ACTA ENTOMOLOGICA
MUSEI NATIONALIS PRAGAE
ISSN 1804-6487 (online) – 0374-1036 (print)

2023
63(2): 305–322
doi: 10.37520/aemnp.2023.019
www.aemnp.eu

RESEARCH PAPER

Larval morphology of *Crephelochares* and *Peltochares* (Coleoptera: Hydrophilidae)

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Accepted:
5th November 2023

Published online:
24th November 2023

Abstract. The larval morphology of *Crephelochares* Kuwert, 1890 and *Peltochares* Régimbart, 1907 is described in detail based on specimens of *C. abnormalis* (Sharp, 1890) and *P. atropiceus* (Régimbart, 1903) collected in Taiwan; the chaetotaxy of the head capsule and head appendages of both genera is described for the first time. Larvae were obtained by laboratory rearing and field collecting; field collected larvae were identified by morphological comparison with those reared in the laboratory. Larval morphology of *C. abnormalis* corresponds with that of *C. nitescens* (Fauvel, 1883) except for the serrate margin of the nasale in *C. abnormalis*. We confirm that *Crephelochares* does not construct the usual egg case, and report active behaviour of its larvae, possibly indicating they are not ambush predators. Larval morphology of *P. atropiceus* corresponds with that of *P. foveicollis*; on the other hand, we conclude that the larvae previously described as *Peltochares* from Madagascar belong to *Tritonus* Mulsant, 1844 of the tribe Laccobiini. The egg-carrying behaviour of *Peltochares* is confirmed. We briefly summarize the state of the knowledge of immature stages of the Acidocerinae, indicating that a special effort in discovering and describing immature stages from northern South America and Brazil is needed.

Key words. Coleoptera, Hydrophilidae, Acidocerinae, aquatic beetles, chaetotaxy, immature stages, Taiwan, Oriental Region

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Introduction

Crephelochares Kuwert, 1890 and *Peltochares* Régimbart, 1907 are genera of the hydrophilid subfamily Acidocerinae, which is one of the largest lineages of the family Hydrophilidae. Members of both genera are aquatic, distributed in the Old World and Australasia, with 29 valid species in *Crephelochares* and eight in *Peltochares* (Girón & Short 2021). The immature stages of both genera are poorly known (Table 1): Anderson (1976) described the egg-case, larval stages and pupa of the Australian *C. nitescens* (Fauvel, 1883) based on larvae reared in the laboratory. Bertrand (1962) described larvae collected on wet rocks in Madagascar as belonging to *Peltochares*, based on co-occurring adults similar to *P. conspicuus* Régimbart, 1907, but this identification seems doubtful (Girón & Short 2021). Watts (2002) described the third instar larva of the Australian *P. foveicollis* (Montrouzier, 1860); this is the only larval study of *Peltochares* using reliably identified material. All three descriptions are relatively superficial when compared to the current standard of hydrophilid larvae systematics and lack the description of the chaetotaxy.

We recently obtained larvae of *Crephelochares abnormalis* (Sharp, 1890) and *Peltochares atropiceus* (Régimbart, 1903) by laboratory rearing and field collecting in Taiwan; they represent the second species with reliably known larvae of both *Crephelochares* and *Peltochares*. In this paper, we describe their morphology in detail and

report biological observations of their immature stages under rearing conditions, improving the knowledge of Acidocerinae larvae and allowing comparisons with other genera.

Material and methods

Specimens and identification. All larvae of *Crephelochares abnormalis* were obtained by laboratory rearing. Some of the *Peltochares atropiceus* larvae were obtained by rearing from an egg-case carried by a field-collected female, others were collected directly in the field at the same locality as the egg-carrying female. The identity of the field-collected larvae was confirmed by comparison with the laboratory-reared specimens. Rearings were carried out by MF in the laboratory at the National Sun Yat-sen University, Kaohsiung City, Taiwan.

Adults and larvae of both genera were collected at the following locality: Taiwan, Kaohsiung City, E part of Fongshan District, E Cheng Zhengli, former military park, 22.609054, 120.379831, 50 m; 16.vii.2021; M. Fikáček leg., #TW2021-14; small shallow sun-exposed pools with lots of plant debris. We examined the following larvae (deposited in the Kitakyushu Museum of Natural History and Human History, Kitakyushu and in the Department of Entomology, National Museum, Prague): *Crephelochares abnormalis*: Four L3 (third instar larvae); all larvae were found in the rearing box with adults on 8.viii.2021 when the rearing was terminated. Two specimens were examined as slide preparations using a compound microscope. *Peltochares atropiceus*: More than ten L1, four L2 and three L3. L1 and L2 specimens were reared from an egg-case carried by a female caught in the wild. Four L1, four exuviae of L1, and two L2 specimens were examined as slide preparations using a compound microscope. Additional L3 specimens were collected in the field at the same place where adults were found; one L3 specimen was examined as a slide preparation using a compound microscope.

Morphological study. Some larvae were cleared using 10% KOH solution (ca. 50 minutes at 50°C) or proteinase K (20 µl proteinase K solution and 180 µl ATL Buffer, for ca. 5 hours at 55°C; Qiagen, Hilden). Specimens cleared by KOH solution were subsequently soaked in lactic acid for at least 10 minutes; then, these parts were rinsed in 70% ethanol or distilled water. These specimens were mounted on HS-Slides (Shirayama et al. 1993) with Euparal (Waldeck, Münster) after dehydration, or mounted on standard glass slides in glycerol for temporal examination.

The specimens were mainly examined by an MZ16 stereoscopic microscope (Leica Microsystems, Wetzlar) and a BX50 compound microscope (Olympus, Tokyo) equipped with differential interference contrast optics. Illustrations were made using a drawing tube attached to the BX50 microscope. Drawings were scanned and digitally prepared using the Clip Studio Paint software (CELSYS, Inc., Tokyo). Photographs of whole specimen were taken with Olympus OM-D E-M5 Mark II and E-M1 Mark II digital cameras (Olympus, Tokyo) with an MP-E 65mm f/2.8 1-5× Macro Photo lens (Canon Inc., Tokyo) attached with the aid of lens mount adapter, followed by stacking combination in the Helicon Focus software (Helicon Soft Ltd, Kharkov). Photographs were edited using Adobe Lightroom Classic CC and Photoshop CC (Adobe Inc., San Jose) when necessary.

Terminology and abbreviations. Morphological terminology follows Archangelsky (1997) and Minoshima & Hayashi (2011) for general morphology, and Fikáček et al. (2008) and Bytebier &Torres (2009) for primary chaetotaxy of the head.

Abbreviations used in this paper are as follows:

AN	antenna;
FR	frontale;
gAN	group of the apical antennal sensilla;
gAPP	group of sensilla on inner appendage of maxilla;
gFR	group of sensilla on frontale;
gLA	group of the apical sensilla on labial palpus;
gMX	group of the apical sensilla on maxilla;
LA	labium;
MN	mandible;
MX	maxilla;
PA	parietale;
SE	sensorium.

Results

Crephelochares Kuwert, 1890

Diagnosis. Body slender (Fig. 1A). Coronal line very short (Fig. 3A). Nasale irregularly serrated, with one small tooth on left side (Fig. 3C) or with five distinct teeth (Anderson 1976: fig. 9). Head capsule and head appendages with paddle-shaped setae (Fig. 3A). Mandibles (Figs 4B–C) asymmetrical; left mandible with one inner tooth bifurcate apically; right mandible with two inner teeth, distal tooth bifurcate apically, proximal tooth simple, slightly

Table 1. Knowledge of immature stages of *Crephelochares* and *Peltochares*, including this study.

Species	Association	Stages	Reference	Remarks
Crephelochares abnormalis	rearing	L	this study	
*Crephelochares nitescens**)	rearing	E, L, P	Anderson (1976)	as *Helochares nitescens*
Peltochares atropiceus	rearing from eggs carried by adults / collection in the field and association with reared larvae	L	this study	
Peltochares foveicollis	rearing from eggs carried by adults	L	Watts (2002)	as *Helochares foveicollis*
Peltochares sp.	collection in the field and association with adults	L	Bertrand (1962)	misidentification, likely representing *Tritonus* (see Discussion)

*) The species identification needs to be confirmed by the revision of *Crephelochares* in Australia, since the preliminary study of Australian species revealed that they may not be conspecific with *C. nitescens* described from New Caledonia (see also Fikáček 2019).

Fig. 1. Third instar larvae of *Crephelochares abnormalis* (Sharp, 1890). A – habitus, dorsal, lateral, ventral view; B – abdominal apex, ventral view (a: proleg on segment 7, b: ventral lobe, c: acrocercus); C – left proleg, abdominal segment 6, ventral view; D, E – alive individual.

smaller than distal one. Maxillary palpomere 1 completely cylindrically sclerotized. Mentum (Figs 4F–G) rounded laterally. Cervical sclerites subdivided into small lateral sclerite and large mesal sclerite. Legs well developed (Fig. 2C). Abdominal prolegs present (Figs 1A–C). Acrocercus very long (Fig. 2D).

Comparative notes. *Crephelochares* larvae are similar to those of *Agraphydrus* Régimbart, 1903; they can be distinguished from *Agraphydrus* by the following characters: completely cylindrically sclerotised maxillary palpomere 1 (Figs 4D–E) (incompletely sclerotized in *Agraphydrus*); maxillary palpomere 1 longer than wide (wider than long in *Agraphydrus*); much longer acrocercus (Fig. 2D) (short in *Agraphydrus*) (Table 2). For detailed morphology of *Agraphydrus*, see Minoshima & Hayashi (2011) and Minoshima et al. (2013, 2021).

Crephelochares abnormalis (Sharp, 1890)

(Figs 1–4)

Third instar larva. ***General morphology.*** Body slender, almost parallel-sided until abdominal segment 5, then tapering posteriad (Fig. 1A). Nine pairs of spiracles, one on anterolateral area of mesothorax and eight on abdomen; last abdominal pair enclosed within spiracular atrium.

Colour. Body milky white with yellowish head and sclerotised parts on thorax and abdomen in ethanol-fixed specimen (Fig. 1A). In living specimens, sclerotized parts reddish brown, and body partly transparent, with contents of digestive system visible as dark stripe or spots (Figs 1D–E).

