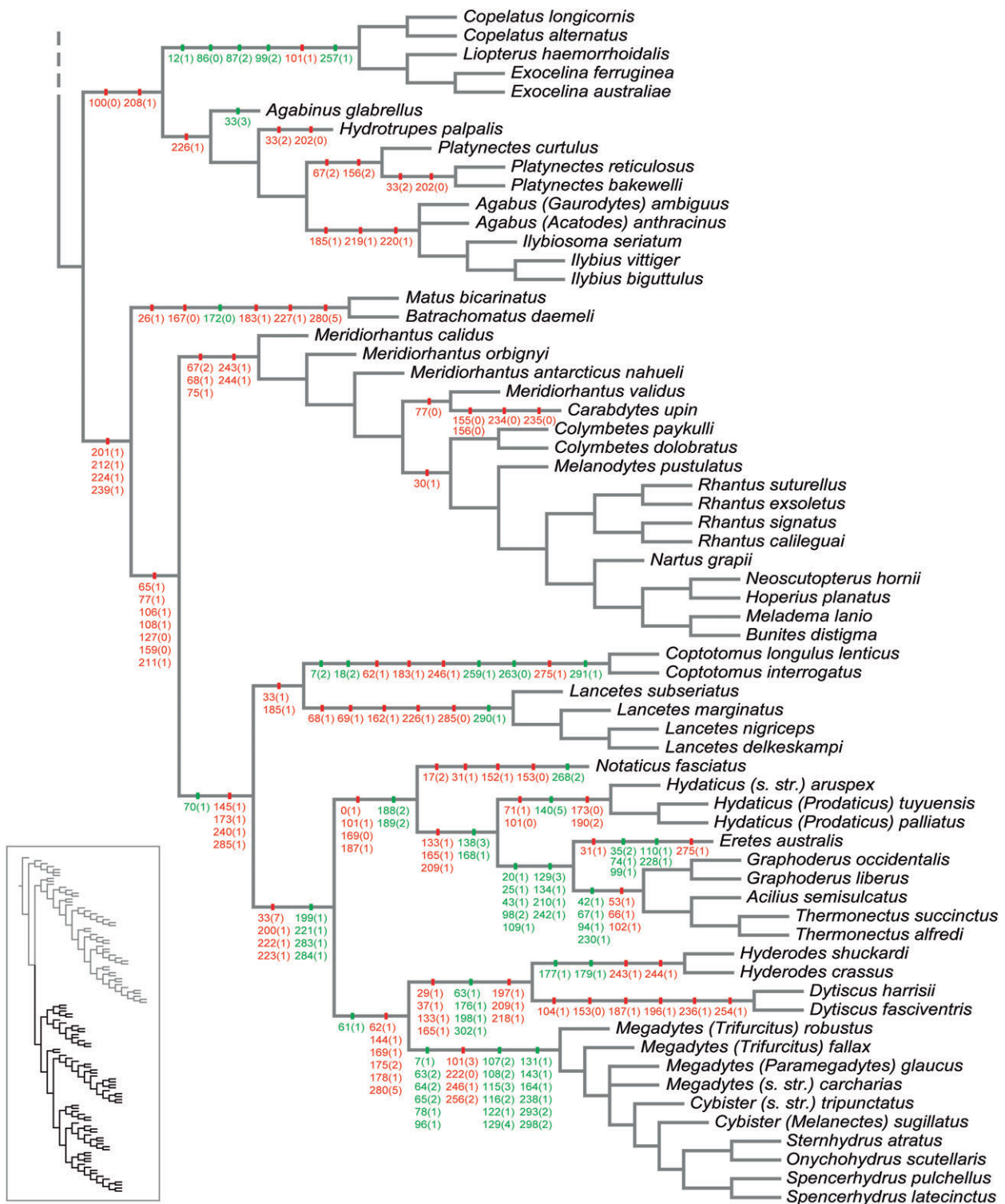
Fig. 6. Portion of one of the most parsimonious trees with selected character changes mapped for each clade. Characters in green indicate unique transformations; characters in red indicate homoplasious transformations.
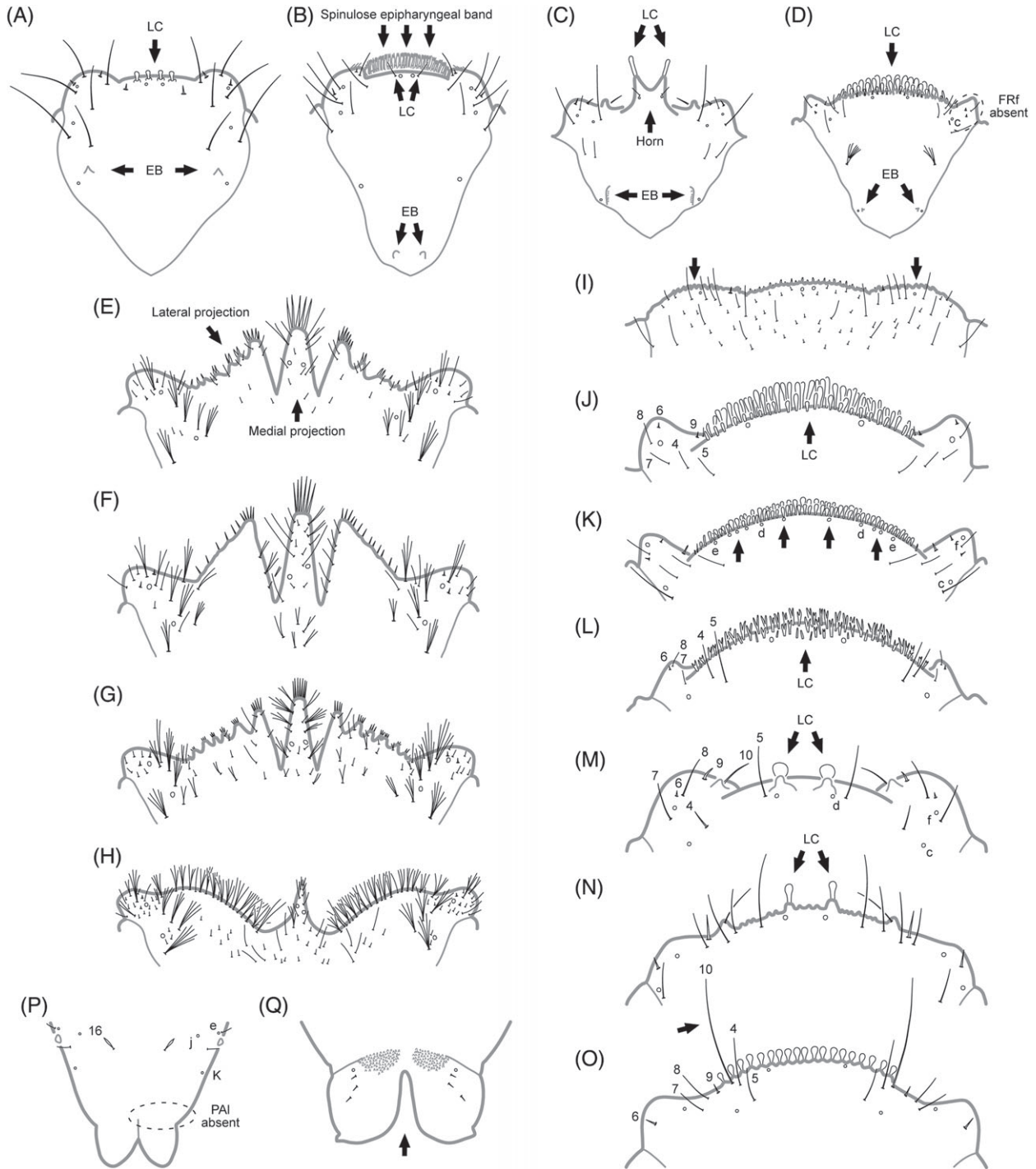
Fig. 7. Cephalic capsule of larval Dytiscidae. (A–D) Instar I, frontoclypeus, dorsal view: (A) *Hydrotrupes palpalis* Sharp; (B) *Laccomimus distinctus* Toledo & Michat; (C) *Coptotomus longulus lenticus* Hilsenhoff; (D) *Notaticus fasciatus* Zimmermann. (E–O) Anterior margin of frontoclypeus, dorsal view: (E) *Sternhydrus atratus* (Fabricius), instar I; (F) *Cybister tripunctatus* (Olivier), instar I; (G) *Onychohydrus scutellaris* (Germar), instar I; (H) *Megadytes fallax* (Aubé), instar I; (I) *Exocelina ferruginea* (Sharp), instar III; (J) *Acilius semisulcatus* Aubé, instar I; (K) *Hyderodes shuckardi* Hope, instar I; (L) *Eretes australis* (Erichson), instar I; (M) *Lancetes marginatus* (Steinheil), instar I; (N) *Neptosternus ornatus* Sharp, instar I; (O) *Batrachomatus daemeli* (Sharp), instar I. (P) *A. semisulcatus*, instar I, posterior portion of cephalic capsule, ventral view. (Q) *Thermonectus alfredi* Griffini, instar III, posterior portion of cephalic capsule, dorsal view. EB, egg bursters; LC, lamellae clypeales.
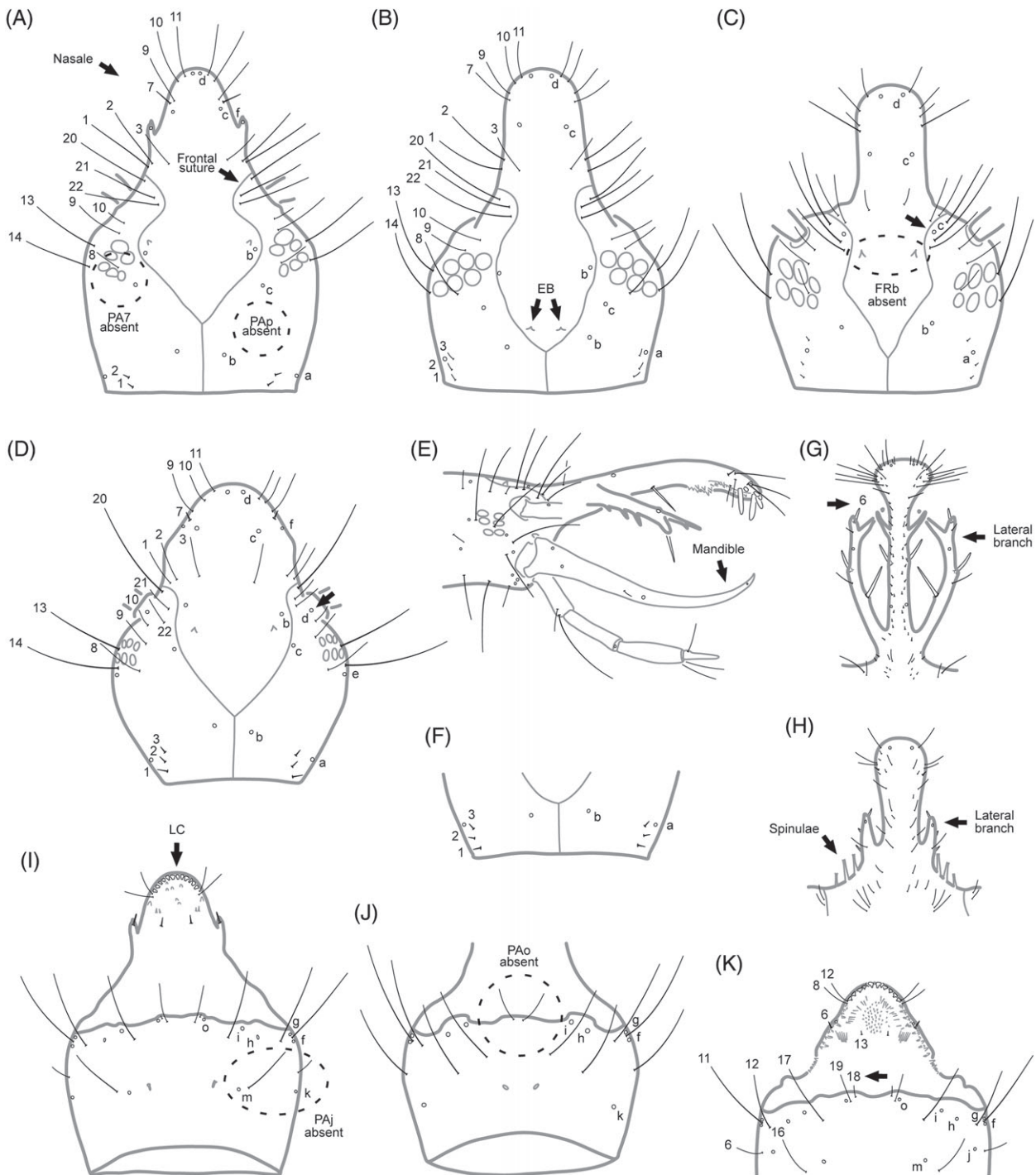
Fig. 8. Cephalic capsule of larval Dytiscidae. (A–D) Instar I, cephalic capsule, dorsal view: (A) *Brachyvatus acuminatus* (Steinheil); (B) *Hydrovatus caraibus* Sharp; (C) *Desmopachria concolor* Sharp; (D) *Laccornis latens* (Fall). (E) *Derovatellus lentus* (Wehncke), instar I, cephalic capsule, lateral view. (F) *Laccornellus lugubris* (Aubé), instar I, posterior portion of cephalic capsule, dorsal view. (G, H) Instar III, nasale, dorsal view: (G) *Vatellus haagi* Wehncke; (H) *Pachydrus obesus* Sharp. (I–K) Instar I, cephalic capsule, ventral view: (I) *B. acuminatus*; (J) *H. caraibus*; (K) *Celina parallela* (Babington). EB, egg bursters.
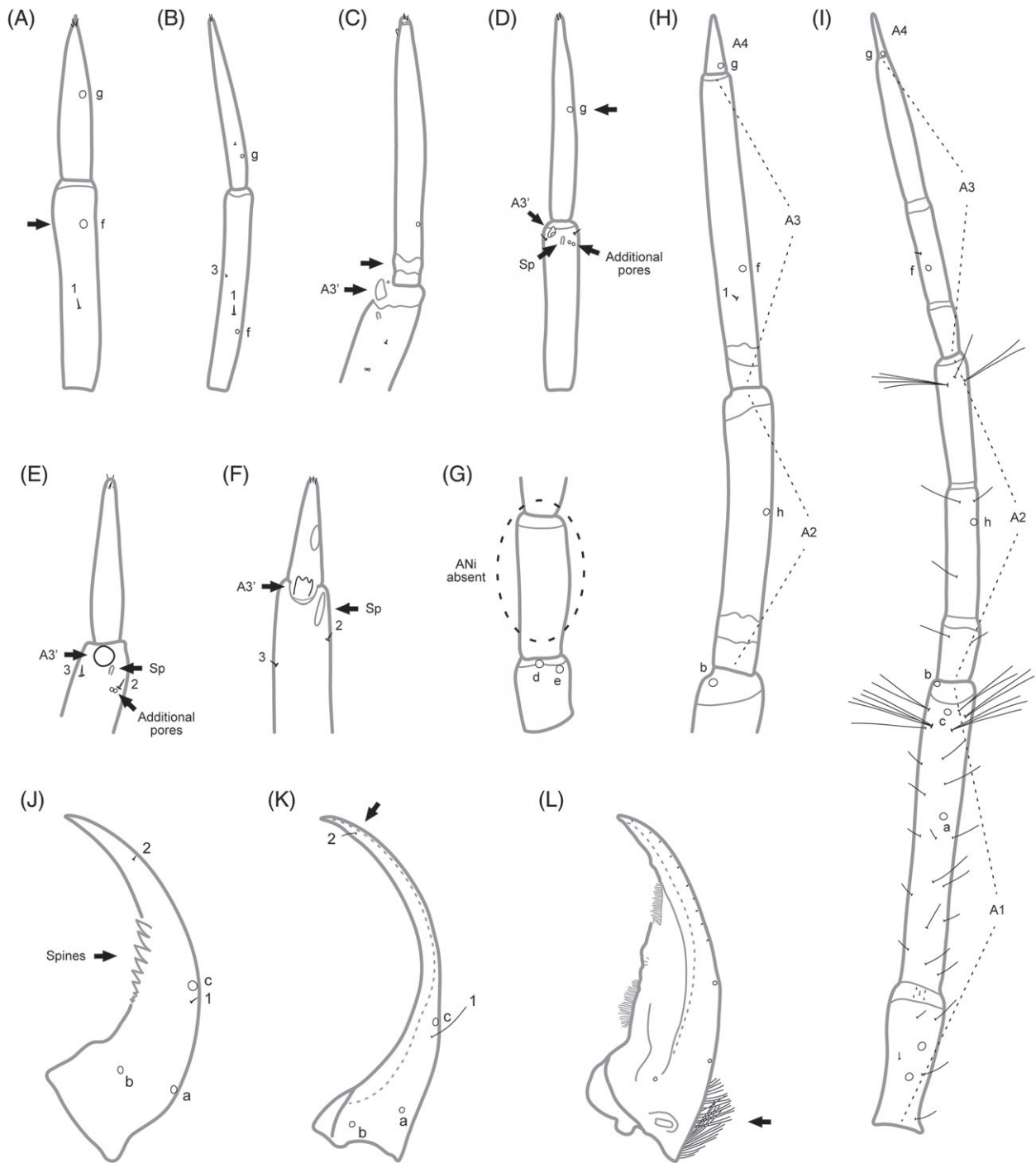
Fig. 9. Antenna and mandible of larval Dytiscidae. (A, B) Instar I, right antennomeres 3 and 4, dorsal view: (A) *Eretes australis* (Erichson); (B) *Lancetes marginatus* (Steinheil). (C–F) Left antennomeres 3 and 4, ventral view: (C) *L. marginatus*, instar III; (D) *Hoperius planatus* Fall, instar I; (E) *Platynectes curtulus* (Régimbart), instar I; (F) *Graphoderus occidentalis* Horn, instar I. (G) *Hydrovatus caraibus* Sharp, instar I, left antennomeres 1 and 2, ventral view. (H) *Dytiscus harrisii* Kirby, instar I, right antennomeres 2, 3 and 4, dorsal view. (I) *Sternhydrus atratus* (Fabricius), instar I, right antenna, dorsal view. (J–L) Right mandible, dorsal view: (J) *Copelatus longicornis* Sharp, instar I; (K) *H. caraibus*, instar I; (L) *Thermonectus alfredi* Griffini, instar III. A1–4, antennomeres 1–4; A3′, apical lateroventral process of antennomere 3; Sp, spinula.
