The head of *Heterogyrus milloti* (Coleoptera: Gyrinidae) and its phylogenetic implications

Rolf G. Beutel *,[1], Evgeny Yan[1], Adrian Richter[1], Sebastian Büsse[2], Kelly B. Miller[3], Margarita Yavorskaya[1] & Benjamin Wipfler[1]

[1] Institut für Spezielle Zoologie and Evolutionsbiologie, Friedrich-Schiller-Universität Jena, Erbertstr. 1, 07743 Jena, Germany; Rolf Georg Beutel * [rolf.Beutel@uni-jena.de] — [2] Zoological Institute, Functional Morphology and Biomechanics, Kiel University — [3] Department of Biology, University of New Mexico, Museum of Southwestern Biology, 167 Castetter Hall, MSC03 2020 Albuquerque, NM 87131-0001 — * Corresponding author

Abstract

Head structures of *Heterogyrus milloti* Legros, 1953 are described in detail and documented with different morphological techniques, including μ-computed tomography and computer-based 3D reconstructions. The results are compared with cephalic conditions found in other gyrinid taxa and the observed characters are interpreted and analysed phylogenetically. Nine unambiguous cephalic apomorphies support the monophyly of Gyrinidae. In addition to well-known characters like the subdivided compound eyes and highly modified antennae, this includes the very tight connection of the dorsal surface of the head with the anterior pronotal margin, the presence of a clypeofrontal gland, the separation of a lateral portion from the clypeus, and the loss of the dorsal tentorial arm. Unambiguous synapomorphies of *Heterogyrus* and Gyrininae sensu Miller and Bergsten (2012) are the relatively widely separated dorsal and ventral ocular subunits, the absence of tactile setae on the head capsule, the shortened mesal mandibular edge, widely separated mandibular incisivi, three rows of setae on the labrum, the enlargement of the lateral mental lobes, and the loss of the stipital muscle attached to the galea. The monophyly of Gyrininae (excl. *Heterogyrus*) is supported by the widened bridge between the dorsal and ventral ocular subunits, the reduced size and dorsal shift of the dorsal eye, its distinct separation from the anterior pronotal margin, the detachment of the lateral frontal ridge from the supraocular bead, the almost completely reduced setation of the antennal flagellum, and a one-segmented galea. The steep frontal side of the head, a transverse regular field of setae on the frontal region, and the fused laminatentoria are autapomorphies of *Spanglerogyrus* Folkerts, 1979. A field of sensilla on the interocular antennal groove is a potential cephalic autapomorphy of *Heterogyrus*. The cephalic characters we analysed remain ambiguous about the interrelationships among the three tribes currently recognized in Gyrininae.

Key words

Head, mouthparts, 3D reconstruction, musculature, *Heterogyrus*, Gyrinidae.

1. Introduction

Gyrinidae are a fascinating and intensively investigated family of highly specialized adephagan beetles (e.g., Hatch 1925a,b, 1926; Brink 1955; Larsén 1954, 1966; Beutel 1989a,b, 1990; Beutel & Roughley 1988, 1994; Miller & Bergsten 2012; Gustafson et al. 2017). The distribution is world-wide. With ca. 750 described species they are a relatively small family compared to the aquatic Dytiscidae (ca. 5,000 spp.) or the terrestrial Carabidae (ca. 30,000 spp.) (Beutel & Roughley 2016; Balke & Hendrichs 2016; Arndt et al. 2016). However, with their highly specialized surface-swimming habits (e.g., Hatch 1925a; Omer-Cooper 1934; Nachtigall 1961; Larsén 1966) they are a unique group of Coleoptera. Numerous morphological modifications are linked

with the very unusual lifestyle. This does not only include highly specialized features of the thoracic locomotor apparatus, which was investigated intensively by Nachtigall (1961) and Larsén (1966), but also far-reaching modifications of cephalic structures, including completely subdivided compound eyes and a unique type of antenna suitable for detecting movements of the water surface (Bendele 1986). The phylogenetic relationships of Gyrinidae are still discussed controversially. A placement in a clade Hydradephaga was suggested by Shull et al. (2001) based on molecular data, but a sistergroup relationship between Gyrinidae and the remaining adephagan families is supported by morphological characters (Beutel 1993; Beutel & Roughley 1988; Beutel & Haas 1996; Beutel et al. 2013) and recent molecular studies (McKenna et al. 2015; Baca et al. 2017).