Head. Head capsule (Figs 2A, 3) subquadrate. Frontal lines vestigial, only posterior part recognizable, converging basally; coronal line very short. Number of stemmata hardly recognizable but apparently six; anterior row with stemmata closely grouped; posterior row with three small stemmata. Clypeolabrum (Fig. 3A) asymmetrical. Nasale serrate with one small tooth on left side, not distinctly projecting anteriorly. Lateral lobes of epistome asymmetrical, weakly projecting anteriorly. Left epistomal lobe not projecting further than right lobe and nasale, anterior margin sinuate. Right epistomal lobe projecting further than nasale, anterior margin sinuate. Gular and submental sulci hardly visible. Posterior tentorial pits very small, near presumable junction of gular sulcus and submental sulcus. Cervical sclerites transverse, subdivided into small lateral sclerite and large mesal sclerite.

Antenna (Fig. 4A) 3-segmented, slender, moderately long. Antennomere 1 longest, slightly wider than antennomere 2. Antennomere 3 small, elongate, shortest and narrowest.

Mandibles (Figs 4B–C) distinctly asymmetrical, with different number of inner teeth. Left mandible with one inner tooth, apex of inner tooth weakly bifurcate; posterior two-thirds of incisor area distinctly serrate. Right mandible with two inner teeth; distal inner tooth bifurcate at apex, similar to inner tooth on left mandible but slightly narrower; proximal inner tooth simple, slightly smaller than distal one; posterior two-thirds of incisor area distinctly serrate.

Maxilla (Figs 4D–E). Counting cardo, 6-segmented, distinctly longer than antenna; stipes and palpus cylindrically sclerotized. Cardo small, irregularly triangular. Stipes

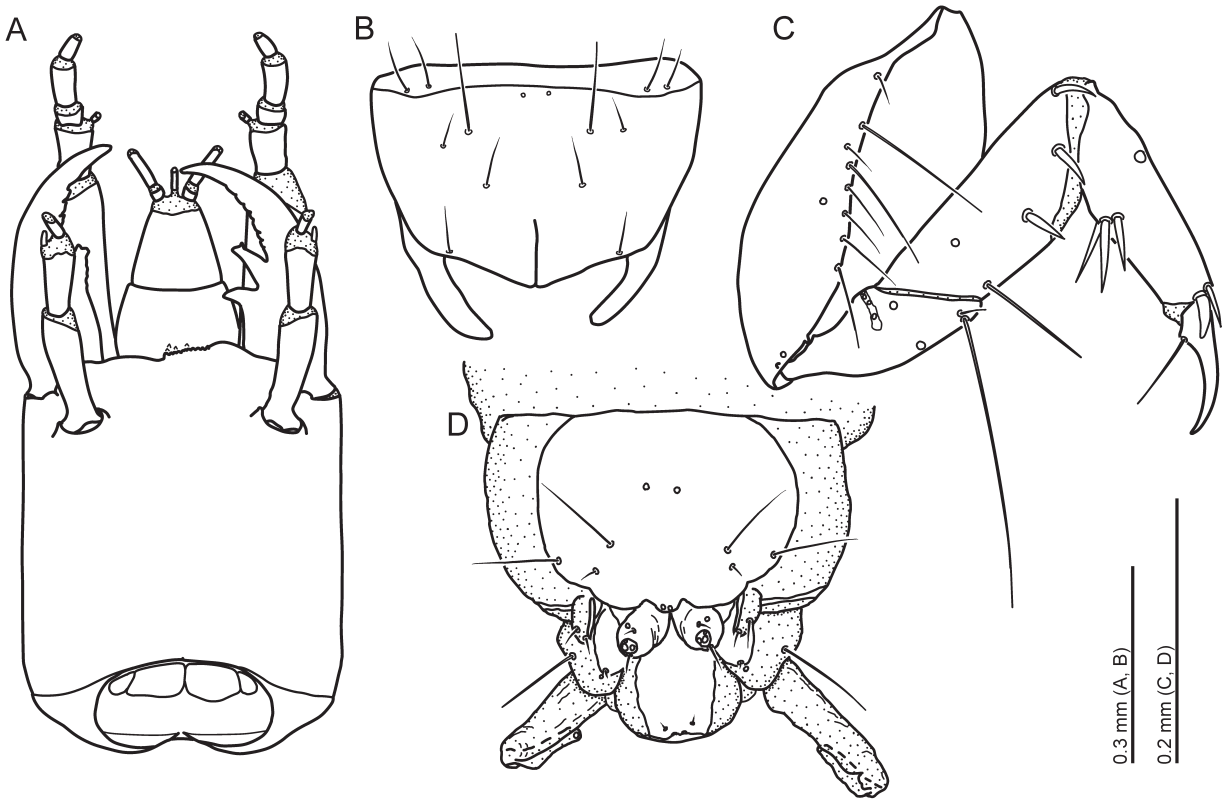

Fig. 2. Third instar larva of *Crephelochares abnormalis* (Sharp, 1890). A – head, dorsal view; B – prosternum, ventral view; C – mesothoracic leg; D – spiracular atrium, dorsal view, many setae missing in the specimen.

longest and widest segment, longer than palpomeres 1–4 combined. Palpomere 1 wider than palpomeres 2 and 3, as long as palpomere 3. Palpomere 2 slightly wider than palpomere 3, slightly shorter than palpomere 4. Palpomere 4 narrowest. Inner process small, cylindrically sclerotized.

Labium (Figs 4F–G) well developed. Submentum large. Mentum subquadrate with rounded lateral face, widest at midlength. Dorsal surface bearing small but strong cuticular teeth on posteromesal area. Prementum trapezoid. Ligula slender, cylindrically sclerotized, distinctly shorter than labial palpus. Labial palpus slender, shorter than prementum; palpomere 1 short, palpomere 2 long.

Thorax. Prothorax slightly wider than head capsule (Fig. 1A). Proscutum formed by one large plate subdivided by fine sagittal line, anterior and posterior parts weakly sclerotised. Prosternum (Fig. 2B) subpentagonal, with incomplete sagittal line posteriorly. Meso- and metanotum with large, subquadrate plate divided by fine sagittal line. Legs (Figs 1A, 2C) well developed, visible from dorsal view, 5-segmented, bearing setae of variable length and pores but without swimming hairs; trochanter with one very long seta; setae on femur and tibiotarsus stout. All pairs similar in shape.

Abdomen (Fig. 1A) 10-segmented, mostly membranous; segments 1–7 similar in shape and size. Abdominal segment 1 with one pair of subquadrate dorsal sclerites moderate in size anteromesally. Ventral surface of abdomen with pair of well-developed prolegs composed by hook-like spines on segments 3–7. Prolegs on segments 3–5 present on projected area of abdomen (Figs 1A–C).

Spiracular atrium (Figs 1B, 2D). Segment 8 with oval dorsal plate with sinuate posterior margin. Procercus short, incompletely cylindrically sclerotized, with two short setae. Segment 9 trilobed, partly sclerotised; median lobe of spiracular atrium large; lateral lobes smaller than median lobe. Acrocercus membranous, very long. Urogomphi one segmented, short, sclerotised. Ventral face with pair of rather long lateral lobes; base of lobes connected by transverse ridge.

Chaetotaxy of head. ***Third instar.*** *Frontale* (Figs 3A, C). One pair of short setae (FR1) at midlength of central area of head capsule. Three pairs of sensilla (FR2, FR3, FR8) anteromesal to FR1, between antennal sockets; FR2 pore-like, posterior to FR3 and FR8; FR3 very short seta, between FR8 and FR2; FR8 seta (broken in the examined specimens) anterior to FR3. Two short setae (FR5, FR6) posteromesal to antennal socket; FR5 behind FR6. FR7 short seta on inner margin of antennal socket. Sensilla FR4, FR9, and FR10 close and mesal to antennal socket; FR4 pore, posteromesal to FR10; FR10 short seta between FR4 and FR9; FR9 rather short seta anterolateral to FR9. Pore FR14 anterolateral to FR9. Very short seta FR12 and pore-like sensillum FR13 on inner part of epistome; FR12 behind FR13. FR11 pore-like, behind lateral part of nasale. Pore-like sensillum FR15 behind nasale. Nasale with six short setae (gFR1); four setae arranged equidistant on anterior margin of nasale, two setae behind mesal two setae. Epistome with three setae (gFR2) on anterior margin; two small setae on inner area of epistome; one short scale-like seta at about midlength between inner two setae and mandibular articulation.

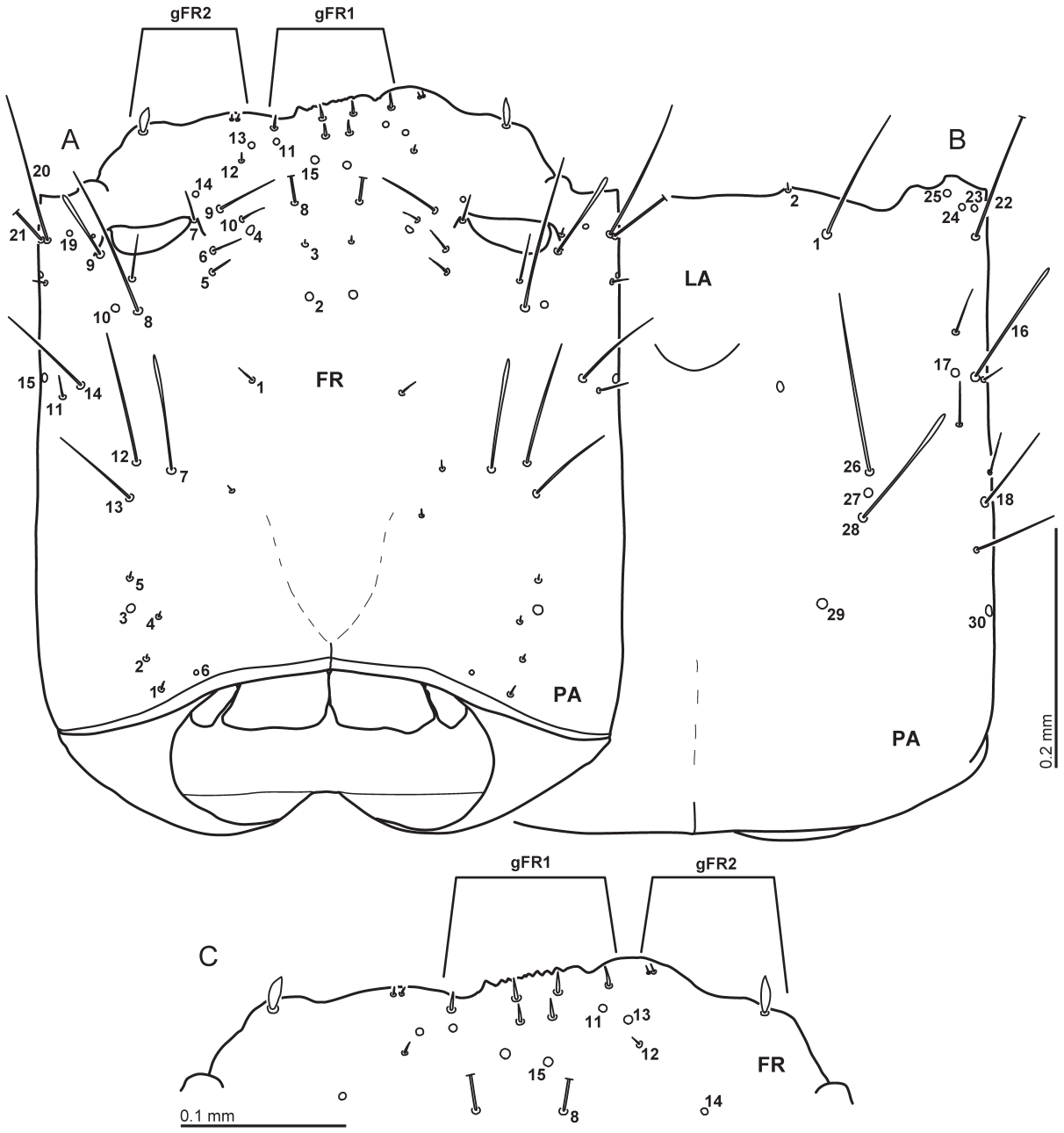

Fig. 3. Head capsule of third instar larva of *Crephelochares abnormalis* (Sharp, 1890). A – dorsal view; B – ventral view; C – detail of anterior margin, dorsal view.