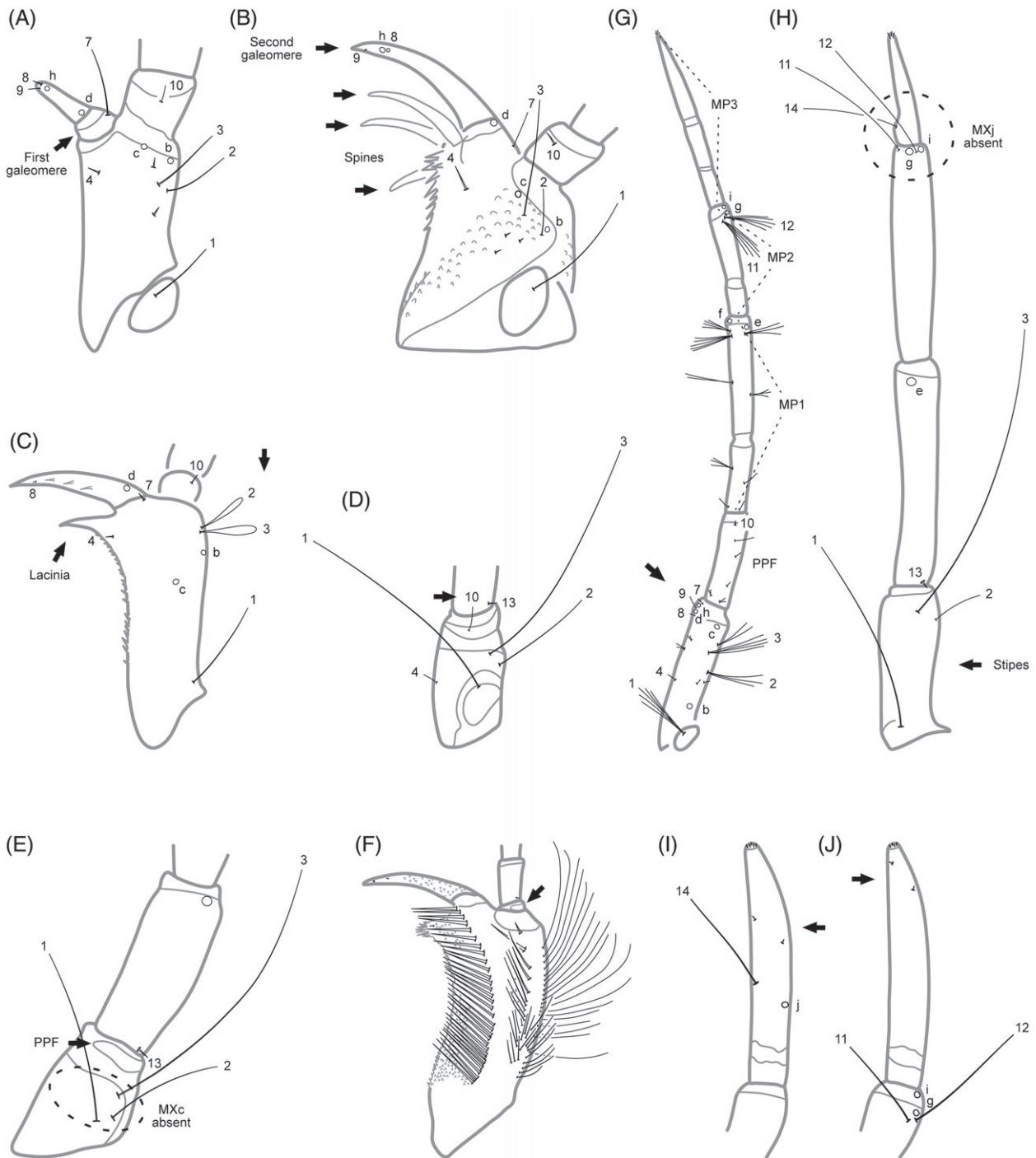
Fig. 10. Maxilla of larval Dytiscidae. (A–E) Left stipes, ventral view: (A) *Platynectes curtulus* (Régimbart), instar III; (B) *Exocelina ferruginea* (Sharp), instar III; (C) *Eretes australis* (Erichson), instar I; (D) *Celina parallela* (Babington), instar I; (E) *Desmopachria concolor* Sharp, instar I. (F) *Thermonectus alfredi* Griffini, instar III, right stipes, dorsal view. (G, H) Instar I, left maxilla, ventral view: (G) *Onychohydrus scutellaris* (Germar); (H) *Derovatellus lentus* (Wehncke). (I, J) *Dytiscus harrisii* Kirby, instar I: (I) right maxillary palpomere 3, dorsal view; (J) left maxillary palpomere 3, ventral view. MP1–3, maxillary palpomeres 1–3; PPF, palpifer.
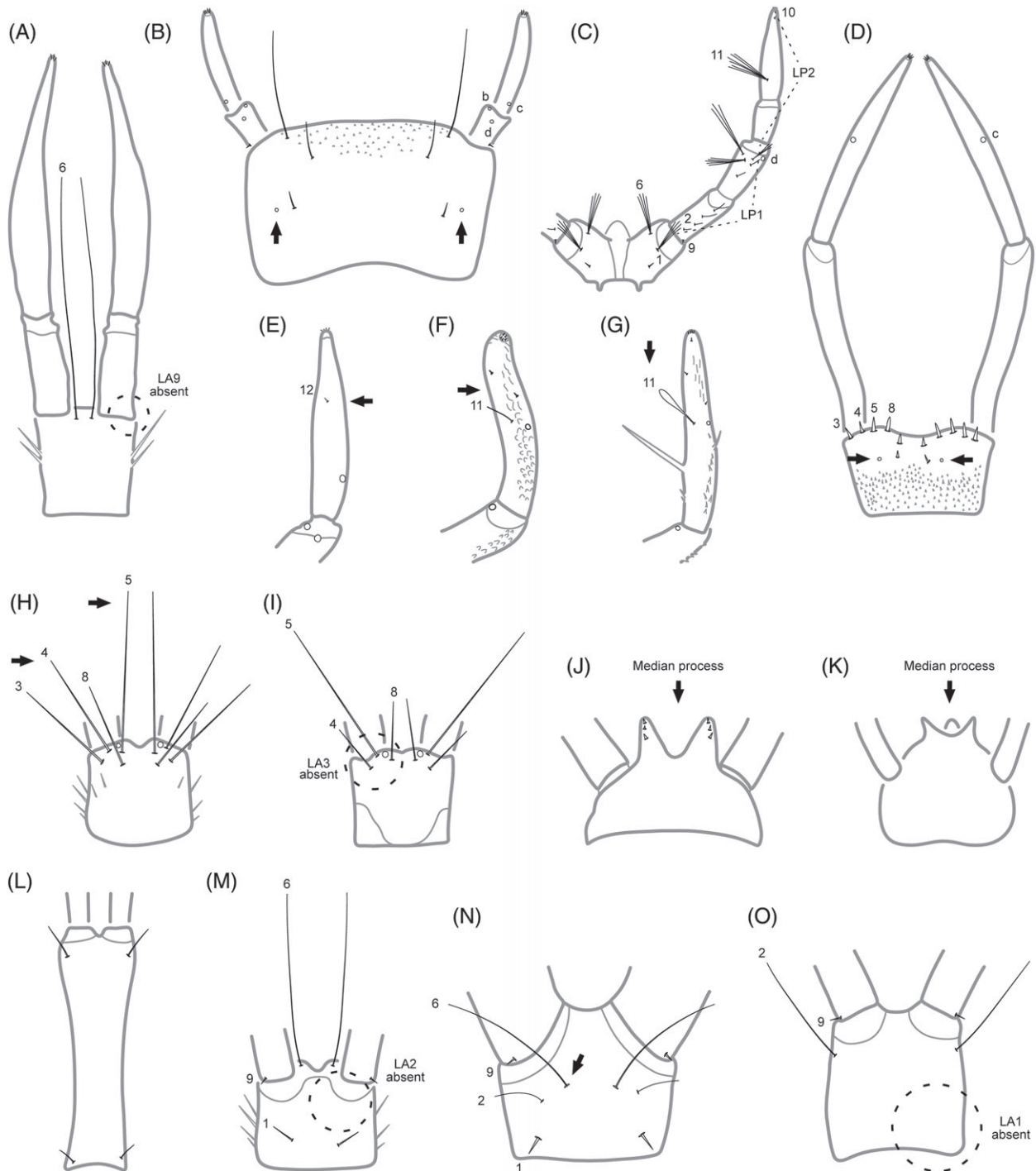
Fig. 11. Labium of larval Dytiscidae. (A–C) Instar I, labium, ventral view: (A) *Derovatellus lentus* (Wehncke); (B) *Agabetes acuductus* (Harris); (C) *Cybister tripunctatus* (Olivier). (D) *Lancetes marginatus* (Steinheil), instar I, labium, dorsal view. (E) *Platynectes curtulus* (Régimbart), instar I, left labial palpomere 2, ventral view. (F, G) Instar I, right labial palpomere 2, dorsal view: (F) *Graphoderus occidentalis* Horn; (G) *Eretes australis* (Erichson). (H–J) Instar I, prementum, dorsal view: (H) *Laccornellus lugubris* (Aubé); (I) *Celina parallela* (Babington); (J) *Hydaticus tuyuensis* Trémouilles. (K–O) Prementum, ventral view: (K) *Hydaticus leander* (Rossi), instar III (modified from Dettner, 1984); (L) *Hyphydrus ovatus* (Linnaeus), instar I; (M) *L. lugubris*, instar I. (N) *Notaticus fasciatus* Zimmermann, instar I; (O) *Pachydrus obesus* Sharp, instar I. LP1, 2; labial palpomeres 1 and 2.
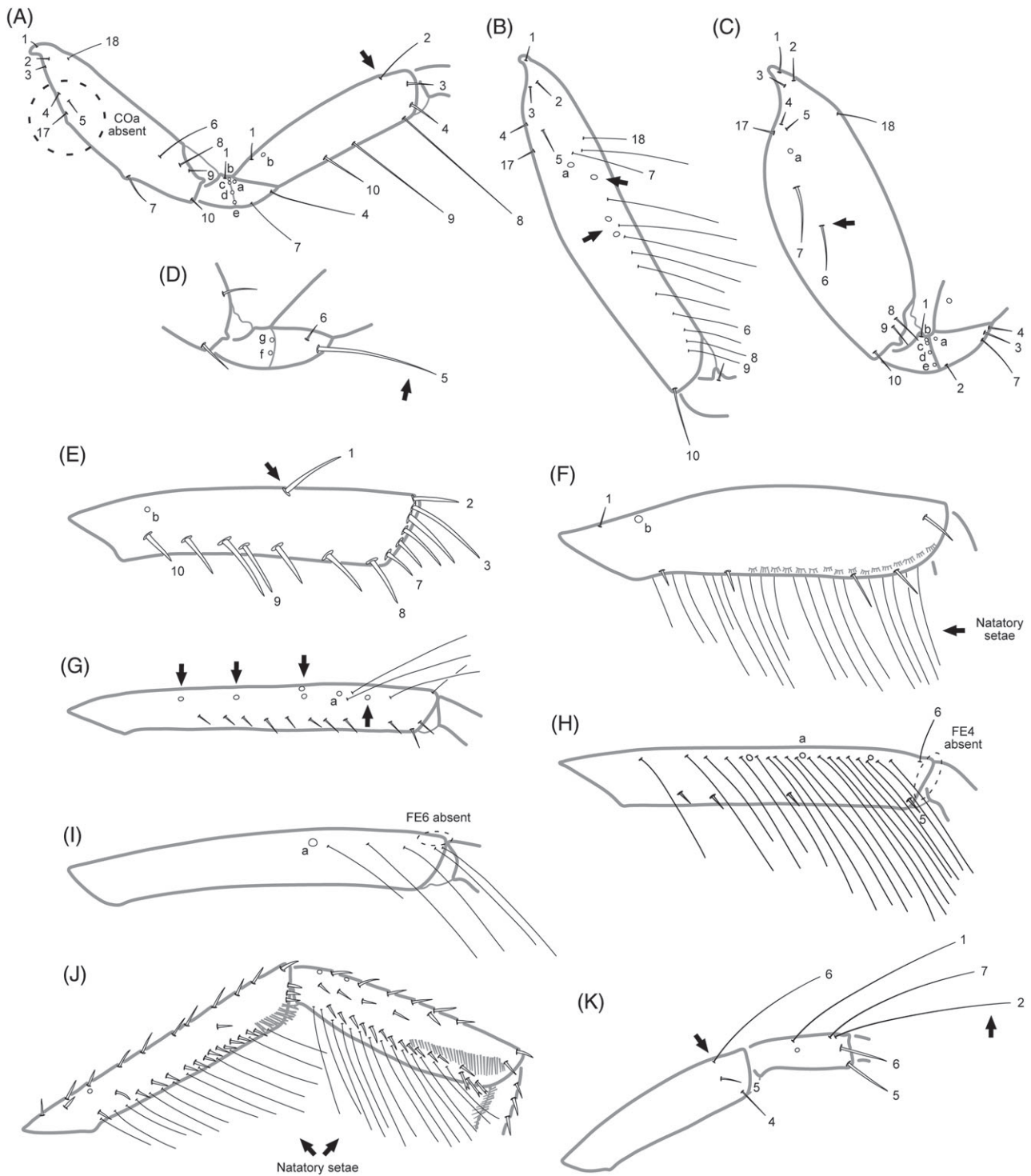
Fig. 12. Legs of larval Dytiscidae. (A) *Pachydrus obesus* Sharp, instar I, left metacoxa, metatrochanter and metafemur, anterior view. (B) *Hyderodes shuckardi* Hope, instar I, left metacoxa, anterior view. (C) *Matus bicarinatus* (Say), instar I, left procoxa and protrochanter, anterior view. (D) *M. bicarinatus*, instar I, right metatrochanter, posterior view. (E) *Ilybius angustior* (Gyllenhal), instar I, left metafemur, anterior view. (F) *P. obesus*, instar III, left profemur, anterior view. (G–I) Instar I, right metafemur, posterior view: (G) *H. shuckardi*; (H) *Dytiscus harrisii* Kirby; (I) *Hydaticus tuyuensis* Trémouilles. (J) *Notaticus fasciatus* Zimmermann, instar III, left profemur and protibia, anterior view. (K) *P. obesus*, instar I, right metafemur and metatibia, posterior view.