The Malagasy species *Heterogyrus milloti* was described relatively recently (Legros 1953). It was also briefly treated in a monograph on Gyrinidae from the Ethiopian region (Brink 1955) and in a recent primarily molecular study (Miller & Bergsten 2012). Just recently a study on the general morphology and behaviour was published by Gustafson et al. (2017). However, until now detailed anatomical information was unavailable, even though its potential phylogenetic key-role in Gyrinidae was pointed out (Miller & Bergsten 2012). Head structures of adults of Gyrinidae are relatively well-known. Skeletal structures of a broad spectrum of species of Gyrinini, Enhydrini and Orectochilini were described, illustrated and phylogenetically interpreted by Hatch (1925b, 1926). Detailed anatomical studies were presented by Honomichl (1975) and Beutel (1989a), on *Gyrinus substriatus* and the apparently ancestral species *Spanglerogyrus albiventris*, respectively. The present study on external and internal characters of the head of *Heterogyrus* closes an important gap of knowledge. The observed cephalic characters are documented with different morphological techniques and evaluated with respect to their phylogenetic implications, especially a suggested sistergroup relationship between *Heterogyrus* and a clade comprising Gyrinini, Enhydrini and Orectochilini, i.e. Gyrininae (Miller & Bergsten 2012).

2. Material and methods

Material. *Heterogyrus milloti* Legros, 1953 (Heterogyrinae; Miller & Bergsten 2012), fixed and stored in 97% ethanol; collected at Fianarantsoa, small stream ~ 8 km W Ranomofana, Ranomofana NP, Madagascar, 21°14.992′S 47°24.332′E, 2 November 2014, Miller, Gustafson and Bergsten. — ***Spanglerogyrus albiventris*** Folkerts, 1979 (Spanglerogyrinae), fixed in FAE [formaldehyde-ethanol-acetic acid], stored in 75% ethanol, dissected specimens, SEM micrographs; collected at Evergreen, Conecuh county, Alabama, USA. — ***Gyrinus substriatus*** Stephens, 1829 (Gyrinini), fixed in FAE and stored in ethanol. — ***Dineutus assimilis*** Kirby, 1837 (Enhydrini), fixed in Kahle's fluid and stored in ethanol; collected at Evergreen, Conecuh county, Alabama, USA. — ***Macrogyrus australis*** (Brullé, 1835) (Enhydrini), 70% ethanol; collected at Greeveston, Arve Forest Drive, SW Tasmania, Australia (donated by Zoologische Staatssammlung München). — ***Orectochilus villosus*** (Müller, 1776) (Orectochilini) fixed in FAE and stored in ethanol; collected in Saale river, 8 km south of Jena, Thuringia, Germany. — ***Orectogyrus* sp.** (Orectochilini), fixed in Dubosq Brazil and stored in ethanol; collected in Maasai Mara National Reserve, Kenya by M.S. Fischer. — ***Gyretes iricolor*** Young, 1947 (Orectochilini), fixed in Kahle's fluid and stored in ethanol; collected at Evergreen, Conecuh county, Alabama, USA.

Specimens of the two enhydrine species and of *Gyrinus substriatus*, *Gyretes iricolor* and *Orectogyrus* sp. were only manually dissected. Micro-CT data sets were available of *Orectochilus* and *Heterogyrus*, and additionally a microtome sections series of the latter.