Parietale (Figs 3A–B). Dorsal surface with irregularly arranged longitudinal row of five sensilla (from posterior to anterior, PA1–5) posteriorly at midwidth between coronal line and lateral margin; PA3 pore-like, remaining four very short setae. PA6 pore-like sensillum, anteromesal to PA1. Three long setae (PA7, PA12–13) forming triangular group at midlength between antennal socket and posterior margin of head capsule; PA7 paddle-shaped, mesal to PA12 and PA13, PA13 behind PA12. One to two very short secondary setae mesal to PA7, close to frontal line. Three sensilla (PA8, PA10, and one secondary seta) behind antennal socket; PA8 long; PA10 pore-like, lateral to PA8; secondary seta anterior to PA8 and PA10. Seta PA9 rather short, paddle-shaped, on outer margin of antennal socket. One small secondary seta anterior to PA9. Anterolateral corner of head capsule with one pore-like sensillum (PA19), three setae (PA20–22) and three ventral pore-like sensilla (PA23–25) close to ventral mandibular acetabulum. PA19 dorsal, between PA9 and PA20; long seta PA20 and seta PA21 (broken in the examined specimens) situated laterally; long seta PA22 ventral. PA23 lateral to PA24 and PA25, PA24 between PA23 and PA25. One short secondary seta and one pore-like sensillum behind PA20. Two pore-like sensilla (PA15 and PA17) and six setae (PA11, PA14, PA16, and three secondary setae) laterally on anterior one-third of parietale; PA14 long, located more dorsally than remaining sensilla; PA15 lateral to PA11 and PA14; PA11 short, between PA14 and PA15; PA16 long, paddle-shaped, lateroventral; PA17 on ventral face, mesal to PA16; one very short secondary

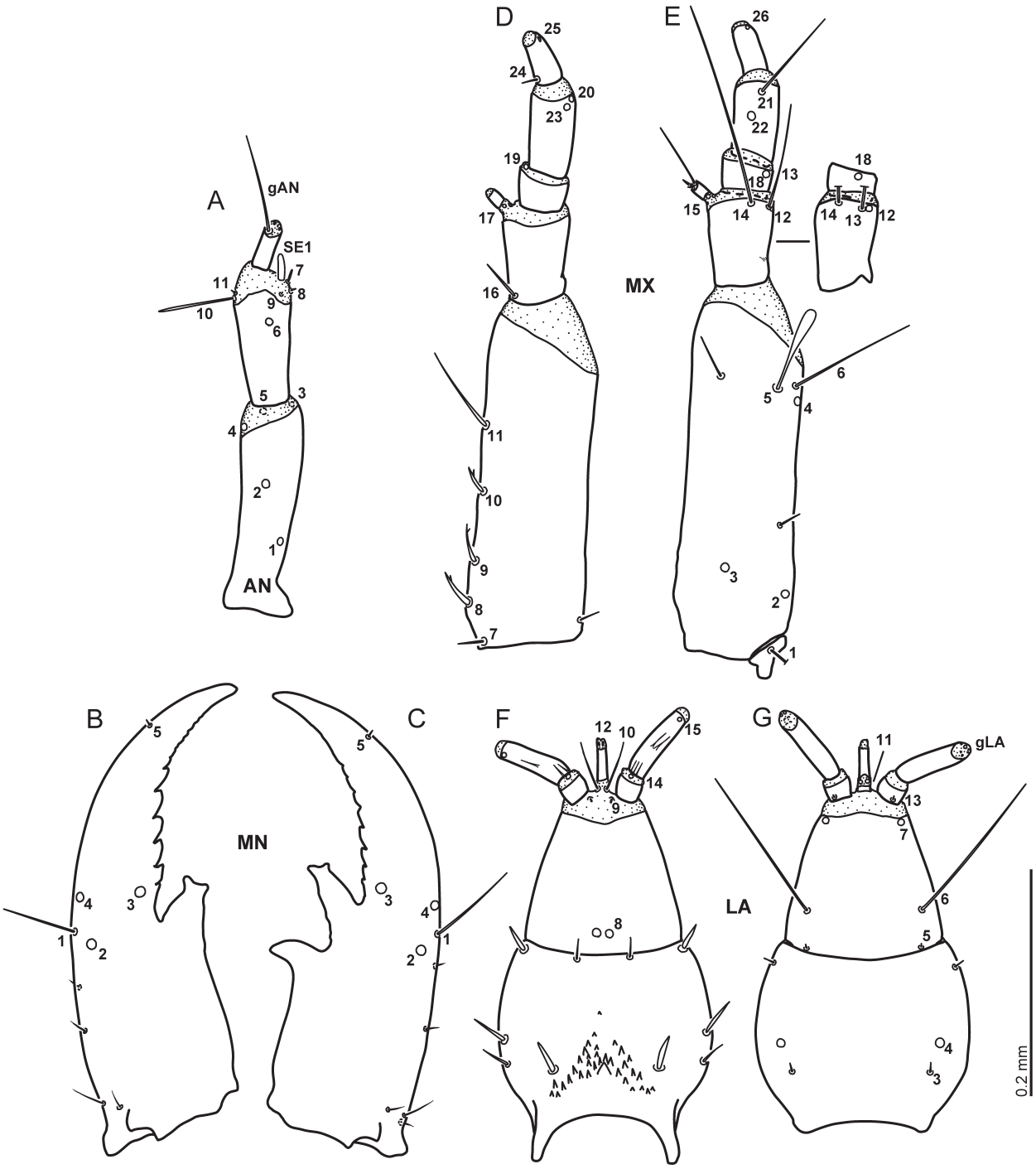

Fig. 4. Head appendages of third instar larva of *Crephelochares abnormalis* (Sharp, 1890). A – antenna, dorsal view, most sensilla of gAN missing; B, C – mandibles, dorsal view; D, E – maxilla, dorsal view (D) and ventral view (E); F, G – labium, dorsal view (F) and ventral view (G).

seta close to PA16; one short secondary seta in front of PA17; one short secondary seta behind PA17. Long seta PA18 and two lateroventral secondary setae at midlength of parietale; one short secondary seta anterior to PA18; one long secondary seta behind PA18. Pore-like sensillum PA30 lateroventral, on posterior third of parietale. Two ventral setae (PA26 and PA28) and one pore-like sensillum PA27 at midlength of parietale; PA26 very long, anterior to PA27 and PA28; PA28 long, paddle-shaped; PA27 between PA26 and PA28. Pore-like sensillum PA29 posteromesal to PA28.

Antenna (Fig. 4A). Antennomere 1 with five pore-like sensilla (AN1–5). AN1 dorsolateral on basal third; AN2 dorsal on anterior two-fifths; AN3–5 apical; AN3 on outer face, AN4 on inner face, AN5 on ventral face. Antennomere 2: pore-like sensillum AN6 dorsal on anterior fourth of sclerite. Six sensilla (AN7–11, SE1) on intersegmental membrane between antennomeres 2 and 3. Antennal sensorium SE1 and setae AN7–9 on outer face; SE1 slender, rounded apically, shorter than antennomere 3; AN7 short, stout, between SE1 and AN8; AN8 very short, posterior to AN7; AN9 small, mesal to AN8. Long, narrow pad-

dle-shaped seta AN10 and very short seta AN11 on inner face. Antennomere 3 bearing a group of sensilla (gAN) on apical membranous area (most sensilla of gAN missing in the examined specimens).

Mandibles (Figs 4B–C). Mandible with five primary sensilla (MN1–5) and secondary setae. MN1 long, at about midlength on outer face. Pore-like sensilla (MN2–4) dorsally at midlength, forming triangular group. MN2 posteromesal to MN1, MN4 anterior to MN1 on outer face, MN3 mesal and slightly anterior to MN4. Seta MN5 very short, subapical on outer face. MN6 undetectable. Two short secondary setae behind MN1. Three rather short secondary setae basally on outer face.

Maxilla (Figs 4D–E). Cardo with one ventral seta (MX1) (broken in the examined specimens). Stipes with five stout setae (MX7–10) dorsally on inner face; MX7–10 almost equidistant; MX7 short, at base; MX8–10 short, bearing one small subapical tooth; MX11 much longer than MX7–9. MX2 and MX3 pore-like, subbasal on ventral face; MX2 on outer face, MX3 on median part. Subapical area of ventral face bearing three primary sensilla (MX4–6) laterally; MX4 pore-like, MX5 long seta, wide paddle--shaped, MX6 long seta; MX4 posterior to MX6, MX5 mesal to MX4 and MX6. Stipes with three short secondary setae; one subapical on inner part of ventral face; one on lateroventral face at posterior third; one basal on outer face. Palpomere 1 with three setae (MX13–14, MX16) and three pore-like sensilla (MX12, MX15, MX17). MX16 rather short basal seta on inner face. MX12–14 apical; MX12 and long seta MX13 on lateroventral face; very long seta MX14 mesal to MX13. MX15 and MX17 on intersegmental membrane behind inner appendage; MX17 dorsal, MX15 ventral. Inner appendage with one long seta and few small sensilla (gAPP). Palpomere 2 with two apical pore-like sensilla (MX18, MX19); MX27 undetectable. MX18 on lateroventral face; MX19 dorsal on inner face of intersegmental membrane between palpomeres 2 and 3. Palpomere 3 with four primary sensilla (MX20–23); MX21 moderately long seta, MX22 pore-like, character states (seta or pore-like) of MX20 and MX23 unclear due to limited number of specimens, but MX23 may be a seta (missing in the examined specimens) and MX20 may be a pore based on comparison with other Acidocerinae genera. MX23 dorsal and subapical, on lateral face; MX20 lateral and apical; MX21 and MX22 on ventral face; MX21 subapical; MX22 behind MX21. Palpomere 4 with two apical pore-like sensilla (MX25 and MX26) on outer face and one short seta (MX24) at base on inner face; MX25 dorsal, digitiform; MX26 ventral. Apical membranous area of palpomere 4 with several small sensilla (gMX).