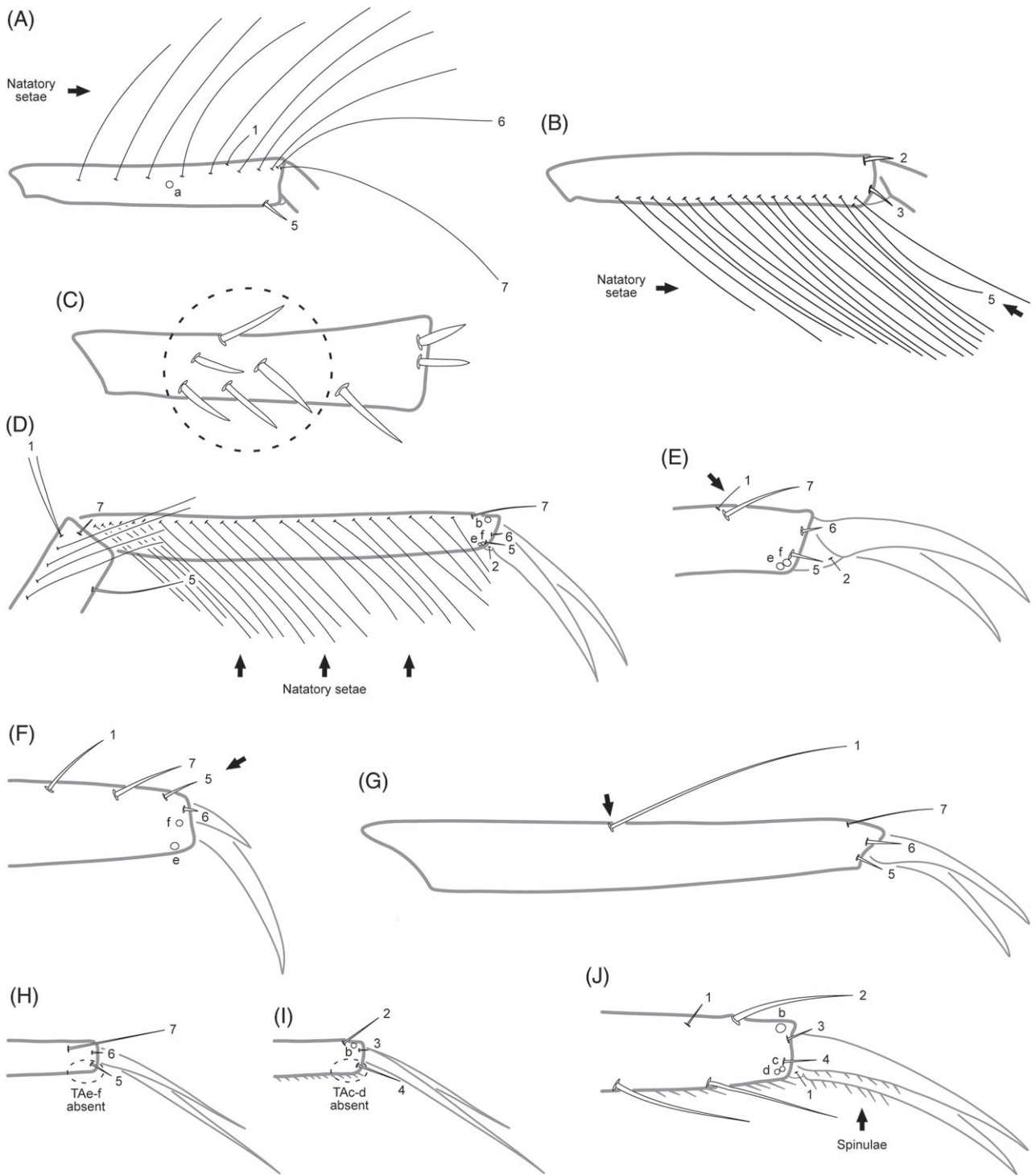
Fig. 13. Legs of larval Dytiscidae. (A) *Notaticus fasciatus* Zimmermann, instar I, right metatibia, posterior view. (B, C) Left metatibia, anterior view: (B) *Hydaticus tuyuensis* Trémouilles, instar I; (C) *Ilybius biguttulus* (Germar), instar III. (D–H) Instar I, right metatarsus and claws, posterior view: (D) *Sternhydrus atratus* (Fabricius); (E) *Platynectes curtulus* (Régimbart); (F) *Eretes australis* (Erichson); (G) *Batrachomatus daemeli* (Sharp); (H) *Graphoderus occidentalis* Horn. (I, J) Instar I, left metatarsus and claws, anterior view: (I) *G. occidentalis*; (J) *Hoperius planatus* Fall.
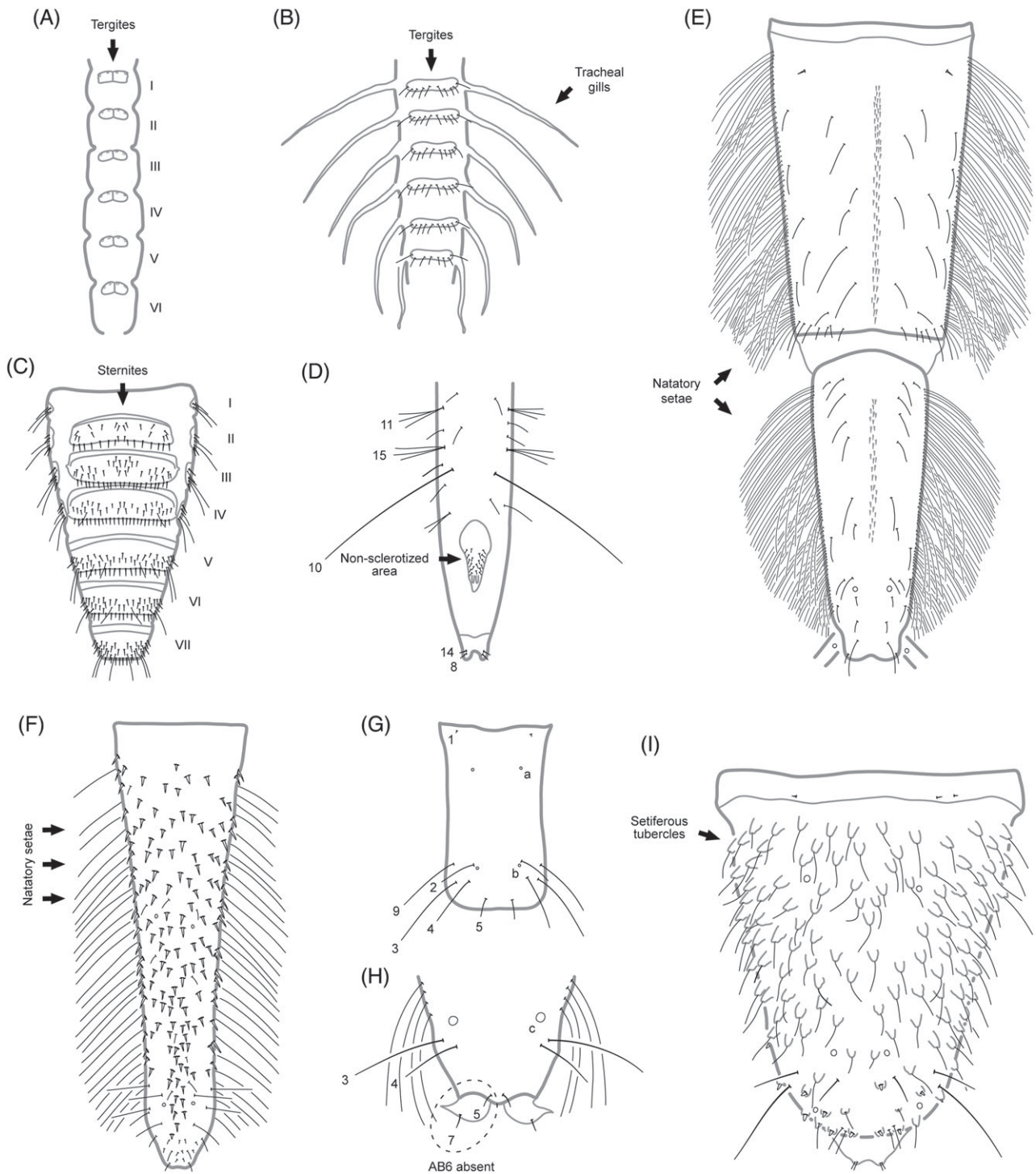
Fig. 14. Abdomen of larval Dytiscidae. (A, B) Instar I, abdominal segments I–VI, dorsal view: (A) *Megadytes glaucus* (Brullé); (B) *Coptotomus longulus lenticus* Hilsenhoff. (C) *Hydrovatus caraibus* Sharp, instar III, abdominal segments I–VII, ventral view. (D) *Sternhydrus atratus* (Fabricius), instar I, abdominal segment VIII, ventral view. (E) *Hydaticus tuyuensis* Trémouilles, instar III, abdominal segments VII–VIII, dorsal view. (F–I) Abdominal segment VIII, dorsal view: (F) *C. l. lenticus*, instar III; (G) *C. l. lenticus*, instar I. (H) *Notaticus fasciatus* Zimmermann, instar I; (I) *Copelatus longicornis* Sharp, instar III.
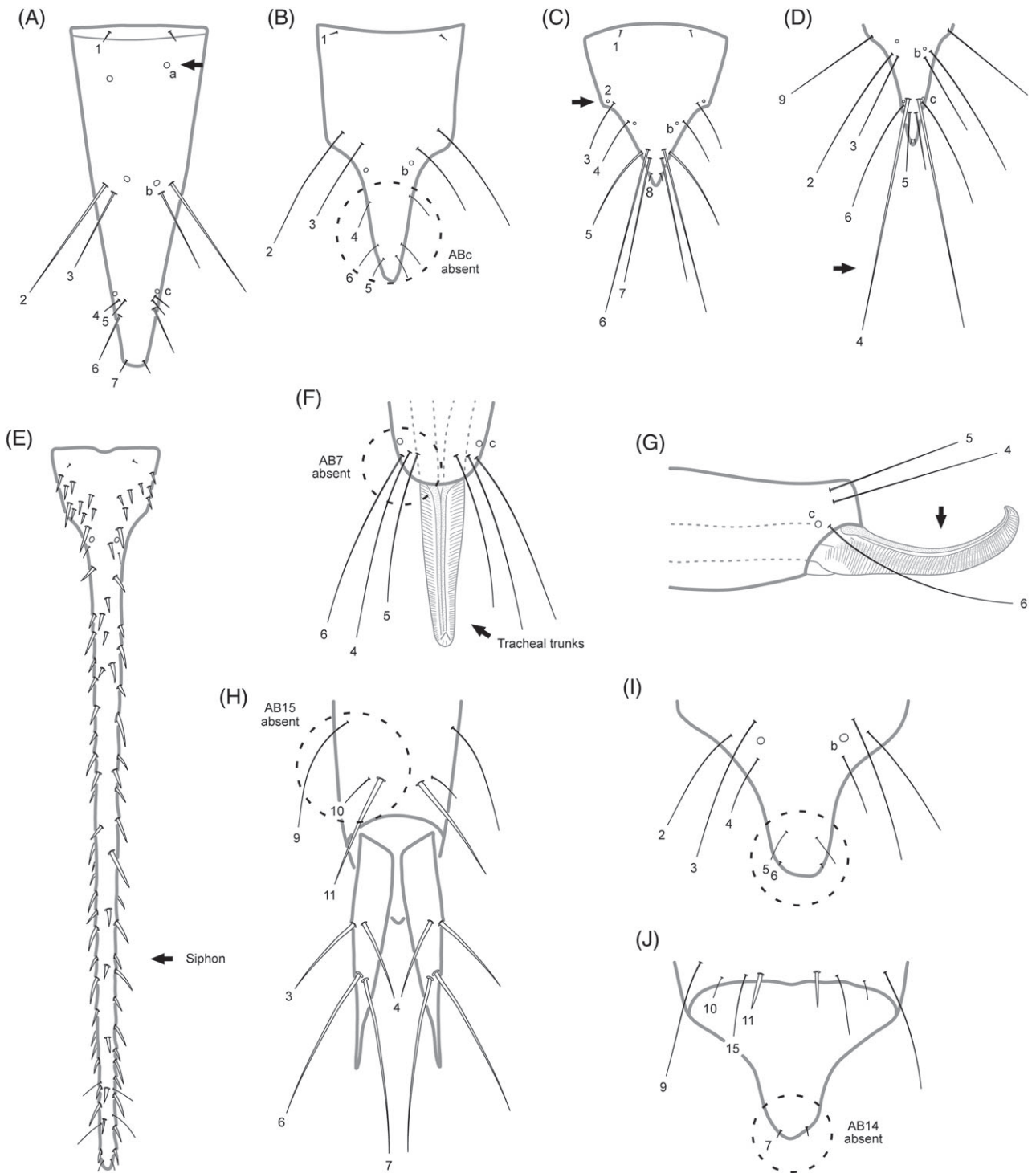
Fig. 15. Abdomen of larval Dytiscidae. (A–F) Abdominal segment VIII, dorsal view: (A) *Laccornis latens* (Fall), instar I; (B) *Anodocheilus maculatus* Babington, instar I; (C) *Andex insignis* Sharp, instar I; (D) *Laccornellus lugubris* (Aubé), instar I; (E) *Vatellus haagi* Wehncke, instar III; (F) *Celina parallela* (Babington), instar I. (G) *C. parallela*, instar I, abdominal segment VIII, lateral view. (H) *Agabetes acuductus* (Harris), instar I, abdominal segment VIII and urogomphi, ventral view. (I, J) *Laccophilus obliquatus* Régimbart, instar I, abdominal segment VIII: (I) dorsal view; (J) ventral view.
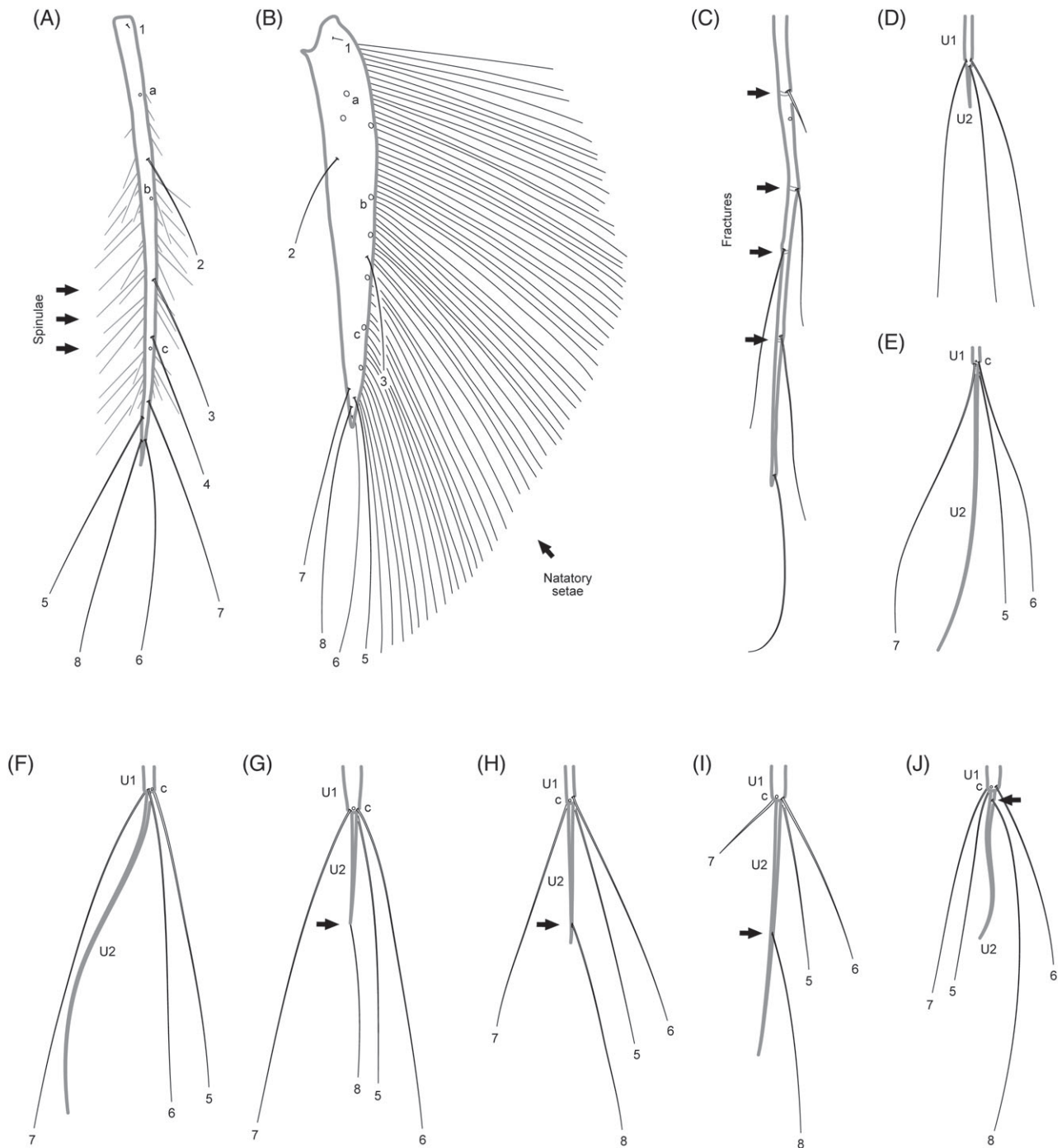
Fig. 16. Urogomphus of larval Dytiscidae. (A, B) Instar I, right urogomphus, dorsal view: (A) *Coptotomus longulus lenticus* Hilsenhoff; (B) *Dytiscus harrisii* Kirby. (C–J) Instar I, distal portion of right urogomphus, dorsal view; (C) *Lancetes biremis* Říha; (D) *Neptosternus ornatus* Sharp; (E) *Hydrovatus caraibus* Sharp; (F) *Laccornellus lugubris* (Aubé); (G) *Laccornis latens* (Fall); (H) *Hydroporus* Clairville sp.; (I) *Hygrotus masculinus* (Crotch); (J) *Microdytes uenoi* Satô.