Micro-Computed Tomography (μCT). One female specimen of *Heterogyrus milloti* was dehydrated in an ethanol series, dried at the critical point (EmiTech K850 Critical Point Dryer) and scanned with a SkyScan 1172 (Bruker micro-CT, Kontich, Belgium) desktop μCT (Zoological Institute, Functional Morphology and Biomechanics, Kiel University) at 40 kV and 250 μA with images taken every 0.25°. A female of *Orectochilosus villosus* was also dehydrated and dried at the critical point (EmiTech K850 Critical Point Dryer). It was scanned in a small Eppendorf tube at Beamline BW2 of German Electron Synchrotron Facility (DESY, Hamburg) using a stable low photon energy beam (8 kVP) and absorption contrast.

The data sets are stored in the collection of the Phyletisches Museum, Jena, Germany and can be accessed by contacting the corresponding author.

Computer-based 3D-reconstruction. Based on the μCT-image stack the head of *Heterogyrus milloti* was reconstructed using Amira 6.0 (Visage Imaging, Berlin, Germany). Musculature, nervous system and gut were manually outlined using the interpolation function. Subsequently the individual materials were separated (using the algorithm function of Amira) and imported into VG studio Max 2.0.5 (Volume Graphics, Heidelberg, Germany) where volume rendering was performed with Scatter HQ. Image plates were assembled and arranged with Adobe Photoshop and Illustrator (Adobe Inc., California, USA).

Histology. One specimen of *Heterogyrus milloti* (head and thorax) was embedded in araldite CY 212® (Agar ScientiWc, Stansted/Essex, UK) and cut at 1 μm using a microtome HM 360 (Microm, Walldorf, Germany) equipped with a diamond knife. Sections were stained with toluidine blue and pyronin G (Waldeck GmbH and Co.KG/Division Chroma, Münster, Germany). Selected images of the series were photographed using a light

microscope (Zeiss Axioplan, Germany) equipped with a camera (PixeLink Capture OEM, Ottawa, Canada).

Digital microscopy. A specimen of *Heterogyrus milloti* was cleaned with ultrasonic sound, dried with acetone and mounted on a needle. Digital microscopy was performed with a Keyence VHX 2000 (Keyence, Osaka, Japan). Mandibles were embedded in glycerin and then photographed under a light microscope (Zeiss Axioplan, Germany) equipped with a camera (PixeLink Capture OEM, Ottawa, Canada). Image plates were assembled and arranged with Adobe Photoshop and Illustrator (Adobe Inc., California, USA).

Scanning electron microscopy. Specimens of *Heterogyrus milloti* and *Gyrinus substriatus* were cleaned with ultrasonic sound, dehydrated in an ethanol series, dried and coated with gold (EmiTech K500 sputter coater). SEM micrographs were taken with a Philips XL 30 ESEM equipped with Scandium software.

Terminology. The terminology for skeletal cephalic structures is mainly based on the detailed description of *Spanglerogyrus* by Beutel (1989a) (see also Dressler & Beutel 2010). The nomenclature of v. Kéler (1963) is used for cephalic muscles but the muscle designations of Wipfler et al. (2011) are given in brackets.

Phylogenetic analyses. The data (44 characters of the head) were entered in a matrix with Winclada (Goloboff 1995) and parsimony analyses were carried out with NONA (ratchet, 1000 replicates) (Goloboff 1995) and TNT using the exact search algorithm (implicit enumeration) (Goloboff et al. 2008). All characters had equal weight, and all were treated as unordered, with the exception of character 6 (subdivision of compound eyes). The Bremer support values were calculated with NONA (Goloboff 1995).