Labium (Figs 3B, 4F–G). Submentum with long seta LA1 on lateral area and very short seta LA2 on anterior corner. Mentum with two pairs of primary sensilla (LA3–4) on lateroventral face and six pairs of secondary setae. LA3 very short seta, on posterior third; LA4 pore-like, at about midlength. Four pairs of short, stout dorsal secondary setae; one pair posterior, three pairs on lateral face. One pair of short dorsoapical setae on median part; one pair of very short ventral secondary setae on anterolateral corners of mentum. Dorsal face of prementum with one pair of subbasal pore-like sensilla (LA8) on mesal area of sclerite; pore-like sensillum LA9 on median part of membrane between prementum and palpi; rather long seta LA10 at base of ligula. Ventral face of prementum with three lateral sensilla (LA5–7); LA5 very short basal seta; LA6 very long seta, anterior to LA5; LA7 pore-like, on anterior margin of sclerite. Ligula with one pair of small sensilla (LA12) on apical membranous area; one pair of ventral pore-like sensilla (LA11) on membrane in basal area of ligula. Palpomere 1 with very short seta LA13 and pore-like sensillum LA14; LA13 basal, on ventral face; LA14 dorsal, on intersegmental membrane between palpomeres 1 and 2. Palpomere 2 with apical pore-like sensillum LA15; gLA composed of setae of variable length on apical membranous area.

Biology. Anderson (1976) described the egg-case of *Crephelochares nitescens*, which is in a hollow made by the adult, bag-like and without mast and cap. This is an unusual type of egg-case in the Hydrophilidae. Unfortunately, we could not find any egg-case of *C. abnormalis* in the rearing box, this was possibly due to the unusual type of egg-case (see Discussion).

Live larvae of *C. abnormalis* had air bubbles in their alimentary canal as observed by Anderson (1976) in the first instar larva of *C. nitescens*. This behaviour has been observed in several previous studies; the bubbles may increase their buoyancy, which helps them float on water surface for respiration (Minoshima & Hayashi 2015). Larvae were very active under rearing conditions: moving relatively quickly and stopping from time to time, keeping the middle and posterior parts of the abdomen in the same position, and actively exploring the area within reach of the anterior body of the larva (Fig. 1E).

Comparative notes. The larva of *C. abnormalis* described here corresponds well with the larval description of *C. nitescens* by Anderson (1976) except for the following differences: the nasale in *C. abnormalis* is irregularly serrated and has only one small lateral tooth on the left (Fig. 3C), whereas that of *C. nitescens* has five rather large teeth of equal size; the epistomal lobes are asymmetrical in *C. abnormalis* (Fig. 3C), whereas symmetrical in *C. nitescens*, and apex of distal inner tooth on right mandible is bifurcate in *C. abnormalis* (Fig. 4B), whereas simple in *C. nitescens* (Anderson 1976: fig. 9).

Peltochares Régimbart, 1907

Diagnosis. Nasale with distinct teeth, almost symmetrical (Fig. 9B). Epistomal lobes present, rounded, almost symmetrical (Fig. 9B). Frontal lines convergent at base, coronal line short (Fig. 6A). Head capsule and head appendages with paddle-shaped setae (Figs 6–7). Antennomere 1 with small subapical projection on inner face (Fig. 10A). Antennal sensorium SE1 present on intersegmental membrane between antennomeres 1 and 2 (Fig. 10A). Mandibles symmetrical, with two inner teeth (Figs 7D–E, 10B). Anterior corners of mentum projecting anteriorly, this projection sharply pointed in first instar (Fig. 7F), rounded in second and third instar (Figs 8F, 10F). Legs

Fig. 5. Larvae of *Peltochares atropiceus* (Régimbart, 1903). A – first instar larva, dorsal view; B – second instar larva, dorsal view; C – third instar larva, dorsal, lateral, ventral view; D – first instar larva, alive individual; E – third instar larva, alive individual.

well developed (Fig. 9F). Dorsal part of posterior margin of segment 8 strongly projected posteriorly, forming trilobate projection (Fig. 9G). Abdominal prolegs absent. Acrocercus long (Fig. 9G).

Comparative notes. Larvae of *Peltochares* are similar to those of *Helochares* Mulsant, 1844; however, *Peltochares* may be distinguished from *Helochares* by the anteriorly projecting corners of mentum (Figs 7F, 8F, 10F); anterolateral corners are angulate and not projecting anteriad in *Helochares* (e.g., Archangelsky 1997, Minoshima & Hayashi 2011) (Table 2). In this character, *Peltochares* resembles representatives of the tribe Hydrophilini and the genus *Amphiops* Erichson, 1843 of the subfamily Hydrophilinae. The larva of *Peltochares* differs from that of *Amphiops* by the presence of ligula, narrower mentum, very short maxillary palpomere 1, and antennomere 1 much shorter than antennomere 2 (see Minoshima & Hayashi 2012). It differs from the larvae of Hydrophilini in the well-developed antennal sensorium (reduced in size and hence inconspicuous in all Hydrophilini larvae), the very short maxillary palpomere 1, and antennomere 1 without numerous setae or spines (see e.g., Archangelsky 1997, Torres et al. 2008, Minoshima & Hayashi 2011).

Peltochares atropiceus (Régimbart, 1903)

(Figs 5–10)

First instar larva. ***General morphology*** (compared with second instar larva). Similar to second instar larva (Figs 5A, D). Antenna (Fig. 7A) proportionally stouter than that of second instar larva; subapical projection of antennomere 1 projected more strongly than in second instar larva. Anterior corners of mentum projected more strongly than in second instar larva; projections pointed apically (Fig. 7F).

Primary chaetotaxy of head. *Frontale* (Figs 6A, C). Central area with three pairs of sensilla diverging posteriad; FR1 long seta, close to frontal line; FR2 pore-like, between FR1 and FR3; FR3 very short seta, situated anterior to FR2. Setae FR5 and FR6 behind antennal socket; FR5 very short, behind FR6; FR6 short, paddle-shaped. Short seta FR7 on inner margin of antennal socket. FR4, and FR9–10 close and mesal to antennal socket; FR4 pore, behind FR10; FR10 short seta, mesal to FR9; FR9 long, paddle-shaped seta. Pore FR14 anterior to FR7. Very short seta FR12 and pore-like sensilla FR11 and FR13 on inner margin of epistome, forming small triangular group; FR12 lateral to FR11, FR13 behind FR11 and FR12. Long seta FR8 and pore-like sensillum FR15 on mesal part behind nasale; FR15 anterior to FR8. Nasale with group of six short, stout dorsal setae and two very short ventral setae (gFR1); dorsal setae equidistant, lateralmost ones shorter than mesal ones; ventral setae anterior to FR15. Epistome with four setae (gFR2) on anterior margin; two small setae on inner area of epistome; two short scale-like setae on median area of epistome.

Parietale (Figs 6A–B). Dorsal surface with basal longitudinal row of five sensilla (from posterior to anterior, PA1–5) at midwidth between coronal line and lateral margin; PA3 pore-like, remaining four very short setae. PA6 pore-like sensillum, close to coronal line. Three setae (PA7, PA12–13) at midlength of head capsule, anterior to PA5; PA7 long, paddle-shaped, mesal to PA12 and PA13; PA13 very long, behind PA12; PA12 short. Very long, paddle-shaped seta PA14 anterolateral to PA12, equidistant between PA12 and PA10. Pore-like sensillum PA10

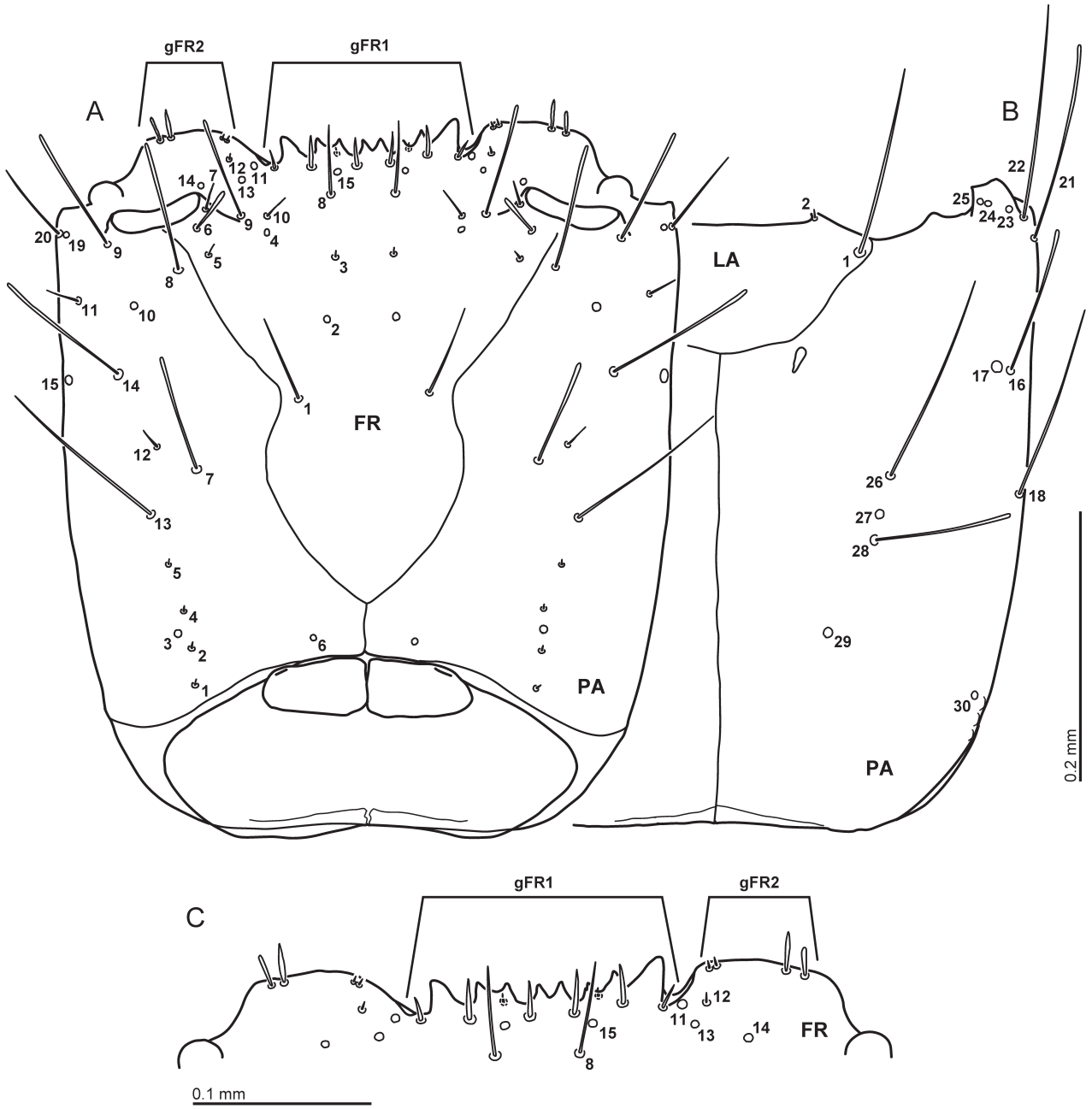

Fig. 6. Head capsule of first instar larva of *Peltochares atropiceus* (Régimbart, 1903). A – dorsal view; B – ventral view; C – detail of anterior margin, dorsal view.