larva were labelled by comparison with the ground-plan of chaetotaxy recently developed for the Dytiscidae (see earlier references). Homologies were recognized using the criterion of similarity of position (Wiley, 1981). This procedure is supported by the relative stability of the character system considered within higher taxa, even subfamilies (Nilsson, 1988). Setae located at the apices of the antenna and the maxillary and labial palpi (see Alekseev *et al.*, 2006) were extremely difficult to distinguish due to their position and small size. Accordingly, they are not well represented in the drawings and are not considered as character sources.

Unlike primary setae and pores, secondary setae and pores are not labelled individually because they vary considerably in both number and position, and are difficult to homologize among species of different subfamilies and tribes. Secondary leg setae, however, are generally arranged in more or less linear series. These series are named according to their position on the leg articles, following the system proposed by Wolfe & Roughley (1985) and modified by Nilsson (1986, 1987a,1987b) and Alarie (1991).

Phylogenetic analysis

The phylogenetic relationships within Dytiscidae were analysed cladistically using the program TNT (Goloboff *et al.*, 2008) and considering the character set provided by the larval morphology and chaetotaxy. Data were scored directly from the observation of the specimens (Table S1), except for *Liopterus haemorrhoidalis* (Fabricius) and *Melanodytes pustulatus* (Rossi). Data for these two species were scored from the literature (De Marzo, 1974, 1976; Nilsson, 1988; Nilsson & Hilsenhoff, 1991). A broad range of outgroup taxa were considered, including members of all hydradephagan families, with the exception of Amphizoidae. The tree was rooted using the family Gyrinidae [*Andogyrus seriatopunctatus* (Régimbart)], which is clearly distantly related to Dytiscidae (e.g. Beutel *et al.*, 2012). In order to avoid the problem of comparing different semaphoronts (see Meier & Lim, 2009), the same instars were compared in the codification of each character, except for *Hydrovatus crassulus* Sharp, *Carabdytes upin* Balke, Hendrich & Wewalka, *Megadytes robustus* (Aubé), *Exocelina* Broun spp., *Meru phyllisae* Spangler & Steiner (only third instars were available) and *Bunites distigma* (Brullé) (only first instar available). All characters were treated as equally weighted. Multistate characters were treated as nonadditive except for characters 63, 64, 65, 107 and 108 which were treated as additive. Three search strategies were implemented to find the most parsimonious trees. First, a common heuristic search was performed under 'Analyze/Traditional search', using 'tree bisection reconnection' (TBR) as algorithm, with 200 replicates and saving 100 trees per replication, previously setting 'hold 20 000' (Analysis 1). A second search involved additional heuristics, with 100 random addition sequences and TBR followed by 'tree drifting', using the commands 'hold 20 000' and 'mult 100=tbr drift ;' (Analysis 2). Finally, a third option was implemented using 100 random addition sequences and TBR followed by 'ratchet',

using the commands 'hold 20 000' and 'mult 100=tbr ratchet ;' (Analysis 3). Bremer support values were calculated using the commands 'hold 20 000', 'sub n' and 'bsupport', where 'n' is the number of extra steps allowed. The process was repeated, increasing the length of the suboptimal cladograms by one step until ten (Kitching *et al.*, 1998). Bremer support values higher than ten are indicated by the symbol >10. Bootstrap values were calculated using the following parameters: 'standard (sample with replacement)'; 1000 replicates.

Interactive key

The interactive key was constructed using LUCID 3.5 BUILDER software and compiled for internet deployment. It is available to download as File S2, and also freely available at: http://www .entomo.com.ar. When opening the key file (using LUCID PLAYER software) the user will find four panes. The upper left pane shows the list of available characters, whereas the upper right pane shows the list of taxa arranged into a hierarchical tree, i.e. with tribes nested within subfamilies. At the beginning of the identification process, the upper right pane contains all the taxa, and during the identification it contains the set of remaining taxa, i.e. those that match the used character states. The lower left pane contains the character states that were selected during the identification, and the lower right pane lists the discarded taxa, i.e. those that do not match the used character states.

Results

List of characters

In total, 304 characters were included, of which 201 were coded as binary and 103 as multistate (Table S3).

Results of the parsimony analysis

The data matrix is shown in Table S4. Seven characters included in the matrix were uninformative (autapomorphies); although their presence produces no changes in topology, they were retained and considered in the discussion. The searches with TNT using different parameters (Analyses 1–3, see Material and methods) found eight equally most parsimonious trees of 1398 steps. The strict consensus (of similar topology in the three analyses) is well resolved and informative, with only a few polytomous nodes within Colymbetinae and Cybistrinae (Fig. 3). A condensed cladogram showing higher-level relationships within Dytiscidae is presented in Fig. 4.

Dytiscidae is presently composed of 11 subfamilies (Miller & Bergsten, 2014), all of which were examined in this study, with the exception of Hydrodytinae. We found evidence of monophyly of nine subfamilies: Agabinae, Colymbetinae, Copelatinae, Coptotominae, Cybistrinae, Hydroporinae, Laccophilinae, Lancetinae and Matinae. For the majority of these, the support obtained was strong, the exceptions being the weakly supported

Agabinae and Laccophilinae (Fig. 3). The support for Colymbetinae and Lancetinae was moderate. The subfamily Dytiscinae is paraphyletic with respect to Cybistrinae, but monophyly of a clade comprising both subfamilies (previous concept of Dytiscinae) was strongly supported.

The statistical support was variable throughout the tree, with several subfamilies and tribes showing strong support, and inter-subfamily and inter-tribe relationships generally less supported. Characters were ACCTRAN optimized and those of interest were mapped in one of the most parsimonious cladograms (Figs 5, 6).

Key to larvae of the subfamilies and tribes of world Dytiscidae

A key to separate the larvae of all instars of the subfamilies and tribes of Dytiscidae (sensu Miller & Bergsten, 2014, see also Morinière *et al.*, 2015) is presented, summarizing our current knowledge of dytiscid higher taxa. More extensive diagnoses for all suprageneric taxa are presented in Table S5. An emphasis has been placed on including easily seen and identifiable characters and on avoiding (as much as possible) the use of minute or difficult structures, although this is not possible in several places. Previous keys for higher dytiscid taxa are either geographically limited (e.g. Barman, 1998; Boobar *et al.*, 1998; Larson *et al.*, 2000) or restricted to a particular subfamily (Matta, 1983; Michat & Alarie, 2009) or larval instar (Nilsson, 1988). All of them are also outdated with respect to current higher-level classification. An exception is a key to subfamilies presented by Miller & Bergsten (2016), which reflects current classification but does not include tribes. The key presented here can be applied to all three instars on a worldwide scale, keeping in mind that only the subfamily Hydrodytinae (a mainly Neotropical group which larva is unknown) was not included. The key in traditional (paper) format is available as File S1. It is also provided as an online LUCID interactive identification key (available as File S2 and at: http://www.entomo.com.ar). The characters and data matrix used for the construction of the interactive key are shown in Tables S6 and S7, respectively.

Discussion

Previous studies based on different datasets supported a monophyletic origin of the family Dytiscidae (e.g. Ruhnau, 1986; Miller, 2001; Ribera *et al.*, 2008; Miller & Bergsten, 2014). In our study, the monophyly of diving beetles is moderately supported (Fig. 3) and is indicated by the presence of secondary spine-like setae on the lateral margin of the parietals [character (ch.) 58.1, reversed in some groups of Hydroporinae and Dytiscinae sensu lato], the presence of a vertical row of secondary hair-like setae posteriorly to the stemmata in instar III (ch. 60.1, difficult to distinguish in some taxa), the presence of a distinct row of spinulae (although sometimes short and weakly developed) on the ventral surface of the protibia in instar III (ch. 205.1, reversed in Dytiscinae sensu lato and some Hydroporinae), the membranous ventral surface of the abdominal segment IV in instar III (ch. 249.1, convergent with Gyrinidae and reversed in some Laccophilinae and Hydroporinae), and the terminal position of the spiracles on the abdominal segment VIII (ch. 261.1, convergent with Noteridae) (see also Ruhnau, 1986) (Fig. 5). The reversals observed in most of these characters are probably due to the large diversity of dytiscids, which represent one of the most extensive radiations of aquatic coleopterans.

Subfamily Agabinae

Agabines were recovered as the sister group of the remaining Dytiscidae by Ribera *et al.* (2004). Their larvae exhibit many plesiomorphic character states and the subfamily is therefore difficult to diagnose based on apomorphies. Nonmonophyly of Agabinae was suggested by previous studies based on molecular characters (Ribera *et al.*, 2002, 2004, 2008). Adult characters (Miller, 2001) supported the monophyly of Agabinae, although evidence for this conclusion was weak. In the most recent phylogenetic analysis of Dytiscidae, Miller & Bergsten (2014) found Agabinae monophyletic with moderate support. These authors divided the subfamily into two tribes: Agabini (including the genera *Agabus* Leach, *Agabinus* Crotch, *Hydronebrius* Jakovlev, *Ilybiosoma* Crotch, *Ilybius* Erichson and *Platambus* Thomson) and Hydrotrupini (comprising *Agametrus* Sharp, *Andonectes* Guéorguiev, *Hydrotrupes* Sharp, *Leuronectes* Sharp and *Platynectes* Régimbart). Subsequently, in a comprehensive phylogeny of Agabinae, Toussaint *et al.* (2017) erected the tribe Platynectini for the genera previously included in Hydrotrupini, with the exception of *Hydrotrupes*, thus raising to three the number of currently recognized tribes.

In previous iterations of our analysis, Agabinae was never monophyletic with its component genera dispersed at the base of Dytiscidae and as a whole not supported by clear synapomorphies. Final analyses, including new taxa and characters, recovered agabines as monophyletic, but no apomorphic characters were discovered to define the group unambiguously, and therefore it appears as one of the most poorly supported (Fig. 3). The genera *Agabus*, *Ilybiosoma* and *Ilybius* are together monophyletic and sister to *Platynectes*. The genus *Agabinus* is sister to the remaining Agabinae genera studied, rendering Agabini paraphyletic. None of these relationships, however, is strongly supported. There is no evidence of a close relationship of Agabinae with Colymbetinae sensu stricto, as historically considered based on adult morphology (e.g. Brinck, 1948; Burmeister, 1976), or for a sister-group relationship with the remaining Dytiscidae as postulated by Ribera *et al.* (2004). The subfamily is here recovered as sister to Copelatinae with moderate support.

Based on characters of the head of the third-instar larva, Beutel (1994, 1997) postulated a sister-group relationship of the genus *Hydrotrupes* with the remaining Dytiscidae excluding *Copelatus* Erichson. However, in a more comprehensive analysis of larval characters, Alarie *et al.* (1998) suggested that *Hydrotrupes* is closely related to other agabine genera, a conclusion later supported by adult (Miller, 2001) and molecular (Ribera *et al.*, 2008; Miller & Bergsten, 2014) data. In our study, *Hydrotrupes*

is recovered as sister to the remaining agabines, except for *Agabinus*.

Overall, although evidence based on both adult and larval morphology as well as most recent analyses based on DNA sequence data suggest a monophyletic origin of Agabinae, the reduced number of synapomorphies provided by adults (Nilsson, 2000; Miller & Bergsten, 2014) and the absence of unambiguous synapomorphies from larvae indicate that the group as presently conceived is rather weakly defined. Monophyly of the currently recognized tribes is also weakly or not supported by larval characters. Additional work combining as many character systems as possible is needed to clarify the status and delimitation of this group. The subfamily and its currently recognized tribes are tentatively diagnosed based on ambiguous characters (Table S5; Figs 7A, 9E, 10A, 11E, 12E, 13C, E).

Subfamily Colymbetinae

The subfamily Colymbetinae has been subject of several changes in composition. The taxa Agabini, Copelatini, Coptotomini, Lancetini, Matini, *Agabetes* Crotch and *Hydrodytes* Miller (as *Agaporomorphus* Zimmermann), previously included in Colymbetinae, have been removed and either raised to subfamily rank or placed in other subfamilies (Böving & Craighead, 1931; Ruhnau & Brancucci, 1984; Burmeister, 1990; Miller, 2001). These exclusions are justified according to our study, as all these groups are recovered as monophyletic, outside of Colymbetinae sensu stricto (*Hydrodytes* not examined here).

In our analysis Colymbetinae were recovered as monophyletic in agreement with previous studies (Burmeister, 1990; Miller, 2001; Ribera *et al.*, 2002, 2008; Beutel *et al.*, 2008; Miller & Bergsten, 2014; Morinière *et al.*, 2016) (Fig. 3). The support obtained was moderate, probably reflecting the absence of unambiguous characters for the group. Members of this subfamily, however, exhibit several larval synapomorphies that may indicate monophyly (Fig. 6). The apical lateroventral process of the third antennomere does not protrude (Fig. 9D; ch. 67.2, reversed in *Meladema* Laporte), which was only observed in a few Agabinae and Dytiscinae. The presence of basoventral spinulae on the claws (Fig. 13J; chs 243.1 and 244.1) is also found in some Dytiscinae and Cybistrinae and in the first instar of Hydrovatini. A fourth antennomere subequal in length or even somewhat longer than the third antennomere in instar I (Fig. 9D; ch. 68.1) is observed in Lancetinae and *Batrachomatus* Clark (Matinae). The submedial position of the pore ANg on the fourth antennomere in instar I (Fig. 9D; ch. 75.1) is shared with Eretini and *Batrachomatus*. Although these character states are not restricted to Colymbetinae, they are homoplasious in members of well supported, probably natural groups such as Dytiscinae, Cybistrinae, Hydroporinae, Matinae and Lancetinae, suggesting convergent evolution. Colymbetines can be relatively easily diagnosed based on these characters (Table S5).

The first branches within Colymbetinae separate the genera *Meridiorhantus* Balke, Hájek & Hendrich and *Carabdytes* Balke, Hendrich & Wewalka from a large clade including the remaining genera (*Melanodytes* Seidlitz, *Colymbetes* Clairville, *Rhantus* Dejean, *Nartus* Zaitzev, *Neoscutopterus* Balfour-Browne, *Hoperius* Fall, *Meladema*, *Bunites* Spangler). Although weakly supported, this clade is characterized by the presence of the pore FRe (ch. 30.1), a highly distinctive feature (Fig. 6). The presence of this pore in the tribe Dytiscini (Alarie *et al.*, 2011a) is evidently convergent. Whereas the presence of FRe in *Carabdytes* could not be evaluated owing to lack of first instars, it is absent in all examined species of *Meridiorhantus*.

The position of *Carabdytes* deeply within Colymbetini supports inclusion in this tribe and reaffirms the recent synonymy of Carabdytini with Colymbetini (Miller & Bergsten, 2014; Morinière *et al.*, 2015). This result is compatible with previous studies (Alarie & Balke, 1999; Balke, 2001; Balke *et al.*, 2007b). Within Colymbetinae, *Carabdytes* is characterized by the more distal position of the pore ANi on the second antennomere [ch. 77.0, shared with *Meridiorhantus validus* (Sharp)], the absence of secondary posteroventral setae on the pro- and metatarsus in instar III (chs 234.0 and 235.0, convergent with some *Rhantus* and *Meridiorhantus* species), and the absence of the setae LA10 and LA12 on the labial palpus (chs 155.0 and 156.0). All these characters, however, are present in several taxa outside Colymbetinae, which reduces their phylogenetic value. The discovery of the still unknown first instar of *Carabdytes* would shed light on its phylogenetic position.