3. Morphological results

3.1. External head capsule
Figs. 1, 2A, 3

The prognathous head is distinctly broader than long, with a maximum width (ca. 1.8 mm) at the lateral edge of the interocular bridge separating the upper and lower part of the compound eyes. A short posterodorsal part of the head capsule is retracted into the prothorax. A deep lateral emargination of the anterior pronotal margin precisely fits with the posterior edge of the dorsal subunit of the compound eye, and a broad median pronotal projection reaches the middle dorsal ocular region. The nearly straight anterior edge of the projection appears almost merged with the dorsal surface of the frontal region, with the border almost obliterated. On the ventral side, the

prosternum covers the posterior gular region and adjacent genal areas. Its lateral part almost reaches the hind margin of the ventral subunit of the compound eye, which is laterally adjacent with the anteriorly pointed lateral pronotal projections. The colouration of the head capsule is almost black on the posterodorsal region, along the lateral frontal edges and between the subunits of the compound eyes, partly with a metallic greenish hue. It is brownish on the remaining dorsal region and posteroventral parts, but yellowish on most areas of the ventral side, like on most parts of the postcephalic body (Fig. 1). The surface is almost completely glabrous, without granulation, specific sculpture, pubescence, or long setae (Fig. 3). Only an extremely fine wrinkled pattern is recognizable on the dorsal side at high magnification (SEM). The compound eyes are completely subdivided into a ventral and a dorsal subunit. Both are moderately convex and not protruding beyond the lateral edge of the head, which is formed by the sharp ventrolateral edge of the interocular bridge (Fig. 2A). The dorsal surface of this structure is distinctly concave, thus providing an elongate groove for reception of the antennal flagellum in posterior orientation, with a group of medium length sensorial setae (sensilla trichodea) on its anterior portion. It is less than half as wide as the dorsoventral diameter of the ventral ocular subunit. The dorsal eye is about as large as the ventral ocular subunit and not shifted to the dorsal surface of the head (Fig. 2A). The numerous small and slightly convex cuticular lenses are very similar on both subdivisions. The ventral edge of the dorsal eye is almost straight whereas the dorsal border is evenly rounded and delimited by an indistinct supraocular bead. The slightly convex upper margin of the ventral eye is adjacent with a sharp lateral edge of the interocular bridge. The anterior and ventral margins are more rounded. The exposed part of the dorsal side of the head is largely formed by the frons but frontal sutures are missing. The converging lateral edges form slightly rounded ridges (lfrr in Fig. 2A) anterior to the compound eyes and a distinct lateral bead, continuous with the dorsal supraocular bead. The lateral edge delimits a shallow, roughly triangular groove between the lateral clypeus, the antennal foramen, and the anterior margin of the upper portion of the compound eye. The groove serves for reception of the basal part of the antennal flagellum in posterodorsal orientation. It is subdivided by a curved longitudinal furrow anteriorly and a nearly straight, oblique furrow posteriorly. The interocular suture (Hatch 1925b, 1926: exoculata) is continuous with the anterior curved furrow. It is quite indistinct dorsally and obliterates ventrally. The anterior frontal margin is nearly straight. A thin but distinct transverse furrow corresponds with an internal clypeofrontal strengthening ridge. The transverse clypeus appears evenly rounded and slightly convex along its anterior edge in dorsal view, but is in fact slightly concave. A horizontal lateral part is very distinctly separated from the main middle clypeal portion. The lateral clypeal part bears a distinct bulge and a group of medium length setae is inserted on its surface. On the ventral side of the head the posterior tentorial grooves are visible as distinct fissure-shaped openings. The gula is