anterior and slightly mesal to PA14. Long, paddle-shaped seta PA8 behind antennal socket, close to frontal line. Long, paddle-shaped seta PA9 on outer margin of antennal socket. Anterolateral corner of head capsule with one pore-like sensillum (PA19) and three setae (PA20–22) on lateral area and three pore-like sensilla (PA23–25) close to ventral mandibular acetabulum. PA19 dorsal to PA20; rather long seta PA20 and very long, paddle-shaped seta PA21 lateral; PA20 dorsal to PA21; very long seta PA22 on lateroventral face. PA23 lateral to PA24 and PA25, PA24 between PA23 and PA25. Short seta PA11 between PA19 and PA14. Two pore-like sensilla (PA15, PA17) and very long, paddle-shaped seta PA16 on anterior fourth of lateral face; PA15 dorsal, PA17 ventral, PA16 between PA15 and PA17. Very long seta PA18 at midlength of lateroventral face. Pore-like sensillum PA30 lateroventral, on posterior fifth of parietale. Two setae (PA26, PA28) and one pore-like sensillum PA27 ventral, at midlength of parietale; PA26 very long, anterior to PA27 and PA28; PA27 between PA26 and PA28; PA28 very long, paddle-shaped. Pore-like sensillum PA29 posteromesal to PA28.

Antenna (Fig. 7A). Antennomere 1 with five pore-like sensilla (AN1–5). AN1 situated dorsolaterally on posterior third; AN2 dorsal, at midlength; AN3–5 apical; AN3 on outer face, AN4 on inner face, AN5 ventral. Antennomere 2: pore-like sensillum AN6 dorsal and subapical. Six sensilla (AN7–11, SE1) on intersegmental membrane between antennomeres 2 and 3. Antennal sensorium SE1 and setae AN7–9 on outer face; SE1 slender, rounded apically, as long as antennomere 3; AN7 short, between SE1 and AN8;

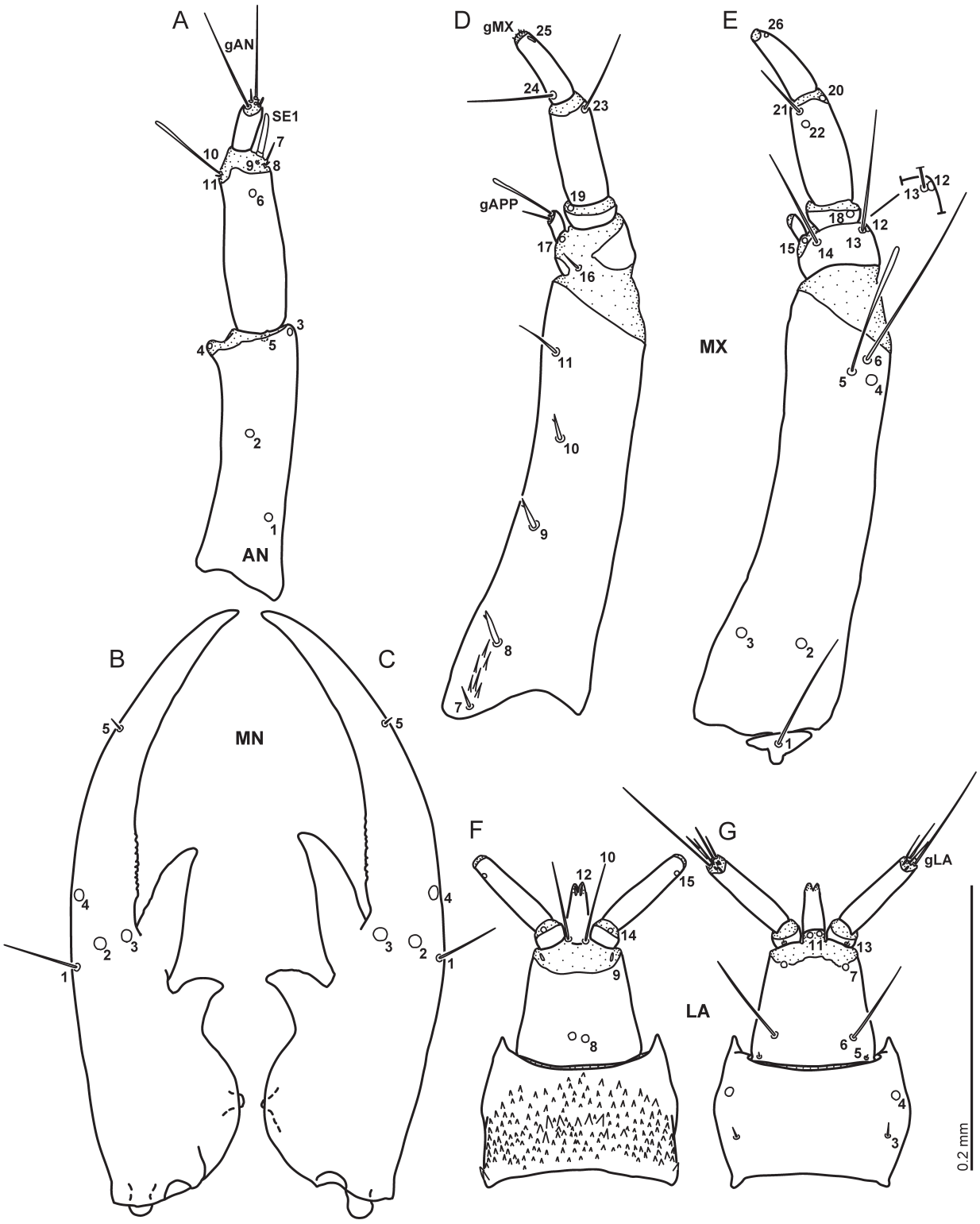

Fig. 7. Head appendages of first instar larva of *Peltochares atropiceus* (Régimbart, 1903). A – antenna, dorsal view; B, C – mandibles, dorsal view; D, E – maxilla, dorsal view (D) and ventral view (E); F, G – labium, dorsal view (F) and ventral view (G).

AN8 very short, posterior to AN7; AN9 small, dorsal to AN8. Long, paddle-shaped seta AN10 and very short seta AN11 on inner face. Antennomere 3 bearing group of sensilla (gAN) on apical membranous area; gAN with two long setae and four short sensilla.

Mandible (Figs 7B–C) with five primary sensilla (MN1–5). MN1 long, on posterior two-fifths of outer face. Pore-like sensilla (MN2–4) dorsal, between midlength and posterior two-fifths. MN2 anteromesal to MN1, between MN1 and MN3; MN3 mesal to MN2; MN4 at midlength, on outer face. Seta MN5 very short, subapical, on outer face. MN6 undetectable.

Maxilla (Figs 7D–E). Cardo with one long ventral seta (MX1). Stipes with five stout dorsal setae (MX7–11) on