Subfamily Copelatinae

Whereas the monophyly of Copelatinae seems well supported on the basis of adult characters (Miller, 2001), molecular data were contradictory and favoured either the monophyly of the group (Balke *et al.*, 2004) or its para- or polyphyly (Ribera *et al.*, 2002, 2008). Since their exclusion from Colymbetinae, the phylogenetic position of copelatines has remained contentious. Some authors (Ruhnau & Brancucci, 1984; De Marzo & Nilsson, 1986; Beutel, 1994, 1995, 1997), based on larval characters, considered *Copelatus* the sister group of the remaining Dytiscidae. Others, based on adult (Miller, 2001), molecular (Ribera *et al.*, 2002, 2008; Balke *et al.*, 2004; Miller & Bergsten, 2014), but also larval (Nilsson, 1988; Michat & Torres, 2009) characters, found no evidence for this relationship and argued in favour of a more derived position of the group. These papers, however, failed to provide a robust hypothesis of the identity of the sister group of the Copelatinae.

Whereas the support obtained in our analysis for the clade Copelatinae is strong (Fig. 3), we recognize the preliminary nature of this result given that only five species in three genera were included from this, one of the largest subfamilies in Dytiscidae. Copelatinae is resolved as sister to Agabinae with moderate support. This result favours the idea that copelatines represent a derived lineage within Dytiscidae, in agreement with some previous studies (see earlier). The absence of a mandibular channel in members of this subfamily is certainly highly distinctive. *Hydrotrupes* (Agabinae) and, to a lesser extent, *Agabetes* (Laccophilinae) exhibit more or less widely open channels, but not absent. If we assume that the lack

of a channel is plesiomorphic (Ruhnau, 1986; Alarie *et al.*, 2011b), our results favour the hypothesis that copelatines have secondarily lost the channel. The presence of a foregut with a crop and the ingestion of solid food in members of this subfamily (De Marzo, 1979) are therefore derived features. The following synapomorphies characterize the Copelatinae in our study (Fig. 6): anterior margin of anterolateral lobes of frontoclypeus dentate in instar III (Fig. 7I; ch. 12.1), absence of a mandibular channel (Fig. 9J; ch. 86.0), presence of two rows of strong spines on the mesal margin of the mandible (Fig. 9J; ch. 87.2), presence of three strong spines projected inward on the anterior mesal margin of the stipes (Fig. 10B; ch. 99.2), and presence of setiferous tubercles on the dorsal surface of the abdominal segment VIII in instar III (Fig. 14I; ch. 257.1). The group is unambiguously diagnosed using these characters (Table S5).

Subfamily Coptotominae

The genus *Coptotomus* Say was for a long time included in the subfamily Colymbetinae sensu lato (e.g. Brinck, 1948; Burmeister, 1976; Ruhnau & Brancucci, 1984). Based on larval morphology, Böving & Craighead (1931) erected the subfamily Coptotominae for this taxon. Another view was presented by Brinck (1948) who, based on adult characters, placed *Coptotomus* in the tribe Coptotomini within Colymbetinae, along with the genus *Lancetes* Sharp. *Coptotomus* larvae are characterized by a unique feature within Dytiscidae, the presence of abdominal tracheal gills that resemble those observed in the larvae of Gyrinidae (Michat & Alarie, 2013). Most recent papers have supported the subfamilial status, but have disagreed regarding the phylogenetic position of the taxon. Miller (2001) postulated that Coptotominae is sister to a clade formed by Copelatinae, Hydrodytinae and Hydroporinae. Ribera *et al.* (2002) found Coptotominae to be closely related to some Agabinae taxa, whereas Ribera *et al.* (2008) found it nested within Copelatinae, sister to *Agaporomorphus*. On the other hand, Michat & Alarie (2013) postulated a clade formed by the subfamilies Coptotominae, Lancetinae and Laccophilinae, whereas Barman (2004) proposed a clade formed by Coptotominae and Laccophilinae. In the most recent analysis, Miller & Bergsten (2014) found Coptotominae sister to Hydrodytinae + Hydroporinae.

In our study Coptotominae is recovered as part of a large clade, including the subfamilies Lancetinae, Dytiscinae and Cybistrinae, and within this clade it is resolved as sister to Lancetinae with relatively strong support (Fig. 3). Both subfamilies share several character states, including the presence of two lamellae clypeales in instar I (Fig. 7C; ch. 33.1, convergent with Laccophilinae), the seta FE1 inserted submedially on the femur in instar I (ch. 185.1, convergent with some Agabinae), and the second labial palpomere subdivided into two articles in instar III (ch. 145.1, convergent with Cybistrinae and some Dytiscinae). Michat & Alarie (2013), considering a more restricted set of larval characters and taxa, found a close relationship between the subfamilies Coptotominae, Lancetinae and Laccophilinae based on the presence in these taxa of an unusually low number of

lamellae clypeales in the first instar, a condition related to the 'four-peg pattern' suggested by Ruhnau & Brancucci (1984). These authors postulated that the four-peg pattern has arisen independently in Laccophilinae, Coptotominae and Lancetinae, and that it represents a reversal to the ancestral condition found in the Carabidae and Gyrinidae, but also present in Aspidytidae, Meruidae and Haliplidae (Alarie & Bilton, 2005; Alarie *et al.*, 2011b, this study). Following Nilsson (1988), Michat & Alarie (2013) supported a common origin of the four-peg pattern within the Dytiscidae, and not an independent acquisition in the three subfamilies. Our study, however, partly contradicts both views and supports a common origin of the four-peg pattern in Coptotominae and Lancetinae, and a convergent evolutionary reappearance in Laccophilinae.

Larvae of Coptotominae share several apomorphic character states with members of Dytiscinae and Cybistrinae, suggesting a close relationship of the three subfamilies. The first antennomere is subdivided into two articles in instar III of *Coptotomus*, Cybistrinae and Dytiscini (ch. 62.1); a basoventral patch of dense slender spinulae is present on the protarsus in *Coptotomus*, Cybistrinae, and in the dytiscine taxa Aubehydrini, Hydaticini and *Hyderodes* Hope (ch. 240.1); the abdominal tergites I–VI are strongly reduced in *Coptotomus* and Cybistrinae (Fig. 14B; ch. 246.1); and the abdominal segment VIII of instars II and III bears natatory setae on the lateral margins in the three subfamilies (Fig. 14F; ch. 285.1). However, the clade Dytiscinae + Cybistrinae is one of the most strongly supported in our analysis, characterized by several unique features (see later) and also by several adult characters (Miller, 2000, 2001). Therefore, the presence of all these character states in members of this clade and in Coptotominae should be considered the result of convergent evolution. Larvae of Coptotominae are nonetheless highly characteristic within Dytiscidae and bear several unique character states (Fig. 6), including the anterior margin of the frontoclypeus projected forward in a short bifid horn in instar I (Fig. 7C; ch. 7.2), the elongation of the egg bursters in instar I (Fig. 7C; ch. 18.2), the presence of tracheal gills on the abdominal segments I–VI (Fig. 14B; ch. 259.1), the absence of a siphon (Fig. 14G; ch. 263.0), and the presence of very long spinulae on the urogomphus in instar I (Fig. 14F; ch. 291.1). This subfamily is therefore easily diagnosed using the aforementioned characters (Table S5).

Subfamily Cybistrinae

There is little doubt that cybistrines are monophyletic (Miller, 2000, 2001, 2003; Miller *et al.*, 2007; Ribera *et al.*, 2008; Michat, 2010; Alarie *et al.*, 2011a; Miller & Bergsten, 2014; Michat *et al.*, 2015). In our study, this group is very well supported (Fig. 3) and characterized by a large number of synapomorphies (Fig. 6): anterior margin of the frontoclypeus trilobed (Fig. 7E–H; ch. 7.1); second and third antennomeres subdivided into three articles (Fig. 9I; chs 63.2, 64.2 and 65.2); presence of additional setae on the first and second antennomeres (Fig. 9I; ch. 78.1); premaxillary lobes well developed, projected forward (ch. 96.1); third maxillary palpomere subdivided into

three articles in instars II and III (Fig. 10G; chs 107.2 and 108.2); setae MX8 and MX9 and pore MXd inserted on the stipes (Fig. 10G; chs 115.3, 116.2 and 122.1); presence of several hair-like additional setae on the ventral surface of the stipes (Fig. 10G; ch. 129.4); presence of additional setae on the palpifer and on the first maxillary palpomere (Fig. 10G; ch. 131.1); first and second labial palpomere subdivided into two articles in instar I (Fig. 11C; ch. 143.1); presence of additional setae on the first labial palpomere (Fig. 11C; ch. 164.1); presence of natatory posterodorsal setae on the tarsus in instar I (Fig. 13D; ch. 238.1); presence of a nonsclerotized ventrodistal rounded area around the anus on the abdominal segment VIII (Fig. 14D; ch. 256.1–2); and presence of a single primary pore on the urogomphus (ch. 293.2). Larvae of Cybistrini are highly characteristic within Dytiscidae, and can be unambiguously and easily diagnosed with the characters given above (Table S5). The group is, however, deeply nested within the subfamily Dytiscinae and therefore the subfamilial status is not supported (see later).

Subfamily Dytiscinae

Dytiscinae sensu lato (i.e. including cybistrines) is a morphologically well-defined group, for which strong evidence of monophyly has been presented based on adult (Burmeister, 1976; Miller, 2000, 2001, 2003) and larval (Ruhnau & Brancucci, 1984; Alarie *et al.*, 2011a) characters. The genus *Notaticus* Zimmermann was excluded from Dytiscinae and placed in its own subfamily (Aubehydrinae) by Guignot (1942), and followed by several authors (e.g. Spangler, 1973a; Pederzani, 1995; Trémouilles *et al.*, 1995; Trémouilles, 1998). On the other hand, the tribe Cybistrini has been recognized at the subfamily rank by some researchers (e.g. Michael & Matta, 1977; Trémouilles *et al.*, 1995; Gundersen, 1996). More recent studies based on DNA sequence data (Ribera *et al.*, 2002, 2008) found cybistrines not closely related to other Dytiscinae. Miller & Bergsten (2014), based on molecules and adult morphology, supported this hypothesis and formally excluded cybistrines from Dytiscinae, giving them subfamily rank. The identity of the sister group of Dytiscinae is also controversial. Ruhnau & Brancucci (1984) suggested a close relationship with Lancetinae, but were unable to produce a conclusive statement. Subsequent authors postulated a sister relationship of Dytiscinae and Lancetinae (Miller, 2001; Alarie *et al.*, 2002a). Others, however, related Dytiscinae either to Copelatinae and Hygrobiidae (Ribera *et al.*, 2002), Agabetini (Ribera *et al.*, 2008), or to Matinae (Michat & Torres, 2009; Michat, 2010; Michat & Alarie, 2013).

Our results indicate that the clade Dytiscinae + Cybistrinae is monophyletic and strongly supported by several unique characters (Figs 3, 6) (see also Alarie *et al.*, 2011a). Larvae of this group bear rows of natatory setae on the anteroventral surface of the protibia in all instars (Fig. 12J; ch. 221.1), on the femur and the lateral margin of the abdominal segment VIII in instar I (chs 199.1 and 284.1), and on the lateral margin of the abdominal segment VII in instars II and III (Fig. 14E; ch. 283.1). All these characters have not been reported from any other diving beetle larva, and therefore the group is easily

diagnosed based on larval morphology (Table S5). Dytiscinae sensu lato are also characteristic in the presence of rows of natatory setae on the posterodorsal margin of the tibia in instar I (Fig. 13A; ch. 223.1), on the anteroventral margin of the femur in instars II and III (Fig. 12J; ch. 200.1), and on the lateral margin of the abdominal segment VIII in instars II and III (Fig. 14E; ch. 285.1). The presence of similar character states in *Batrachomatus*, Pachydrini and Coptotominae, respectively, is most likely a convergence. These arrangements of natatory setae probably originated as secondary developments which later on became primary (Nilsson, 1988), and are responsible for the strong propulsion organ used by dytiscine and cybistrine larvae in their characteristic shrimp-like escape and jerky prey capture behaviours (Ruhnau & Brancucci, 1984). It is worth mentioning that Dytiscinae sensu lato larvae are also characterized by the presence of one or two ventral sclerites on the prothorax in instar III (ch. 169.0–1) and a large number of lamellae clypeales in instar I (Fig. 7J; ch. 33.7). Larvae of some colymbetine genera (*Colymbetes*, *Neoscutopterus*, *Hoperius*) share these conditions, most likely evolved independently.

Larval characters do not give support to the exclusion of cybistrines from Dytiscinae. Actually, this group is nested well within Dytiscinae, as part of a clade including the tribe Dytiscini, with strong support (Fig. 3) (see also Michat *et al.*, 2015). The clade Dytiscini + Cybistrini contains large to very large species characterized by a first antennomere subdivided into two articles in instars II and III (chs 61.1 and 62.1, the latter convergent with Coptotominae) and the first and second labial palpomere subdivided into two articles in instar III (chs 144.1 and 145.1, the former reversed in *Hyderodes crassus* Sharp and the latter convergent with Coptotominae + Lancetinae) (Fig. 6). Based on our results, to recognize cybistrines at the subfamily rank results in Dytiscinae paraphyletic with respect to Cybistrinae unless an additional subfamily for the tribes Aubehydrini, Hydaticini, Eretini and Aciliini is recognized. This view supports previous results of Ribera *et al.* (2008), who suggested a split of Dytiscinae sensu lato into three groups: Cybistrini, Dytiscini and Hydaticini sensu lato (including the remaining tribes). Larval characters in support of Dytiscinae sensu stricto (i.e. exclusive of Cybistrini) are scarce and therefore the group is diagnosed only on the basis of ambiguous characters (Table S5; Figs 10I, J, 11F, 12H, 13B). The clade Dytiscinae sensu lato, for its part, is monophyletic and strongly supported by adult and larval morphology (Burmeister, 1976; Miller, 2000, 2001, 2003; Alarie *et al.*, 2011a; this study). Clearly, broader analyses combining as many character systems as possible are needed to clarify the phylogenetic position of cybistrines.

The tribe Dytiscini, comprising the genera *Dytiscus* Linnaeus and *Hyderodes* after Miller & Bergsten (2014), is strongly supported (Fig. 3). Larvae of this group share a number of synapomorphies (Fig. 6), including the second and third antennomeres subdivided into two articles in instar I (Fig. 9H; ch. 63.1), the presence of a row of natatory setae on the external margin of the urogomphus in instar I (Fig. 16B; ch. 302.1), and the presence of additional pores on the anterior margin of the frontoclypeus (Fig. 7K; ch. 37.1, convergent with *Hoperius*), the anterodorsal surface of the coxa (Fig. 12B; ch. 176.1) and the posterodorsal

surface of the meso- and metafemur (Fig. 12G; ch. 198.1). Other characters supporting this grouping are the pore FRc inserted contiguously to the frontal suture (ch. 29.1, convergent with *Neptosternus* Sharp), and the presence of additional pores on the posterodorsal surface of the profemur and on the posterior surface of the tibia (chs 197.1 and 218.1, both convergent with *Meladema*). This result is in contradiction with Ribera *et al.* (2008), who placed *Hyderodes* within a clade comprising Aubehydrini, Hydaticini, Eretini and Aciliini, but compatible with Alarie *et al.* (2011a) and Michat *et al.* (2015). Two scenarios have been considered in recent years with respect to the taxa composition of the tribe Dytiscini. One placed *Hyderodes* in its own tribe, Hyderodini (e.g. Miller, 2000, 2001), and the other included *Hyderodes* and *Dytiscus* in a single tribe, Dytiscini (Miller & Bergsten, 2014 and most studies previous to Miller, 2000). In our study, whereas the clade *Dytiscus* + *Hyderodes* (Dytiscini sensu lato) is well supported by several unique features, the exclusion of *Hyderodes* results in Dytiscini (sensu stricto) being defined only by homoplasious characters. It therefore seems more convenient to group both genera in a single tribe (Dytiscini) following Miller & Bergsten (2014). As presently defined, the tribe is well defined and diagnosable using larval characters (Table S5).

Recognition of Aubehydrinae at the rank of subfamily is certainly not supported by this study. *Notaticus* is nested within a large clade including the tribes Hydaticini, Eretini and Aciliini, represented by medium-sized to small Dytiscinae characterized by the absence of the seta FE5 on both the meso- and metafemur (chs 188.2 and 189.2) and of the seta FE4 on all femora (ch. 187.1, convergent with *Dytiscus*) (Fig. 6). The support for this clade was moderate (Fig. 3). A similar result was obtained by Miller (2000) with strong support, and by Ribera *et al.* (2002, 2008), except that in these molecular studies *Hyderodes* was included in this clade. Aubehydrini is unambiguously supported by the absence of the seta AB6 on the abdominal segment VIII (Fig. 14H; ch. 268.2). Other apomorphic characters for this tribe are the proximal position of the egg bursters on the frontoclypeus of instar I (Fig. 7D; ch. 17.2, convergent with Laccophilinae and Hydrovatini), the absence of the pore FRf on the frontoclypeus (Fig. 7D; ch. 31.1, convergent with Eretini), the submedial insertion of the seta LA6 on the prementum (Fig. 11N; ch. 152.1, convergent with *Desmopachria* Babington) and the absence of the labial seta LA8 (ch. 153.0, convergent with *Dytiscus*, *Sternhydrus* Brinck and some *Megadytes* Sharp) (see Table S5 for diagnosis). Aubehydrini is resolved as sister to a clade comprising the tribes Hydaticini, Eretini and Aciliini, in agreement with Miller (2000), Ribera *et al.* (2008) and Alarie *et al.* (2011a). Larvae of this clade share the anterior margin of the prementum projected forwards in instar I (ch. 138.3) and the presence of a setose median process of the prementum (ch. 168.1). However, larvae of *Notaticus* share with those of *Eretes* Laporte and Aciliini a gibbous body shape (Fig. 1E; ch. 0.1) and a well developed and spiniform second galeomere (ch. 101.1, convergent with Copelatinae), two apomorphies absent in Hydaticini and which may argue in favour of a closer relationship between these groups, as suggested by Michat & Alarie (2009); Miller *et al.* (2009) and Miller & Bergsten (2014).

No evidence was found for a placement of *Notaticus* within the tribe Hydaticini, as suggested by Zimmermann (1928) and Ribera *et al.* (2002), although both taxa share the presence of a basoventral patch of dense slender spinulae on the protarsus in instars II and III (Fig. 12J; ch. 240.1), an unusual feature also found in Coptotominae, Cybistrinae and *Hyderodes*.

The tribe Hydaticini is recovered as monophyletic in agreement with previous results of Miller (2000, 2001), Ribera *et al.* (2008), Miller *et al.* (2009) and Miller & Bergsten (2014). The support for the group, however, was weak (Fig. 3). Other analyses recovered Hydaticini as paraphyletic (Ribera *et al.*, 2002; Miller, 2003). Whereas adult characters give strong support to the Hydaticini (Miller, 2000, 2001), there are few larval characters useful to distinguish members of this tribe (Michat & Torres, 2006). Larvae are unique in that the median process of the prementum is bifid from the base, appearing as two separate lobes (Fig. 11J; ch. 140.5). This character clearly differentiates hydaticines from any other dytiscid, and is therefore a strong argument in favour of the monophyly of the group. The median process, however, is apparently dimorphic within the genus, being either bifid or trifid (Fig. 11K) depending on the species considered (Dettner, 1984; Michat & Torres, 2006). The submedial insertion of the seta AN3 on the third antennomere (ch. 71.1) also separates Hydaticini from all other dytiscine taxa, although a similar state is present in several other groups of diving beetles. The absence of the seta FE6 on the femur (Fig. 12I; ch. 190.2) links Hydaticini to *Thermonectus* Dejean. Diagnostic characters for this tribe are summarized in Table S5.

The clade formed by the tribes Eretini and Aciliini stands out as one of the most strongly supported in this analysis (Fig. 3) as well as in most previous analyses (Miller, 2000, 2001; Ribera *et al.*, 2002, 2008; Alarie *et al.*, 2011a; Bukontaite *et al.*, 2014; Miller & Bergsten, 2014). Larvae of both taxa are characterized by several synapomorphies (Fig. 6), including the lanceolate aspect of the setae FR3, PA16, PA19 and AB9 (ch. 20.1), the ventral insertion of the setae FR9 and FR10 with respect to the lamellae clypeales (ch. 25.1), the strong development of the two anterodorsal stemmata (ch. 43.1), an elongate, subtrapezoidal to subtriangular stipes (Fig. 10C, F; ch. 98.2), the presence of a lateroventral subapical process on the third maxillary palpomere (ch. 109.1), the presence of a row of additional spine-like setae on the dorsal surface of the stipes (Fig. 10F; ch. 129.3), the presence of a row of long hair-like secondary setae along the external margin of the stipes in instars II and III (Fig. 10F; ch. 134.1), the more proximal position of the seta TI5 on the metatibia (ch. 210.1), and the absence of the pretarsal setae PT1 and PT2 (Fig. 13F, H, I; ch. 242.1).

Although the monophyly of Eretini was not tested owing to inclusion of only one species of *Eretes*, it is indicated by the presence of apically bifid lamellae clypeales in instar I (Fig. 7L; ch. 35.2), the distal position of the pore ANf on the third antennomere (Fig. 9A; ch. 74.1), the presence of an elongate and spiniform lacinia on the anterior mesal margin of the stipes (Fig. 10C; ch. 99.1), the lanceolate aspect of the setae MX2, MX3 and LA11 (Figs 10C, 11G; ch. 110.1), and the posterodorsodistal insertion of the tarsal seta TA5 (Fig. 13F;

ch. 228.1) (see also Alarie *et al.*, 2011a). All these characters summed to the absence of the pore FRf on the frontoclypeus (ch. 31.1, convergent with Aubehydrini), the absence of the seta AB13 on the abdominal segment VIII (ch. 275.1, convergent with Coptotominae), and the adult characters provided by Miller (2001) contribute to the highly derived condition of Eretini within Dytiscidae, in line with previous studies. Selected diagnostic characters are shown in Table S5.

The tribe Aciliini is recovered as monophyletic with strong support (Fig. 3). Although four genera with unknown larvae could not be included in our analysis, the monophyly of this lineage is suggested by the following unambiguous synapomorphies (Fig. 6): occipital foramen deeply emarginate dorsally (Fig. 7Q; ch. 42.1), apical lateroventral process of the third antennomere protruding, either rounded or dentate apically (Fig. 9F; ch. 67.1), presence of a row of secondary hair-like setae on the basolateral margin of the mandible in instars II and III (Fig. 9L; ch. 94.1), and absence of the tarsal pores TAc, TAd, TAe and TAf (Fig. 13H, I; ch. 230.1). This tribe was consistently recovered as monophyletic in previous studies based on adult, larval and molecular characters (Burmeister, 1976; Miller, 2000, 2001, 2003; Ribera *et al.*, 2002; Alarie *et al.*, 2011a; Bukontaite *et al.*, 2014; Miller & Bergsten, 2014), with the exception of Ribera *et al.* (2008) in which Aciliini was paraphyletic with respect to Eretini. The group is diagnosed based on the characters presented in Table S5.

Subfamily Hydroporinae

The subfamily Hydroporinae is generally recognized as monophyletic (Burmeister, 1976; Wolfe, 1985; Miller, 2001; Ribera *et al.*, 2002; Miller *et al.*, 2006; Michat *et al.*, 2007; Miller & Bergsten, 2014). An exception is Ribera *et al.* (2008) in which Hydroporinae was recovered as paraphyletic with respect to Laccophilini. There is, however, no general agreement with respect to the identity of its sister taxon. Ruhnau & Brancucci (1984) and Wolfe (1985) suggested a sister relationship of Hydroporinae and Laccophilinae. Miller (2001) and Miller & Bergsten (2014) found Hydroporinae more closely related to Hydrodytinae. Ribera *et al.* (2002) proposed a sister-group relationship of Hydroporinae with the remaining Dytiscidae, a result later supported by Michat (2010) and Michat & Alarie (2013). Previous papers based on adult (Wolfe, 1985, 1988; Miller, 2001; Miller *et al.*, 2006) and larval (Alarie & Michat, 2007a; Michat *et al.*, 2007) morphology suggested that Laccornini, Methlini, *Laccornellus* Roughley & Wolfe and *Canthyporus* Zimmermann are ancestral lineages within Hydroporinae. The position of Hydrovatini, however, is more controversial; whereas several studies proposed an ancestral condition (Burmeister, 1976; Wolfe, 1985, 1988; Alarie & Michat, 2007a; Michat *et al.*, 2007), others suggested a more derived position (Miller, 2001; Michat, 2006; Miller *et al.*, 2006); molecular studies suggested other alternatives (Ribera *et al.*, 2002, 2008; Miller & Bergsten, 2014).

Hydroporinae is strongly supported in this analysis (Figs 3, 5), characterized by many synapomorphies, including a strongly

curved frontal suture (Fig. 8A; ch. 5.1), the presence of a well-developed nasale (Fig. 8A; ch. 7.4), obliquely oriented mandibles (Fig. 8E; ch. 83.1), the absence of the frontoclypeal setae FR4 and FR5 (chs 21.1 and 22.2), the absence of the seta PA7 and the pore PAp on the parietal (Fig. 8A; chs 45.1 and 55.0), the absence of the pore ANi on the antenna (Fig. 8A; ch. 77.1), the absence of the pore MXc on the maxilla (ch. 121.0) and the long and hair-like aspect of the labial setae LA4 and LA5 (Fig. 11H; chs 149.1 and 151.1). Other character states defining this subfamily are the elongate aspect of the frontoclypeal seta FR10 (Fig. 8A; ch. 26.1, shared with Matinae), the pore PAb inserted far from the seta PA3 (ch. 47.1, shared with Coptotominae), an inconspicuous palpifer not clearly differentiated from the stipes (Fig. 10E; ch. 102.1, also present in Aciliini), and the absence of the galeal pore MXd, the labial pore LAc and the trochanteral seta TR3 (chs 122.2, 159.2 and 182.1, shared with *Laccomimus* Toledo & Michat). As the Hydroporinae most likely represent a natural group, similar character states found outside this subfamily are considered convergences. Our results support a sister-group relationship of Hydroporinae with the remaining Dytiscidae except Laccophilinae; however, support for this relationship was weak. The subfamily is presently composed of 10 tribes (Miller & Bergsten, 2014). Other than Laccornini (represented by a single species), we found evidence for monophyly of most tribes (Methlini, Hydrovatini, Pachydrini, Vatellini, Hygrotini, Hyphydrini and Bidessini). The support obtained for these groups was generally strong, with the exception of the weakly supported Hygrotini and the moderately supported Bidessini. The only nonmonophyletic tribes in our analysis are Hydroporini (consistent with most previous studies) and Laccornellini. Relationships among tribes, however, were weakly supported in general (Fig. 3).

The most basal branchings within Hydroporinae separate successively into the tribes Methlini, Laccornini and Laccornellini + Hydrovatini, the latter from a large clade including the remaining tribes (Fig. 3). Although the support obtained for these relationships was weak, the hypothesis of an ancestral condition of these taxa is in line with previous studies (see earlier). Larvae of Methlini, Laccornini, Laccornellini (*Canthyporus* needing corroboration) and Hydrovatini bear a small galea (ch. 101.2). The absence of this structure in the remaining Hydroporinae and its presence within most Adephaga and Dytiscidae (exceptions: Paelobiidae and Cybistrinae) support the hypothesis that these taxa retained the plesiomorphic condition (Michat *et al.*, 2007). Another character supporting an ancestral condition of these taxa is the contiguous insertion of the setae PA1, PA2 and PA3 on the dorsoposterior surface of the parietal (ch. 44.0, convergent with *Pachydrus* Sharp and *Microdytes* J. Balfour-Browne). This condition is found extensively within Adephaga, including all nonhydroporine dytiscids, and thus is most likely plesiomorphic. Seta PA3, however, is inserted somewhat more distantly from PA1 and PA2 in larvae of *Celina* Aubé.

The tribe Methlini has been treated at the subfamily rank by some authors (e.g. Bertrand, 1972; Spangler, 1973b; Pederzani, 1995). In our analysis, this group was recovered as sister of the rest of the subfamily with moderate support (Fig. 3), in

agreement with previous larval studies (Michat *et al.*, 2007) but not with analyses based on adult and molecular characters (e.g. Wolfe, 1985; Miller, 2001; Miller & Bergsten, 2014). As stated by Michat *et al.* (2007), giving Methlini subfamily rank would leave Hydroporinae with a single larval synapomorphy, the absence of the seta MX10 on the maxillary palpifer (ch. 117.1). Therefore, there is a great utility in maintaining Methlini as a tribe within Hydroporinae. Characters supporting Methlini as the sister to all other hydroporines include presence of the seta MX10 on the maxillary palpifer (Fig. 10D; ch. 117.0), presence of the seta PA18 on the ventral surface of the parietal (Fig. 8K; ch. 46.0, convergent with Vatellini), and presence of the pore PAd on the dorsal surface of the parietal (ch. 49.0, shared with Laccornini). These character states are found in the outgroup taxa and also in all nonhydroporine dytiscids, supporting the hypothesis that members of Methlini retained the plesiomorphic condition. Larvae of Methlini are unique within Dytiscidae in the posterior projection of the principal abdominal tracheal trunks from the apex of the siphon (Fig. 15F, G; ch. 262.1) (see diagnosis in Table S5).

Our study contradicts previous hypotheses that proposed Laccornini as sister to the rest of Hydroporinae (Wolfe, 1985; Miller, 2001; Miller & Bergsten, 2014). Our results posit *Laccornis* Gozis as sister to all hydroporines except Methlini (Fig. 3). Larvae of Laccornini are characterized by the proximal insertion of the seta FR13 on the ventral surface of the frontoclypeus (ch. 27.2, convergent with *Antiporus* Sharp and *Hyphydrus* Illiger), the presence of the pore PAd on the parietal (Fig. 8D; ch. 49.0, shared with Methlini and all nonhydroporine dytiscids), the presence of the pore ABa on the abdominal segment VIII (Fig. 15A; ch. 278.0, convergent with *Laccornellus* and all nonhydroporine dytiscids), and the apical insertion of the seta UR8 on the second urogomphomere (Fig. 16G; ch. 297.1, shared with Methlini and Vatellini).

The tribe Laccornellini was not resolved as monophyletic in our analysis, with the genus *Canthyporus* sister to Hydrovatini and *Laccornellus* sister to both taxa (Fig. 3). No convincing characters were discovered in favour of a close relationship of *Laccornellus* and *Canthyporus*, despite both genera appear to be closely related based on adult morphology and DNA sequence data (Wolfe, 1985; Ribera *et al.*, 2008; Miller & Bergsten, 2014). The clade formed by Laccornellini and Hydrovatini, although weakly supported, is defined by two larval characters not found in other dytiscids (Fig. 5), i.e. the strong development of the seta AB4 on the abdominal segment VIII (Fig. 15D; ch. 266.1) and the absence of the seta UR8 on the urogomphus (Fig. 16E, F; ch. 297.5). Another character supporting this grouping is the absence of the seta LA2 on the prementum (Fig. 11M; ch. 147.1, convergent with Vatellini) (Alarie & Michat, 2007a). The tribe Laccornellini can be separated from all other dytiscid taxa except Hydrovatini by the combination of characters given in Table S5. It can be separated from Hydrovatini in the shorter and more triangular nasale (ch. 13.2) and in the presence of an occipital suture in instars II and III (ch. 41.1), among other characters.

Based mainly on adult morphology, Wolfe (1985) suggested that *Hydrovatus* Motschulsky may be closely related to Methlini. Miller (2001) and Miller *et al.* (2006) proposed a derived condition of Hydrovatini, more closely related to Hygrotini and Hyphydrini. Larvae of *Hydrovatus* apparently bear a puzzling combination of presumably plesiomorphic characters that relate the genus to some ancestral taxa (see earlier), with apomorphic characters that link it to more derived taxa such as Hyphydrini. In our study, Hydrovatini is sister to *Canthyporus* and both are sister to *Laccornellus*, suggesting a close relationship with the tribe Laccornellini, although one that is weakly supported (Fig. 3). Hydrovatini is highly distinctive within Dytiscidae, being characterized by the proximal position of the egg bursters on the frontoclypeus (Fig. 8B; ch. 17.2, convergent with Laccophilinae and Aubehydrini), the absence of the pores PAj and PAo on the ventral surface of the parietal (Fig. 8J; chs 51.1 and 54.1, convergent with Bidessini and Hyphydrini and some Aciliini, respectively), the hair-like appearance of the mandibular sensillum MN2 (Fig. 9K; ch. 90.1), the presence of basoventral spinulae on the claws in instar I (ch. 243.1, convergent with Colymbetinae and some Dytiscinae sensu lato), and the sclerotized ventral surface of the abdominal segments II–V in instar III (Fig. 14C; chs 248.0, 249.0 and 250.0, convergent with Hyphydrini and some Laccophilinae). Diagnosis of this group is firm, based on several characters (Table S5).