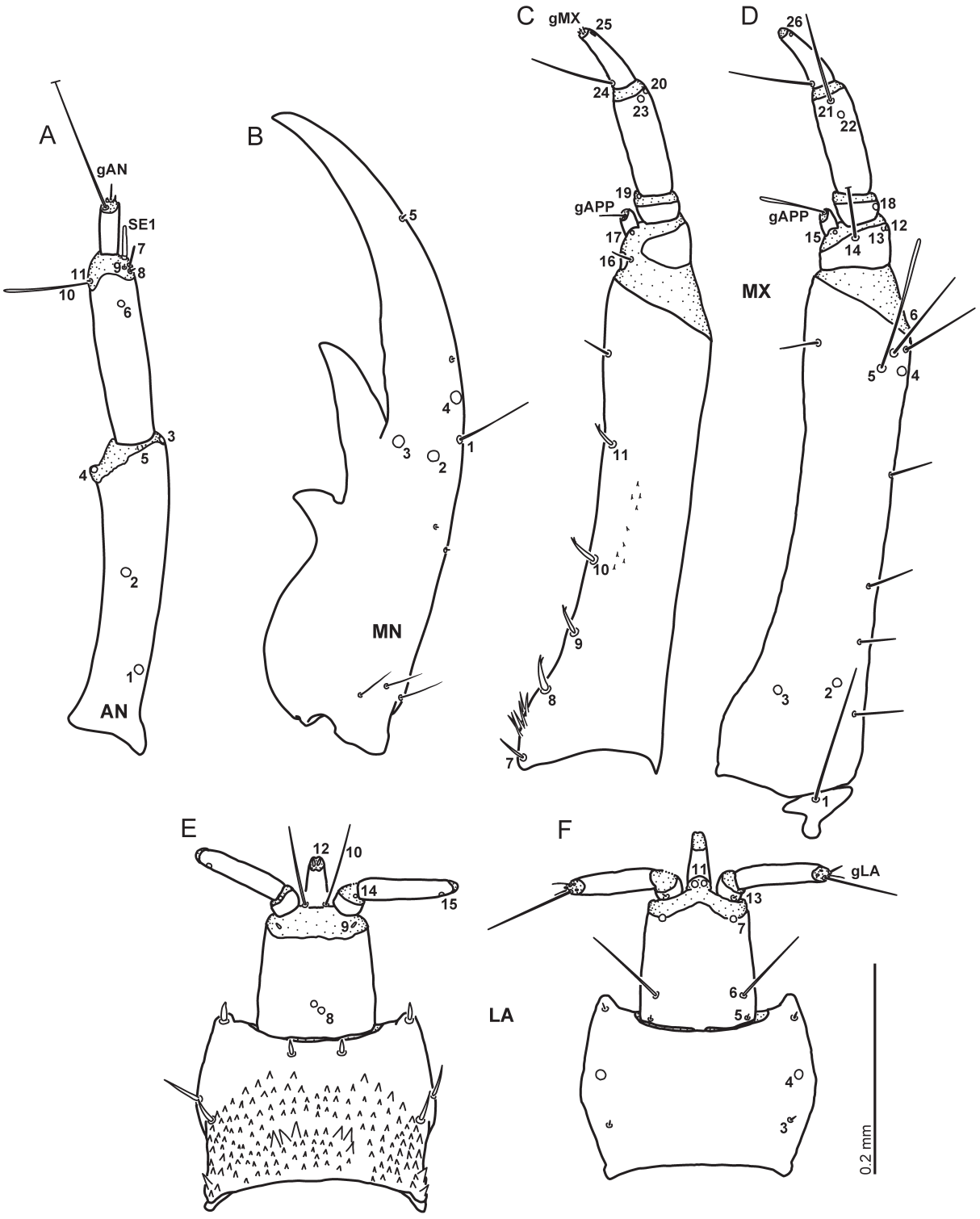

Fig. 8. Head appendages of second instar larva of *Peltochares atropiceus* (Régimbart, 1903). A – antenna, dorsal view; B – mandible, dorsal view; C, D – maxilla, dorsal view (D) and ventral view (E); E, F – labium, dorsal view (F) and ventral view (G).

inner face; MX7 short, basal; MX8–10 short, bearing one small subapical tooth; MX11 much longer and narrower. MX2 and MX3 pore-like sensilla, subbasal, on ventral face; MX2 on outer part, MX3 on inner part. Ventral face bearing three subapical sensilla (MX4–6) laterally; MX4 pore-like, behind MX6; MX5 very long paddle-shaped seta, mesal to MX4 and MX6; MX6 very long seta. Palpomere 1 with three setae (MX13, MX14, MX16) and three pore-like sensilla (MX12, MX15, MX17). MX16 short seta, dorsal on inner area, on membrane. MX12–14 on apical margin of sclerite; MX12 and very long seta MX13 on lateroventral face; very long seta MX14 on inner face. MX15 and MX17 on intersegmental membrane behind inner appendage; MX17 dorsal, MX15 ventral. Appendage with one long paddle-shaped seta, one short seta, and few small sensilla (gAPP). Palpomere 2 with two pore-like

sensilla (MX18–19); MX27 undetectable. MX18 on lateroventral face, close to apical borderline of sclerite; MX19 dorsal, on inner face of intersegmental membrane between palpomeres 2 and 3. Palpomere 3 with two setae (MX21, MX23) and two pore-like sensilla (MX20, MX22); MX20 apical, on lateroventral face; MX23 long, dorsolateral, close to apical margin of sclerite; rather long seta MX21 ventral, on inner face, close to apical margin of sclerite; MX22 behind MX21. Palpomere 4 with two pore-like sensilla (MX25–26) apically on outer face and long seta (MX24) at base of inner face; MX25 dorsal, digitiform; MX26 ventral. Apical membranous area of palpomere 4 with several small sensilla (gMX).

Labium (Figs 6B, 7F–G). Submentum with very long seta LA1 on lateral corner and very short seta LA2 on anterior corner. Mentum with two pairs of ventral sensilla (LA3–4) on lateral face. Short seta LA3 on posterior third; pore-like sensillum LA4 slightly anterior to midlength. Dorsal face of prementum with pair of subbasal pore-like sensilla (LA8) on central part of sclerite. Pore-like sensillum LA9 lateral, on membrane between prementum and palpi. Long seta LA10 at base of ligula. Ventral face of prementum with three lateral sensilla (LA5–7); LA5 very short seta, basal; LA6 long seta, anteromesal to LA5; LA7 pore-like, on margin between sclerite and membrane. Ligula with one pair of small sensilla (LA12) on apical membranous area and one pair of ventral pore-like sensilla (LA11) at base of ligula. Labial palpus with three sensilla (LA13–15) and group of apical sensilla (gLA). Palpomere 1 with very short seta LA13 and pore-like sensillum LA14; LA13 ventral and basal; LA14 dorsal, on intersegmental membrane between palpomeres 1 and 2. Palpomere 2 with subapical pore-like sensillum LA15 on outer face; gLA composed of setae of variable length on apical membranous area.

Second instar larva (compared with third instar larva). Similar to third instar larva (Fig. 5B). ***General morphology.*** Frontal lines, gular sulcus and submental sulcus clearly visible (see Fig. 6A). Frontal lines lyriform, strongly curved at midlength. Prothorax slightly wider than head capsule.

Chaetotaxy. *Head capsule.* Similar to third instar but bearing fewer secondary sensilla as follows. *Frontale.* One short seta in front of FR1. *Parietale*. Three short setae along base of frontal line; one sensillum (possibly seta) between PA8 and PA9; one short seta between PA19 and PA9. *Antenna* (Fig. 8A). Antennal sensorium SE1 shorter than antennomere 3. *Mandible* (Fig. 8B). One small seta in front of MN4. Two small setae behind MN1. Three rather short basal setae on lateral face. *Maxilla* (Figs 8C–D). Stipes with two subapical setae on inner face, one dorsal, one ventral; one long seta close to MX6; four rather long setae on outer face. *Labium* (Figs 8E–F). Mentum bearing four pairs of short, stout dorsal setae; two at midlength on lateral face, one on anterior corner and one apical on mesal part. Ventral face with one very short secondary seta on anterior corner.

Third instar larva. ***General morphology.*** Body (Fig. 5C) slender, widest at abdominal segments 2 and 3, then tapering posteriad.

Colour of fixed larvae light yellowish white with orange-coloured head and sclerotized parts on thorax and abdomen (Fig. 5C); living larvae with sclerotized parts reddish brown, body partly transparent with digestive tract partly visible (Fig. 5E).

Head capsule subquadrate (Fig. 9A). Frontal lines vestigial, only posterior part recognizable, converging at base; coronal line short. Six stemmata closely aggregated on each anterolateral corner of head capsule. Clypeolabrum (Fig. 9B) nearly symmetrical. Nasale with distinct teeth; most lateral teeth larger, six small teeth present between large lateral teeth. Lateral lobe of epistome almost symmetrical, projecting further than nasale and rounded. Gular sulcus only visible basally, submental sulcus hardly visible (Fig. 9D). Posterior tentorial pits oblong oval, near presumable junction of gular sulcus and submental sulcus (Fig. 9D). Cervical sclerites subquadrate (Fig. 9A).

Antenna (Fig. 10A) 3-segmented, slender, moderately long. Antennomere 1 longest and widest; small subapical membranous projection present on inner face of antennomere 1. One minute structure present laterally at base of antennomere 2. Antennomere 3 elongate, shortest and narrowest.

Mandibles (Fig. 10B) symmetrical. Mandible with two inner teeth at about midlength of mandible, distal one larger.

Maxilla (Figs 10C–D). Counting cardo, 6-segmented, distinctly longer than antenna. Cardo small, irregularly triangular. Stipes cylindrically sclerotized, longest and widest segment, slightly longer than twice length of palpomeres 1–4 combined. Palpomere 1 wider than other palpomeres, incompletely cylindrically sclerotised dorsally. Palpomere 2 shortest, as wide as palpomere 3 or slightly wider. Palpomere 3 longest, longer than palpomeres 1 and 2 combined. Palpomere 4 narrowest, slightly longer than palpomere 1.

Labium (Figs 9D, 10E–F) well developed. Submentum large, subtriangular. Mentum subhexagonal, wider than long; anterior corner of mentum projected anteriorly; dorsal surface bearing small but strong cuticular teeth on basal half. Prementum trapezoid, longer than wide. Ligula cylindrically sclerotized, moderately stout, distinctly shorter than labial palpus. Labial palpus slender, similar to prementum in length; palpomere 1 short, palpomere 2 long.

Thorax. Prothorax slightly wider than head capsule (Fig. 5C). Proscutum formed by one large plate divided by fine sagittal line, anterior and posterior areas weakly sclerotised. Prosternum (Fig. 9E) subpentagonal, with incomplete sagittal line posteriorly. Mesonotum with pair of narrow transverse sclerites anteriorly and large, subquadrate plate divided by fine sagittal line behind narrow sclerite (Fig. 5C). Metanotum with pair of small anterior transverse oval sclerites and large irregular plate, anterior part subquadrate, divided by fine sagittal line, posterior part smaller, subtriangular. Legs (Figs 5C, 9F) well developed, visible in dorsal view, 5-segmented, bearing setae of variable length and pores but without swimming hairs; trochanter with one very long seta.

Abdomen ten-segmented, mostly membranous; segment 1 with two pairs of sclerites on anteromedial part; anterior one smaller than posterior one. Ventral surface of abdomen