The clade formed by the tribes Hydroporini, Hygrotini, Bidessini, Vatellini, Pachydrini and Hyphydrini received weak support despite larvae of this group being characterized by the absence of a galea (ch. 101.3, convergent with Cybistrinae), the absence of the galeal setae MX8 and MX9 (chs 115.4 and 116.3, present in Pachydrini), and the insertion of the galeal pore MXh on the stipes (ch. 125.1, convergent with Cybistrinae, absent in Pachydrini). The absence of a galea in Cybistrinae is most likely the result of parallel evolution (Michat, 2010), although Ribera *et al.* (2008) found a close relationship between Cybistrinae and Hydroporinae. The branching pattern within this large clade was poorly supported, preventing the clarification of inter-tribe relationships. Particularly notorious is paraphyly of the tribe Hydroporini with respect to the remaining tribes. A nonmonophyletic Hydroporini is in agreement with most previous analyses except Miller & Bergsten (2014) who found it monophyletic after *Laccornellus* and *Canthyporus* were excluded. No convincing larval characters were discovered in this study that unambiguously support Hydroporini as presently defined, even after refinement. The distal insertion of the seta UR8 on the second urogomphomere (Table S5; Fig. 16H; ch. 297.2) gives some support to this tribe, although a similar state is present in Bidessini.

The monophyly of the tribe Hygrotini suggested by this study should be treated with care. On the one hand, the support obtained for the group as well as for its internal clades was weak (Fig. 3), and on the other hand, previous evidence of a monophyletic origin of members of this tribe is equivocal, with molecular studies (Ribera *et al.*, 2002, 2008; Miller & Bergsten, 2014) suggesting monophyly, and studies based on adult morphology (Miller, 2001; Miller *et al.*, 2006) suggesting para- or polyphyly. In our study, Hygrotini is supported by scarce and homoplasious characters, and we were unable to find unambiguous synapomorphies for the group. The only character state sustaining the tribe is the submedial insertion of

the seta UR8 on the second urogomphomere (Table S5; Fig. 16I; ch. 297.3, see also Alarie & Harper, 1990), convergent with Pachydrini.

Bidessini are also characterized by few and homoplasious characters, although the group was always recovered as monophyletic in this study, with moderate to strong support (Fig. 3). This result is compatible with previous analyses based on various datasets (e.g. Miller *et al.*, 2006; Michat & Alarie, 2008; Miller & Bergsten, 2014). Members of this tribe share the absence of the pore ABc on the abdominal segment VIII (Fig. 15B; ch. 279.1) (Michat & Alarie, 2008). Absence of this pore in Coptotominae, Eretini, Aciliini and *Laccophilus* Leach is deemed a convergence. Absence of the pore PAj on the ventral surface of the parietal (Fig. 8I; ch. 51.1) also gives support to the group, although similar states are present in Hydrovatini and Hyphydrini. This character combination separates bidessines from every other known diving beetle larva (Table S5). The exclusion of *Amarodytes* Régimbart from Bidessini proposed by Biström (1988) based on adult characters is not supported, as this genus is nested well within Bidessini in our study, although with weak support.

There is a general agreement in recognizing Vatellini as a natural group (e.g. Miller, 2001, 2005; Michat & Torres, 2005, 2011; Miller *et al.*, 2006; Ribera *et al.*, 2008). In our study, this tribe is recovered as monophyletic with strong support (Fig. 3). The sister group of Vatellini was found to be a clade formed by Pachydrini and Hyphydrini, all three taxa sharing the absence or pore-like condition of the sensillum AB2 on the abdominal segment VIII (Fig. 15C; ch. 264.1–2) and the hair-like condition of the tibial seta TI7 (Fig. 12K; ch. 213.1, convergent with several nonhydroporine taxa and reversed in *Microdytes*). The Australian genus *Antiporus* (presently included in the tribe Hydroporini) is sister to this clade based mainly on the narrow and parallel-sided nasale (ch. 13.3, convergent with Hydrovatini and reversed in *Microdytes*). Larvae of Vatellini have evolved a large number of synapomorphies (Fig. 5), including the strong development of the lateral branches of the nasale and of the seta FR6 (Fig. 8G; chs 15.3 and 23.1), the absence of the pore MXj on the maxillary palpus (Fig. 10H; ch. 127.2), the absence of the seta LA9 and the pore LAd on the labial palpus (Fig. 11A; chs 154.1 and 160.0), and the strong elongation of the siphon (Fig. 15E; ch. 263.3). Other character states supporting this tribe are the presence of an elongate and subcylindrical stipes (Fig. 10H; ch. 98.1), resembling that of Cybistrinae and some members of Dytiscinae, and the absence of the setae MX7 on the stipes and AB2 on the abdominal segment VIII (chs 114.1 and 264.2), two features linking Vatellini to Pachydrini. Vatellines are a highly characteristic group of diving beetles, easily diagnosed by the characters given in Table S5.

The genera *Pachydrus* and *Heterhydrus* Fairmaire were placed in a separate tribe (Pachydrini) by Biström *et al.* (1997), a hypothesis previously suggested by Young (1980) and later supported by other researchers (Ribera *et al.*, 2002, 2008; Ribera & Balke, 2007; Michat & Torres, 2008; Miller & Bergsten, 2014). Other studies, however, supported the inclusion of *Pachydrus* and *Heterhydrus* in Hyphydrini and rejected the use of Pachydrini (Miller, 2001; Alarie & Challet, 2006; Miller *et al.*, 2006).

In this study, Hyphydrini and Pachydrini are each monophyletic and strongly supported by characters (Fig. 3). Both tribes are resolved as sister groups with weak support, although previous iterations of this analysis including less characters and taxa recovered Pachydrini in a more basal position, closely related to Hydrovatini, Laccornellini, Laccornini and Methlini. Members of Hyphydrini and Pachydrini share the absence of the antennal pore ANh (ch. 76.0), a sclerotized ventral surface of the abdominal segment VI (chs 251.0 and 252.0), and the proximal insertion of the seta LA8 on the prementum (ch. 153.2), all convergent with Hydrovatini (the former three also with some Laccophilinae). Based on our results, the question of including *Pachydrus* in Pachydrini or in Hyphydrini (as the most basal member) remains open. However, evidence of monophyly for each of them is strong, whereas evidence of monophyly for both together is weak. Even if future evidence confirms that they are sister groups, we tend to favour a scenario in which Hyphydrini and Pachydrini are recognized as separate tribes (see also Michat & Torres, 2008).

Larvae of Pachydrini (*Heterhydrus* not studied) are unique within Dytiscidae in the presence of a row of elongate, robust, apically truncate spinulae on the laterobasal margin of the nasale (Fig. 8H; ch. 16.1), the absence of the galeal pore MXh (ch. 125.2), the absence of the coxal pore COa (Fig. 12A; ch. 174.1), the femoral setae FE2 and FE6 not inserted apically (Fig. 12A, K; chs 186.1 and 190.1), and the long and hair-like aspect of the seta TI2 on the meso- and metatibia (Fig. 12K; ch. 207.1). The absence of the seta LA1 on the prementum (Fig. 11O; ch. 146.1) and the presence of natatory setae on the anteroventral margin of the femur in instars II and III (Fig. 12F; ch. 200.1) are also very distinctive, although similar states occur in larvae of *Derovatellus* Sharp and Dytiscinae sensu lato, respectively, most likely evolved independently. On the other hand, larvae of Hyphydrini are unique in the absence of the pore FRb on the frontoclypeus (Fig. 8C; ch. 28.1), the position of the pore PAc anterior to the stemmata (Fig. 8C; ch. 48.1), a prementum clearly longer than it is broad (Fig. 11L; ch. 136.1), the pore-like aspect of the sensillum AB2 on the abdominal segment VIII (Fig. 15C; ch. 264.1), and the proximal insertion of the seta UR8 on the second urogomphomere (Fig. 16J; ch. 297.4). The exclusion of the genus *Desmopachria* from Hyphydrini suggested by Young (1980) based on adult characters is not supported by this study. *Desmopachria* is well placed within Hyphydrini and robustly linked to *Hyphydrus* by the characteristic shape of the second labial palpomere (ch. 142.1) (Alarie *et al.*, 1997).

Subfamily Laccophilinae

The subfamily Laccophilinae was historically composed of a single tribe (Laccophilini). Burmeister (1976, 1990) noted a number of common adult synapomorphies in the genera *Laccophilus* and *Agabetes*, and postulated a sister-group relationship of Laccophilinae and what he considered a distinct subfamily, Agabetinae. Nilsson (1989) relegated Agabetinae to the rank of tribe within Laccophilinae. Miller (2001) supported this hypothesis so that the Laccophilinae presently includes two

tribes, Agabetini and Laccophilini (Nilsson, 2015). Larval studies (Alarie *et al.*, 2000, 2002b) as well as most recent molecular analyses (Miller & Bergsten, 2014) postulated a sister relationship of Agabetini and Laccophilini. Previous molecular studies (Ribera *et al.*, 2002, 2008), however, contradict this view by suggesting a close relationship of Agabetini with Lancetinae and Colymbetinae. The phylogenetic relationships and potential sister group of the Laccophilinae are also contentious. Ruhnau & Brancucci (1984), Wolfe (1985) and De Marzo (1997) suggested that Laccophilinae and Hydroporinae may be closely related. Nilsson (1989) suggested a close relationship of Laccophilinae with Lancetinae. Miller (2001) postulated a sister relationship of Laccophilinae with a clade formed by the subfamilies Coptotominae, Copelatinae, Hydrodytinae and Hydroporinae. Ribera *et al.* (2002, 2008) found Laccophilinae (excluding Agabetini) was closely related to Copelatinae and Hydroporinae, respectively. Michat & Alarie (2013) proposed a close relationship of Laccophilinae with Coptotominae and Lancetinae.

The results of this study are in favour of a sister-group relationship of Laccophilinae with the rest of Dytiscidae. This hypothesis (largely unexpected) received relatively weak support and there are no convincing characters sustaining it (Figs 3, 5). It is also not in line with previous studies based on various datasets. As the larvae of most Laccophilini genera are still unknown, however, this result should not be viewed as firmly based. Laccophilinae sensu lato (i.e. including Agabetini) is recovered as monophyletic although weakly supported. Larvae of this subfamily are characterized by a well developed spinulose epipharyngeal band, which is visible in dorsal view anterior to the lamellae clypeales in instar I (Fig. 7B; ch. 3.1, reversed in *Neptosternus*), the proximal position of the egg bursters in instar I (Fig. 7B; ch. 17.2, convergent with Hydrovatini and Aubehydrini), and the presence of two lamellae clypeales in instar I (Fig. 7B, N; ch. 33.1), an apparently regained plesiomorphic condition convergent with Coptotominae and Lancetinae.

Our results posit Agabetini and Laccophilini as sister groups, in agreement with adult morphological and most recent molecular studies but not with previous molecular analyses (see earlier). The support obtained for this relationship, however, was weak. The tribe Agabetini is characterized by the presence of an additional pore on the ventral surface of the prementum (Fig. 11B; ch. 163.1, convergent with *Neptosternus* and *Spencerhydrus* Sharp), and the absence of the seta AB15 (Fig. 15H; ch. 277.2, convergent with most Hydroporinae). It is also distinguished from other Laccophilinae by the presence of one-segmented urogomphi (Fig. 15H; ch. 289.0). On the other hand, the tribe Laccophilini is supported by the absence of the seta AB14 on the abdominal segment VIII (Fig. 15I, J; ch. 276.2, convergent with Hydroporinae and Copelatinae) and the presence of two-segmented urogomphi (Fig. 16D; ch. 289.1, convergent with Hydroporinae and most Agabinae). The weak support obtained for the Laccophilini is probably caused by the inclusion of the recently described genus *Laccomimus* (Toledo & Michat, 2015). Larvae of this genus (Michat & Toledo, 2015) share several morphological characters with those of the remaining Laccophilini genera, but also with those of *Agabetes* (see strong support for Laccophilini beyond *Laccomimus*).

Some Laccophilini genera (*Laccophilus*, *Neptosternus*) are characterized by an unusual shape of the frontoclypeus in the first instars (Alarie *et al.*, 2000). This character state is not shared by larvae of *Agabetes* (Alarie *et al.*, 2002b) and, as first instars of other Laccophilini genera were unknown, it remained a putative diagnostic feature for the tribe Laccophilini. In first instars of *Laccomimus*, however, the frontoclypeus exhibits the usual triangular shape most commonly encountered within Dytiscidae (Fig. 7B) (Michat & Toledo, 2015). Therefore, this character seems useful for the separation of taxa at the genus level and does not communicate much information at higher taxonomic levels. As a consequence of the absence of unambiguous characters, diagnoses of the subfamily Laccophilinae and its tribes are based on ambiguous characters (Table S5).

Subfamily Lancetinae

Based on adult morphology, the genus *Lancetes* was traditionally included in the subfamily Colymbetinae, either as a distinct tribe, Lancetini (e.g. Pederzani, 1995), or as a member of the tribes Coptotomini (Brinck, 1948) or Colymbetini (e.g. Franciscolo, 1979). This placement, however, was questioned by several authors based on adult (Nilsson, 1989) and larval (Beier, 1928; Ruhnau & Brancucci, 1984; Nilsson, 1988; Alarie *et al.*, 2002a) characters. Ruhnau & Brancucci (1984) proposed a close relationship with the subfamilies Colymbetinae and Dytiscinae, but were unable to elucidate the identity of the sister group of *Lancetes*. Nilsson (1988) raised the question regarding the uncertain position of the group, and suggested that it could be related to *Laccophilus* and *Coptotomus*, a hypothesis later supported by Michat & Alarie (2013). Nilsson (1989) related *Lancetes* with Laccophilinae. Nilsson & Roughley (1997) placed *Lancetes* in its own subfamily, Lancetinae, followed by Miller (2001). A sister-group relationship of Lancetinae and Dytiscinae was proposed based on adults (Miller, 2001) and larvae (Alarie *et al.*, 2002a). Molecular characters, however, placed Lancetinae close to Colymbetinae (Ribera *et al.*, 2002), Agabetini (Ribera *et al.*, 2008) or a clade comprising Colymbetinae and Agabinae (Miller & Bergsten, 2014).

Our study places Lancetinae as sister to Coptotominae with moderate support (Fig. 3). This result agrees with the opinion of Brinck (1948), who related both taxa based on a similar morphology of the male parameres. Larvae of both subfamilies share the presence of only two lamellae clypeales in instar I (Fig. 7M; ch. 33.1), a second labial palpomere subdivided into two articles in instar III (ch. 145.1), and the submedial insertion of the seta FE1 on the femur (ch. 185.1). The former character state, however, suggests that Lancetinae and Coptotominae have regained the plesiomorphic condition found in several taxa outside Dytiscidae and in Laccophilinae, and the latter two are not restricted to these taxa (see earlier under Coptotominae).

The clade Lancetinae + Coptotominae is resolved as sister to the clade Dytiscinae + Cybistrinae, all these taxa supported by three synapomorphies (Fig. 6): the presence of a lateroventral process on the fourth antennomere (Fig. 9F; ch. 70.1), and the secondary subdivision of the second and third

antennomeres (Fig. 9H; ch. 65.1) and of the third maxillary palpomere (Fig. 10I, J; ch. 108.1) in two articles. Further evolution may have resulted in an additional subdivision of these structures in Cybistrinae (Figs 9I, 10G) (Michat, 2010). Another synapomorphy, the secondary subdivision of the second maxillary palpomere into two articles (Fig. 10G; ch. 106.1), also characterizes this group, although apparently the Aciliini have undergone a reversion and bear a nonsegmented second palpomere. It is worth mentioning that similar states for all these characters are present in *Meridiorhantus calidus*. Nilsson (1988) mentioned that larvae of *Lancetes*, *Laccophilus*, *Coptotomus* and Dytiscinae (sensu lato, i.e. including Cybistrini) are related by the proximal position of the coxal seta CO7. This is also true in our study (ch. 173.1), although CO7 is not so proximally inserted in Hydaticini and in some members of Aciliini (Alarie *et al.*, 2011a).

A close relationship of Lancetinae with Colymbetinae is not observed in our study, despite larvae of both groups sharing the presence of an elongate fourth antennomere, which is subequal or even somewhat longer than the third antennomere (Fig. 9B; ch. 68.1). Therefore, the placement of *Lancetes* in its own subfamily seems justified. The monophyly of Lancetinae is relatively well supported by larval morphology. Members of this group bear multifragmented urogomphi (Fig. 15I; ch. 290.1), which is not seen in any other known dytiscid larva (Alarie *et al.*, 2002a). The fourth antennomere secondarily subdivided into two articles in instar III (Fig. 9C; ch. 69.1) is also distinctive given that outside Lancetinae this feature is only known to occur in *Meridiorhantus calidus* (Alarie *et al.*, 2009), most likely as the result of evolutionary convergence. The subfamily Lancetinae can be relatively easily diagnosed by the combination of characters presented in Table S5.

Subfamily Matinae

This group has a long history as a tribe within the subfamily Colymbetinae (Brinck, 1948; Young, 1953; Ruhnau & Brancucci, 1984; Nilsson, 1988; Alarie, 1995, 1998; Alarie *et al.*, 2001). More recently, another view was presented in which Matini was raised to subfamily rank and placed as the sister group of the remaining Dytiscidae (Miller, 2001; Miller & Bergsten, 2014). This hypothesis, however, was not corroborated in other studies (e.g. Ribera *et al.*, 2008; Michat & Alarie, 2013). The monophyly of the group is well established based on adult and larval characters (Alarie *et al.*, 2001; Miller, 2001).

Our results support a position of matines outside Colymbetinae, in agreement with recent analyses. The group, however, is not recovered as sister to the rest of the family but in a more derived position, as part of a large clade including the subfamilies Colymbetinae, Coptotominae, Lancetinae, Dytiscinae and Cybistrinae, with moderate support (Fig. 3). Synapomorphies for this clade are the presence of natatory posterodorsal setae on the femur, tibia and tarsus in instars II and III and the long and hair-like aspect of the seta TI6 on the metatibia in instar I (chs 201.1, 224.1, 239.1 and 212.1, respectively, convergent with several hydroporine and laccophiline taxa and reversed in

some groups) (Fig. 6). Miller (2001) mentioned that members of Matinae share several adult character states with a clade containing Colymbetinae, Lancetinae and Dytiscinae sensu lato. This is similar to our results, with the exception that our clade also includes Coptotominae.

Matinae is sister to a clade comprising Colymbetinae, Coptotominae, Lancetinae, Dytiscinae and Cybistrinae, characterized by the following synapomorphies: pores ANi, MXj and LAc inserted submedially (chs 77.1, 127.0 and 159.0), and seta TI6 long and hair-like on pro- and mesotibia (ch. 211.1, convergent with most Laccophilini). All these characters, however, show reversals in some taxa which decrease their phylogenetic value.

The subfamily Matinae appears as one of the most robust lineages in our analysis. Larvae of this group bear a unique character: the seta CO6 is inserted more proximally on the procoxa (Fig. 12C; ch. 172.0). They are also highly distinctive in that the seta TR5 is strongly developed on the metatrochanter (Fig. 12D; ch. 183.1, convergent with Coptotominae) and the seta TA1 is inserted submedially on the tarsus (Fig. 13G; ch. 227.1, convergent with Methlini and most Laccophilini). The subfamily can be diagnosed using the character combination presented in Table S5. Although larvae of *Batrachomatus* are remarkably different from those of *Matus* Aubé (Alarie *et al.*, 2001), they are closely related phylogenetically (see also Miller, 2001). This, summed to the highly disjunct distribution of the genera (Australia and North America respectively), reinforces Young's (1953) hypothesis that they are relics of a once more extensively distributed group.

Evolution of morphological traits associated with larval swimming behaviour

It is generally held that natatory setae on the legs (and, less commonly, on the abdomen and urogomphi) have evolved to enhance swimming ability in dytiscid larvae. Being the most comprehensive phylogenetic analysis provided so far based on larval characters, our study offers an opportunity to look at the evolution of some morphological features of the larvae commonly associated with an increased performance in the water, and to infer how microhabitat structure may have influenced swimming behaviour.

Dytiscids represent one of the most extensive radiations among aquatic beetles (Hunt *et al.*, 2007), with both adults and larvae inhabiting practically every fresh water environment (Balke & Hendrich, 2016). Among the various factors affecting the occurrence of larvae in a particular microhabitat, the presence and composition of the sediments and of the aquatic vegetation apparently play a major role (Galewski, 1971). Unfortunately, biological information for dytiscid larvae (e.g. Needham & Williamson, 1907; Wilson, 1923; Balduf, 1935; Galewski, 1971) is scant, and the biology of several groups is known only superficially, or not at all. Galewski (1971) divided diving beetle larvae into several groups according to their microhabitat use: (i) larvae mostly creeping among loose bottom sediments, often burrowing among rotting plant debris or detritus (Hydroporinae); (ii) larvae mainly creeping on the

surface of the bottom or on the vegetation (Copelatinae, most Agabinae); (iii) larvae swimming and creeping among the vegetation, relatively independent from the bottom (Colymbetinae, most Laccophilinae) – members of Coptotominae, Matinae and Lancetinae may also belong to this group (Wilson, 1923; Wolfe & Roughley, 1985; M.C. Michat, unpublished observations); (iv) larvae swimming both among the vegetation and in open waters, most of them very good swimmers capable of floating (Dytiscinae). Although Galewski's (1971) study was restricted to the Central European fauna, our observations in America tend to confirm these broad categories.

The most basal branching in our consensus cladogram (Fig. 3) separates Laccophilinae from the rest of the family. Laccophilinae, in turn, splits into two sister tribes: Agabetini, the larvae of which lack natatory setae, and Laccophilini, the larvae of which have developed fringes of natatory setae on the posterodorsal surface of the tibiae and tarsi, although only in instars II and III. The second branching leads to Hydroporinae, the largest subfamily comprising about half of dytiscid species. Most hydroporines, including all earlier diverging lineages (Methlini, Laccornini, Laccornellini, Hydrovatini), lack natatory setae. Some derived groups such as Hyphydrini, Vatellini, and isolated genera within Hydroporini, Hygrotini and Bidessini, however, have developed natatory setae, although similarly to Laccophilini, they are restricted to the posterodorsal surface of the tibiae and tarsi of the later instars. The third branching leads to the clade Agabinae + Copelatinae, the larvae of which lack natatory setae. In the rest of the family (i.e. the clade formed by the subfamilies Matinae, Colymbetinae, Coptotominae, Lancetinae, Dytiscinae and Cybistrinae) the presence of natatory setae seems to be the rule. Larvae of this large group bear natatory setae at least in the same positions and instars mentioned earlier, although further development of additional setal fringes, in all instars, has taken place in members of the subfamilies Dytiscinae and Cybistrinae.

Based on this branching pattern, we suggest the following scenario for the evolution of natatory setae. Absence of natatory setae is plesiomorphic for Dytiscidae. This hypothesis is based on the fact that most outgroups, as well as the earliest diverging lineages within the family (i.e. Agabinae, Copelatinae, Agabetini, most Hydroporinae), lack these structures. The presence of natatory setae in larvae of Hygrobiidae is viewed as the result of parallel evolution. Available biological information indicates that larvae of all these dytiscid taxa mainly inhabit the bottom of water bodies, and lack particular adaptations for swimming (Galewski, 1971; Alarie *et al.*, 1990, 2002b; Alarie, 1995; Michat & Alarie, 2008; Michat & Archangelsky, 2009; Michat & Torres, 2009). Natatory setae on the legs evolved several times independently within Dytiscidae: in the tribe Laccophilini; in the common ancestor of the large group including Matinae, Colymbetinae, Coptotominae, Lancetinae, Dytiscinae and Cybistrinae; and several times within the Hydroporinae. Contrary to earlier diverging lineages, biological data (Wilson, 1923; Galewski, 1971; Michat & Torres, 2011, 2013) suggest that larvae of these taxa live in association with the aquatic vegetation, thus supporting several independent transitions into more vegetated microhabitats. As suggested by Galewski (1971), a

correlation between presence of natatory setae and utilization of more vegetated microhabitats seems evident. Further evolution in the clade formed by Dytiscinae and Cybistrinae resulted in the occurrence of additional setal fringes. Larvae of this group bear natatory setae on both the dorsal and ventral margins of femora and tibiae in all instars, and also on the tarsi in instars II and III. In addition, dense fringes of natatory setae are present on each side of the abdominal segments VII and VIII. Instars II and III of Coptotominae also exhibit these abdominal setal fringes (Michat & Alarie, 2013), most likely evolved independently. Finally, larvae of the tribe Dytiscini have evolved a row of natatory setae on the external margin of the urogomphi. All these morphological adaptations of dytiscine and cybistrine larvae place them as the most efficient swimmers of all dytiscids, being able to exploit not only vegetated microhabitats, but also open water areas free of vegetation, as in Aciliini (Galewski, 1971).

In conclusion, larval swimming ability, and its associated morphological structures, may have evolved in response to transitions to novel microhabitats, such as those structured by the aquatic vegetation, by larvae primitively inhabiting less complex microhabitats such as the sediments of water bodies. Perhaps this capacity of the larvae for exploiting the broad spectrum of microhabitats available in the water has contributed significantly to the extensive radiation experienced by diving beetles.

Evolution of the galea

Presence of a well-developed, two-segmented galea arising from the larval stipes is part of the ground-plan condition of the suborder Adephaga (Bousquet & Goulet, 1984; Alarie *et al.*, 2011b). The two galeal articles (= galeomeres) are most commonly subcylindrical, although the basal one is sometimes short and more or less globose, and the distal one is commonly tronco-conical. According to our cladogram, the plesiomorphic condition for Dytiscidae is presence of a galea, although different degrees of reduction are observed. In the subfamilies Agabinae, Colymbetinae and, to a lesser extent, Copelatinae, the first galeomere can still be distinguished as a short structure partially fused to the stipes (Fig. 10A, B), more evident in later instars, and the primary seta MX7 is inserted on this structure, as in most adephagans. In other dytiscids (except Cybistrinae and most Hydroporinae) and in haliplids, the first galeomere is completely fused and indistinguishable from the stipes, and the seta MX7 appears to be inserted on the stipes, close to the base of the second galeomere (Fig. 10C). Three independent evolutionary losses resulted in the complete absence of the galea in Hygrobiidae and, within Dytiscidae, in the subfamily Cybistrinae and in the common ancestor of a large group in the subfamily Hydroporinae (see also Alarie *et al.*, 2004; Michat *et al.*, 2007; Michat, 2010). As mentioned earlier, absence of this structure is not shared by all hydroporines, as earlier diverging members of this subfamily bear a small galea. All three losses have most likely taken place by a complete fusion of the galea with the stipes, as the primary setae and pores commonly inserted on the galea (or at least some of them) in other groups are inserted on the stipes in these taxa (Alarie *et al.*, 2004;

Michat *et al.*, 2007; Michat, 2010). Finally, the second gale-omere evolved to a more rigid and pointed (spiniform) structure in some dytiscid groups (Fig. 10C). This modification probably occurred two or three times independently: in Copelatinae and, within Dytiscinae, in Aubehydrini and in the common ancestor of Eretini + Aciliini (or, alternatively, in the common ancestor of Aubehydrini + Hydaticini + Eretini + Aciliini, and then reversed in Hydaticini). The functional significance of this structural modification is unknown, although it can be hypothesized that the galea in these groups lost its original sensory function and adopted a more mechanical function in feeding, assisting the mandible in a role more similar to that of the lacinia.

Conclusions and future directions

Nilsson (1989) stated that dytiscid phylogeny will most probably be very difficult to reconstruct due to widespread convergent evolution. This is particularly difficult when trying to resolve relationships at deeper nodes, i.e. among subfamilies and tribes. At this level, it is difficult to find characters that are not affected by homoplasy or apparent secondary loss. As a consequence, the support obtained in our analysis for some subfamilies and subfamilial relationships was weak. This phenomenon, however, is not exclusive of studies based on larval morphology. Lack of resolution and support for most inclusive nodes appears to affect dytiscid phylogenetic analyses irrespective of the character system being considered (see also Miller, 2001; Ribera *et al.*, 2008). One possible solution could be to combine different character systems. Studies doing so in a broader level within Dytiscidae are lacking, with the exception of Miller & Bergsten (2014), who combined adult morphological and molecular characters. It is generally held that the more characters supporting a clade, the more plausible is the hypothesis that the clade represents a natural group (DeSalle & Brower, 1997). When a phylogenetic hypothesis is supported by several independent lines of evidence, we gain confidence in it as an estimate of phylogenetic history (Lanyon, 1993). Despite some attempts to combine different datasets at less inclusive taxonomic levels (Wolfe, 1985; Miller, 2003; Miller *et al.*, 2006, 2007, 2009; Ribera & Balke, 2007), the development of large-scale phylogenetic analyses combining as many character systems as possible is still a challenge for dytiscid taxonomists interested in phylogeny.

Many characters included in our study are affected by homoplasy and appear less meaningful phylogenetically. Some of these were included in previous, more restricted studies within the family (e.g. Michat & Torres, 2005; Michat & Alarie, 2008, 2013 and references therein). The absence of a broader taxon sampling in those analyses meant that certain characters appear as unambiguous synapomorphies for a variety of groups. When analysed in a broader context, however, and considering that convergent evolution appears to be widespread, some of them become homoplasious. These characters are very important in defining relationships within the family, even though some of them appear to be affected by parallel (or convergent) evolution. Therefore, we have maintained them unmodified in our study, although this implies dealing with increased homoplasy levels.

In spite of this, the family-group classification suggested by larval morphology is highly consistent with current classification, with more than 85% (26 out of 30) of taxa recovered in our consensus tree. There are many larval characters supporting higher taxa within Dytiscidae (Table S5). We were able to identify more than 170 characters as useful to define current subfamilies and tribes, 71 of which are unambiguous synapomorphies and the rest being homoplasious yet useful characters (Figs 5, 6). As a consequence, most current suprageneric taxa can be diagnosed by larval characters with moderate to strong support (Fig. 3). We also identified more than 60 characters (23 unambiguous) defining inter-subfamily and inter-tribe relationships, although this was not reflected in strong support, with some exceptions mainly within the subfamily Dytiscinae sensu lato. This clearly emphasizes the great potential of larval morphology and chaetotaxy as a source of phylogenetic information. The subfamilies Laccophilinae and Agabinae and the tribes Laccophilini, Hygrotini and Hydaticini stand out as the most weakly supported clades in our analysis, although they were recovered as monophyletic. Finally, the subfamily Dytiscinae sensu stricto (i.e. excluding cybistrines) and the tribes Agabini, Laccornellini and Hydroporini are not recovered as monophyletic. Even after *Laccornellus* and *Canthyporus* were removed from Hydroporini by Miller & Bergsten (2014), the tribe is still resolved as nonmonophyletic based on larval morphology.

Although our knowledge of larval Dytiscidae has increased considerably during recent decades (Figure S1), it still remains rather poor when considered on a worldwide scale. The larvae of many genera are unknown, and those of other genera are superficially described, which reduces their utility as a source of characters in a phylogenetic context. Our study includes more than 120 taxa and 300 characters and is therefore one of the largest morphological datasets analysed so far for diving beetles. Our results, however, would benefit greatly from being tested in a broader context, including more taxa and additional characters. Future studies on larval dytiscids should focus on describing key taxa that will surely contribute to a better understanding of the phylogenetic position of some difficult groups. The genera *Agaporomorphus* (Copelatinae), *Hydrodytes* (Hydrodytinae), *Carabhydrus* Watts, *Queda* Sharp, *Heterhydrus*, *Methles* Sharp, *Hydrodessus* J. Balfour-Browne and *Peschetius* Guignot (Hydroporinae) are examples of phylogenetically interesting taxa for which an appropriate knowledge of the larvae would probably help in providing a better picture of the relationships within Dytiscidae.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12243

Figure S1. Evolution of dytiscid larval descriptions during the last century, divided by decade (approximate data). Asterisks indicate an emphasis on chaetotaxy in most descriptions.

Table S1. Species of Hydradephaga examined for the phylogenetic analysis.

Table S2. Taxon coverage for subfamilies and tribes of Dytiscidae.

Table S3. Characters used for the phylogenetic analysis.

Table S4. Data matrix used for the phylogenetic analysis.

Table S5. Diagnoses of higher taxa of Dytiscidae based on selected larval characters.

Table S6. Characters used for construction of the interactive key.

Table S7. Data matrix used for construction of the interactive key.

File S1. Key to larvae of the subfamilies and tribes of world Dytiscidae (traditional format).

File S2. Key to larvae of the subfamilies and tribes of world Dytiscidae (interactive format).

Acknowledgements

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