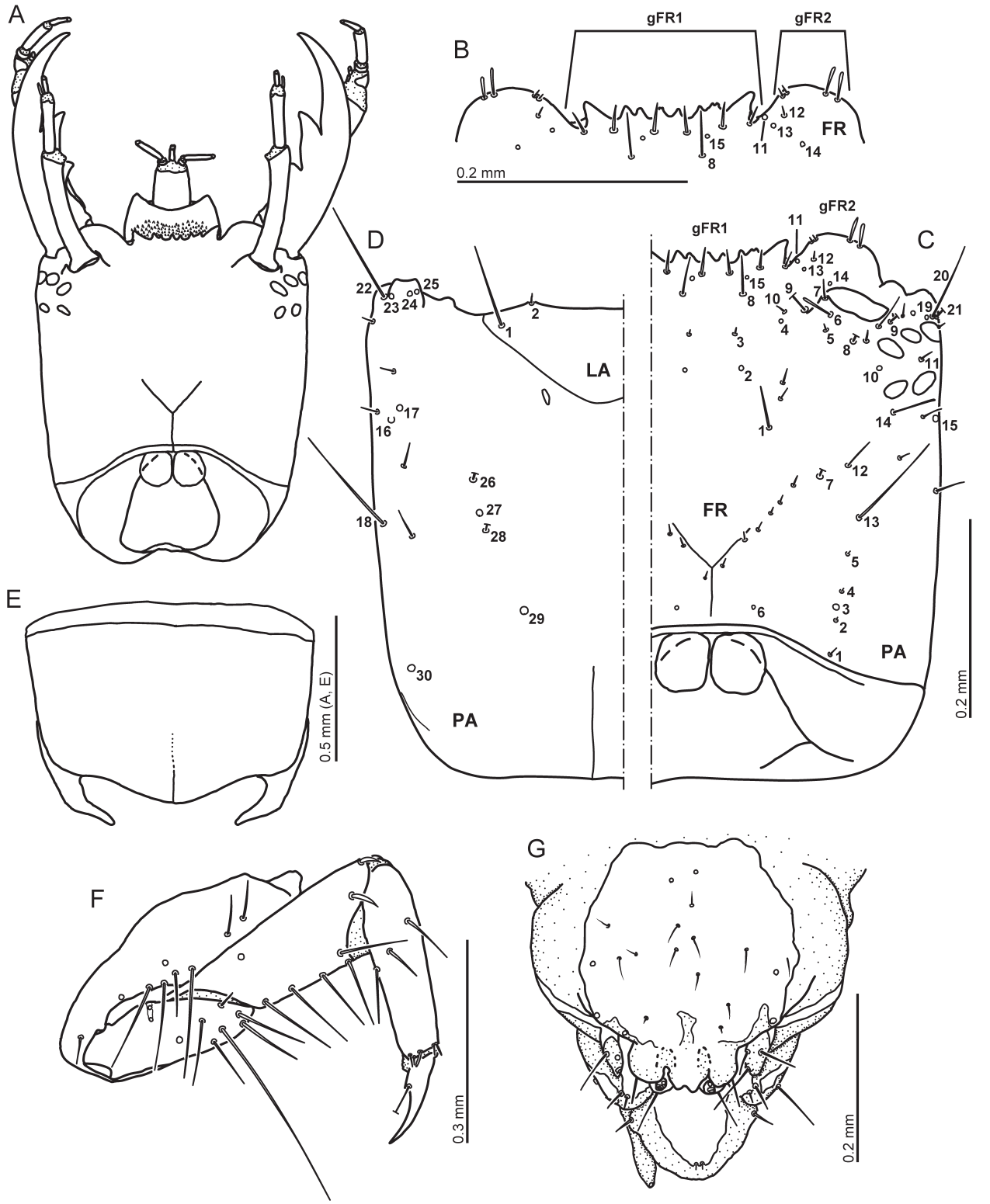

Fig. 9. Third instar larva of *Peltochares atropiceus* (Régimbart, 1903). A – head, dorsal view; B – anterior margin of head capsule; C – head capsule, dorsal view; D – head capsule, ventral view; E – prosternum, ventral view; F – prothoracic leg; G – spiracular atrium, dorsal view.

without prolegs.

Spiracular atrium (Fig. 9G). Segment 8 with suboval dorsal plate with partly membranous posterior margin, borderline between plate and membrane hardly recognizable. Posterior margin of segment 8 strongly projected, trilobate. Procercus short, partly sclerotised with two short and one long seta. Segment 9 trilobed, partly sclerotized, median lobe of spiracular atrium large; lateral lobes smaller than median lobe. Acrocercus membranous, rather short, visible in dorsal view. Urogomphi short, one-segmented, sclerotized.

Chaetotaxy (compared with first instar larvae). Distribution of secondary setae as follows. *Frontale* (Figs 9B–C). Two short setae in front of FR1. *Parietale* (Figs 9C–D).

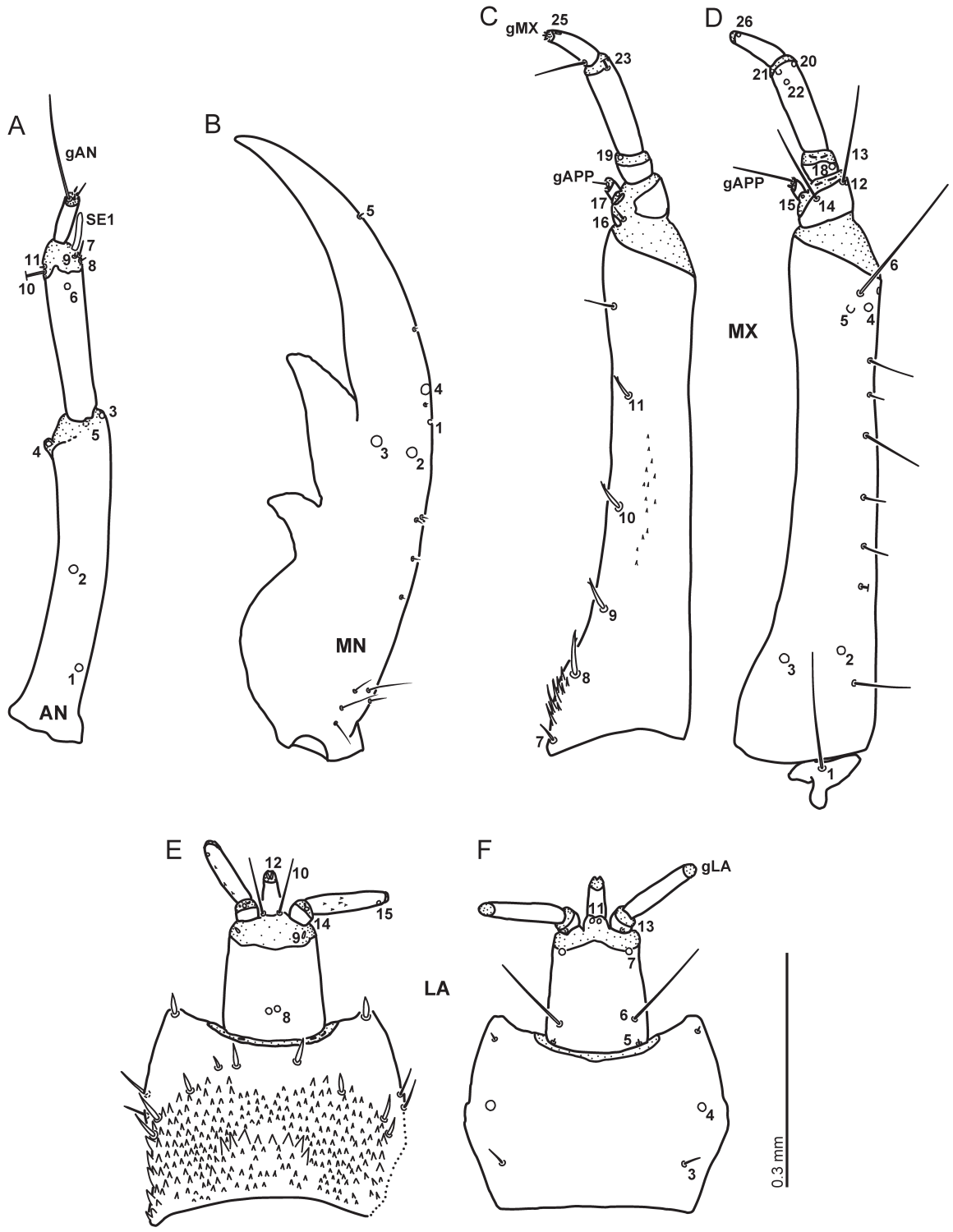

Fig. 10. Head appendages of third instar larva of *Peltochares atropiceus* (Régimbart, 1903). A – antenna, dorsal view; B – mandible, dorsal view; C, D – maxilla, dorsal view (D) and ventral view (E); E, F – labium, dorsal view (F) and ventral view (G).

Six to seven short setae along coronal and posterior half of frontal line. One very short and one short seta behind antennal socket, between PA8 and PA9. One very short seta and one pore-like sensillum on anterior corner, between PA9 and PA19. Anterolateral corner bearing two very short setae behind PA21 and PA22. Anterior half of parietale bearing seven setae laterally. One short seta behind PA14. One short seta between PA14 and PA15. One short seta behind PA15, close to PA18. One short seta anterior to PA17. One short seta lateral to PA16 and PA17. One short seta posterior to PA17. One short seta mesal to PA18. *Mandibles* (Fig. 10B) similar to second instar but bearing additional setae; one small seta between MN1 and MN4; four small setae behind MN2; five rather short on basal part of outer face. *Maxilla* (Figs 10C–D) similar to second instar but stipes bearing seven setae on outer face. *Labium* (Figs 9D, 10E–F) similar to second instar but dorsal surface of mentum with seven setae on right margin and eight on left in illustrated specimen.

Biology. *Peltochares atropiceus* inhabits low-altitude ponds with rich aquatic vegetation and can be collected in Taiwan throughout the year. We observed that females of *P. atropiceus* carry the egg-case under the abdomen, confirming the egg-carrying behaviour of *Peltochares* reported by Watts (2002).

Remarks. The general morphology of larvae of *P. foveicollis*, described by Watts (2002) corresponds well with that of *P. atropiceus* larvae. For the *Peltochares* larvae described by Bertrand (1962), see Discussion.

Discussion

Identity of *Peltochares* larvae by Bertrand (1962)

Bertrand (1962) described some hydrophilid larvae collected in Madagascar as belonging to the genus *Peltochares*, based on the identity of the associated adults, which resembled *Peltochares conspicuus* by their flat and disc-like body. However, *P. conspicuus* or similar species of *Peltochares* have never been recorded from Madagascar, and the identification is therefore doubtful (Girón &

Table 2. Summary of current knowledge of morphology of Acidocerinae larvae. See Table 3 for references.

Characters	***Helochares* group**					***Agraphydrus* gr.**	***Chasmogenus* gr.**
	Helobata	***Helochares***	***Novochares***	***Peltochares***	***Sindolus***	***Agraphydrus***	***Crephelochares***
Frontal lines	lyriform	lyriform	lyriform	lyriform	lyriform	V-shaped	?
Coronal line	absent	very short / short	very short	short	very short	very short	very short
Nasale	without teeth	with teeth	with teeth	with teeth	with teeth	with teeth	serrated / with distinct teeth
Nasale	symmetrical	asymmetrical	asymmetrical	symmetrical	asymmetrical	asymmetrical	symmetrical / asymmetrical
gFR1 composed of	one row	one row	one row	one row	one row	one row	two rows
Epistomal lobes	symmetrical	asymmetrical	asymmetrical	almost symmetrical	asymmetrical	asymmetrical	symmetrical / asymmetrical
Shape of epistomal lobes	projecting and truncate	projecting	weakly projecting	rounded	rounded	weakly projecting	rounded / weakly projecting
Number of setae of gFR2	4	3–4	4	4	4	2–4	3
Long paddle-shaped seta on frontale	?	present	?	present	?	present	absent (shape of FR8 not known)
FR6	?	long, paddle-shaped	?	short, paddle-shaped	?	long, trichoid	short, trichoid
FR9	?	long, trichoid	?	long, paddle-shaped	?	long, paddle-shaped	short, trichoid
Long paddle-shaped seta on parietale	?	present	?	present	?	present	present
Position of PA7, 12, 13	?	forming a row, PA7 between PA12 and PA13	?	loosely forming triangular group	?	forming very closely aggregated triangular group	forming rather closely aggregated triangular group
PA8	?	trichoid	?	paddle-shaped	?	paddle-shaped / ?trichoid	trichoid
PA11	?	distant from PA14	?	distant from PA14	?	close to PA14	close to PA14
Inner projection of antennomere 1	absent	absent	absent	present	absent	absent	absent
Mandibles	symmetrical	symmetrical	almost symmetrical	symmetrical	almost symmetrical	asymmetrical	asymmetrical
Distal inner tooth of right mandible	simple	simple	simple	simple	simple	bifurcate / simple	bifurcate
Dorsal sclerotization of maxillary palpomere 1	complete	incomplete	?complete	incomplete	?complete	incomplete	complete
MX5	trichoid	paddle-shaped	?trichoid	paddle-shaped	trichoid	paddle-shaped	paddle-shaped
Lateral face of mentum	not rounded	not rounded	not rounded	not rounded	not rounded	weakly rounded	rounded
Anterior corner of mentum	angulate	angulate	angulate	projected anteriorly	angulate	angulate	angulate
Cervical sclerites	not subdivided	not subdivided	not subdivided	not subdivided	not subdivided	not subdivided	subdivided
Abdominal prolegs	absent	absent	absent	absent	absent	present	present
Dorsal apex of abdominal segment 8	sinuate	sinuate	?	strongly projected, trilobate	?	sinuate / rounded	sinuate
Acrocercus	?absent	short	?	long	?	long / short	very long

Short 2021). The larvae of *P. atropiceus* described here do not correspond with those described by Bertrand (1962) and we hence consider the identification of the Malagasy larvae to be incorrect.

This fact raises the question of the identity of the larvae. Judging from the description and figures by Bertrand (1962), we consider that the larvae belong to *Tritonus* Mulsant, 1844, of the tribe Laccobiini. This is supported by the following evidence:

(1) *Tritonus* species are flat and disc-like in body shape and superficially similar to *P. conspicuus* (Short 2008, Fikáček et al. 2017).
(2) *Tritonus* is distributed in Madagascar (Short 2008).
(3) The specimens in Bertrand (1962) were collected on wet rock surfaces, which is the habitat of *Tritonus* in Madagascar (Short 2008, Fikáček et al. 2017).
(4) Many characters of the larvae described by Bertrand (1962) correspond with *Tritonus* larvae described by Fikáček et al. (2017): the epistomal lobes are almost symmetrical; the nasale is slightly asymmetrical, with four teeth; the shape of the frontal and coronal lines is identical to that of *Tritonus*; the mandibles are symmetrical and with three inner teeth; the pronotum is oval in shape; the metathorax has a pair of triangular dorsal plates; the legs are well-developed. Bertrand (1962) only did not mention the elongated spiracles, which were described in Fikáček et al. (2017).

The larval and adult morphology, habitat and distributional data of the '*Peltochares*' larvae described by Bertrand (1962) indicate that these larvae most likely belong to the genus *Tritonus*.

Current knowledge of immature stages of Acidocerinae

. The knowledge of the immature stages of the Acidocerinae remains poor. Recent studies on the systematics and phylogeny of the subfamily (Short et al. 2021, Girón & Short 2021) revealed a previously overlooked phylogenetic and morphological diversity and an ancient origin of the group. The deeply diverging lineages of Jurassic to Cretaceous origin were described and reclassified, with 23 genera currently recognized. Immature stages are only known for seven genera (Girón & Short 2021; Table 3). At the genus level, the knowledge is complete for the Nearctic and Palaearctic Realms and Australia, where larvae of all genera are known, sometimes based on species occurring elsewhere. The knowledge is also nearly complete for tropical Asia, where only the larva of *Helopeltarium* Orchymont, 1943 remains unknown (as the genus is rare, known only from historical specimens). In Africa, no data

Table 3. Current knowledge of immature stages of Acidocerinae genera, classified in genus groups defined by Girón and Short (2021). Abbreviations: immature stages: E – egg or egg-case; L – larval stages; P – pupal stage; distribution: AF – Afrotropical, AU – Australian, NA – Nearctic, NT – Neotropical, OR – Oriental, PA – Palaearctic

Genus	Distribution	Immature stages	References (selected)
***Primocerus* group**			
Primocerus Girón & Short, 2019	NT	unknown	
***Helochares* group**			
Acidocerus Klug, 1855	AF	unknown	
Aulonochares Girón & Short, 2019	NT	unknown	
Batochares Hansen, 1991	AF	unknown	
Colossochares Girón & Short, 2021	AF	unknown	
Helobata Bergroth, 1888	NA, NT	E, L	Spangler & Cross (1972), Archangelsky (1997)
Helochares Mulsant, 1844	worldwide	E, L, P	Anderson (1976), Archangelsky (1997), Watts (2002), Minoshima & Hayashi (2011)
Helopeltarium d'Orchymont, 1943	OR	unknown	
Novochares Girón & Short, 2021	NA, NT	E, L, P	Fernández (1983)
Peltochares Régimbart, 1907	AF, AU, OR	L	Watts (2002), this study
Radicitus Short & García, 2014	NT	unknown	Short & García (2014) (egg-carrying behaviour was reported)
Sindolus Sharp, 1882	NT	E, L, P	Fernández (1983, 2004)
***Agraphydrus* group**			
Agraphydrus Régimbart, 1903	AF, AU, OR	E, L	Hayashi (2009), Minoshima & Hayashi (2011), Minoshima et al. (2013), Minoshima et al. (2021)
***Chasmogenus* group**			
Chasmogenus Sharp, 1882	NT	unknown	
Crephelochares Kuwert, 1890	AF, AU, OR, PA	E, L, P	Anderson (1976), this study
***Tobochares* group**			
Crucisternum Girón & Short, 2018	NT	unknown	
Ephydrolithus Girón & Short, 2019	NT	unknown	
Globulosis García, 2001	NT	unknown	
Katasophistes Girón & Short, 2018	NT	unknown	
Nanosaphes Girón & Short, 2018	NT	unknown	
Quadriops Hansen, 1999	NT	unknown	
Tobochares Short & García, 2007	NT	unknown	
Troglochares Spangler, 1981	NT	unknown	

are available for two widespread genera (*Batochares* Hansen, 1991 and *Colossochares* Girón & Short, 2021) and the rare *Acidocerus* Klug, 1855. The main gap in knowledge remains in the Neotropics, the region with the highest diversity of Acidocerinae. Larvae are only known for a single genus endemic to South America (*Sindolus* Sharp, 1882) and three widespread genera, and remain unknown for another 12 genera. Consequently, no larval information is available for two major phylogenetic lineages within the subfamily: the *Primocerus* group (with a single genus in northern South America) and the *Tobochares* group (with eight genera in northern South America and the Atlantic coast of Brazil).

Despite the limited knowledge of immature stages, some preliminary conclusions can be made considering the phylogenetic position of genera with known immatures:

(1) Females of the *Helochares* group carry the egg case until the larvae hatch (Girón & Short 2021); this behaviour reduces predation of the eggs (Nishijima & Hironaka 2023).

(2) All known larvae of the *Helochares* group lack abdominal prolegs, have symmetrical or nearly symmetrical mandibles, and are rather similar in other morphological characters (Table 2). *Peltochares* has an unusual character on the antennomere 1, which is a small inner distal projection. This is not a strong projection as in the tribe Berosini, in which the distal projection (or seta) is an apomorphy (Archangelsky 2008). The strongly projecting and trilobate dorsal apex of abdominal segment 8 and the projecting anterior corners of the mentum are also unique characters in the known larvae of the group, whereas other larvae have a sinuate or rounded dorsal margin on segment 8 and angulate anterior corners of the mentum.

(3) *Agraphydrus* and *Crephelochares* are formally classified in separate genus groups, but both are early-branching lineages of a large clade that also includes the *Tobochares* group of genera (Short et al. 2021). The larvae of *Agraphydrus* and *Crephelochares* are similar in their habitus and morphology including chaetotaxy of the head capsule and asymmetrical mandibles (Table 2). The position of PA11, which is close to PA14, is shared by *Agraphydrus* and *Crephelochares*, whereas PA11 is distant from PA14 in the *Helochares* group (*Helochares* and *Peltochares*). Moreover, both *Agraphydrus* and *Crephelochares* are unusual in the Hydrophilidae in possessing abdominal prolegs. Outside the Acidocerinae, prolegs are only present in *Enochrus* Thomson, 1859 of the subfamily Enochrinae and they are also present in some terrestrial larvae of the subfamily Sphaeridiinae (e.g., Archangelsky 1994, 1997; Minoshima & Hayashi 2011). The presence of prolegs in both *Agraphydrus* and *Crephelochares* indicates that they may also be present in the genera of the *Tobochares* group.

Biology of immature stages of *Crephelochares*

Our rearing of *Crephelochares* confirmed the observation of Anderson (1976) that the genus does not build the egg case as most other Hydrophilidae – she reported that the eggs of *C. nitescens* were laid in a mossy hollow constructed by the adult and only covered by a loose silk. In our experiment, we kept the adults in a container with water and pieces of submerged plants from the collection site, and despite frequent inspections, we never noticed a usual-looking egg case. This is also the reason why larvae were basically overlooked, and only found when the rearing was completed and the plants from the rearing box were inspected.

Our observation of living larvae of *Crephelochares* indicate that *Crephelochares* larvae actively search for prey in a peculiar way: larvae move quickly, stop and inspect the surrounding area, and then move again. When the larvae inspect around, the middle and posterior part of their abdomen is attached to the substrate and they move the anterior half of their body. The presence of abdominal prolegs is possibly an adaptation for such a behaviour, with the prolegs fixing the position of the posterior half of the body. This type of behaviour has not been reported in the genera *Agraphydrus* and *Enochrus*, which have abdominal prolegs. Additional observation of larvae of *Crephelochares* and other genera with larval prolegs (*Agraphydrus* and likely the genera of the *Tobochares* group) would be welcome to understand the function of the abdominal prolegs and the hunting strategy of these larvae in more detail.

Acknowledgements

We are grateful to Miguel Archangelsky (Centro de Investigación Esquel de Montaña y Estepa Patagónica, Argentina) and an anonymous reviewer for their constructive criticism. The work of MF was supported by the Ministry of Culture of the Czech Republic (DKRVO 2019–2023/5.I.e, National Museum, 00023272).

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