Fig. 1. *Heterogyrus milloti*, head and pronotum, LM micrographs. **A**: ventral view; **B**: dorsal view. — Scale bar 0.5 mm.
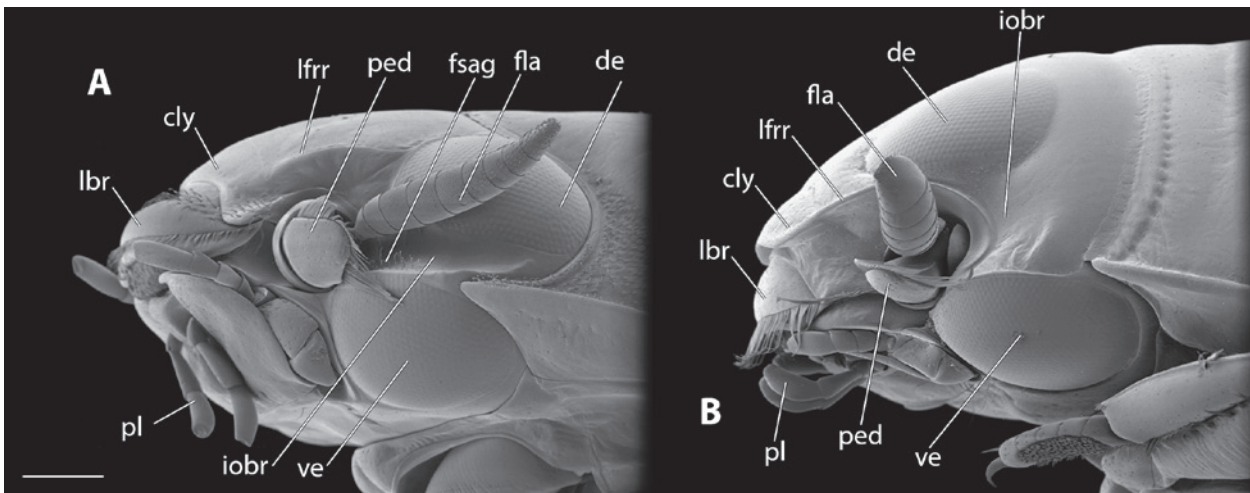
Fig. 2. SEM micrographs, head, lateral view. **A**: *Heterogyrus milloti*; **B**: *Gyrinus substriatus*. — ***Abbreviations***: cly – clypeus, de – dorsal eye, fla – flagellum, fsag – field of sensilla of antennal groove, iobr – interocular bridge, lbr – labrum, lfrr – lateral frontal ridge, ped – pedicellus, pl – palpus labialis, ve – ventral eye. — Scale bar 0.2 mm.

anteriorly completely fused with and externally not delimited from the labial submentum. Internally the gula gives rise to gular ridges that are visible through the partly transparent ventral cuticle.

3.2. Internal skeletal structures
Figs. 6, 7, 10

The transverse clypeofrontal (epistomal) strengthening ridge is low but distinct and complete. The flat longitudinal gular ridges are well-developed and high. Their upper edge is bent laterad. Posterolaterally they are continuous with the well-developed postoccipital ridge, which encloses the foramen occipitale laterally and dorsally and forms a strongly developed apodeme dorsomedially. The gular ridges are anteriorly indistinguishably fused with the equally flat posterior tentorial arms, which are continuous with the distinctly developed anterior arms, which are flat in this transition area but triangular in cross section closer to their origin on the head external capsule. Their anterior end is fused with the nearly vertical lateral wall of the head capsule at the border between the frons and clypeus. An anterior tentorial groove or pit is not re-

cognizable externally. Mesally directed laminatentoria are distinctly developed but not fused medially. The dorsal arms are absent. A typical transverse tentorial bridge is not present but narrow tendons of M. frontobuccalis anterior are probably vestiges of it. A high and long triangular midgular apodeme is present. Extensive internal circumocular ridges are present around both ocular subunits, with separate dorsal and ventral openings for the bipartite optic lobes.

3.3. Labrum
Figs. 1, 2A, 3A, 7

The transverse, short but thick labrum is connected with the clypeus by a membranous fold which is not visible externally. Its basolateral edges reach the posterolateral clypeal edge posteriorly. They are concealed by the horizontal lateral clypeal areas in dorsal view. The basal part of the labrum is bulging except for the lateral area, and fits very closely with the anterior clypeal margin. It is distinctly separated from the main part of the labrum and covered by medium length setae, similar to those of the horizontal lateral clypeal portions. A pair of small, round
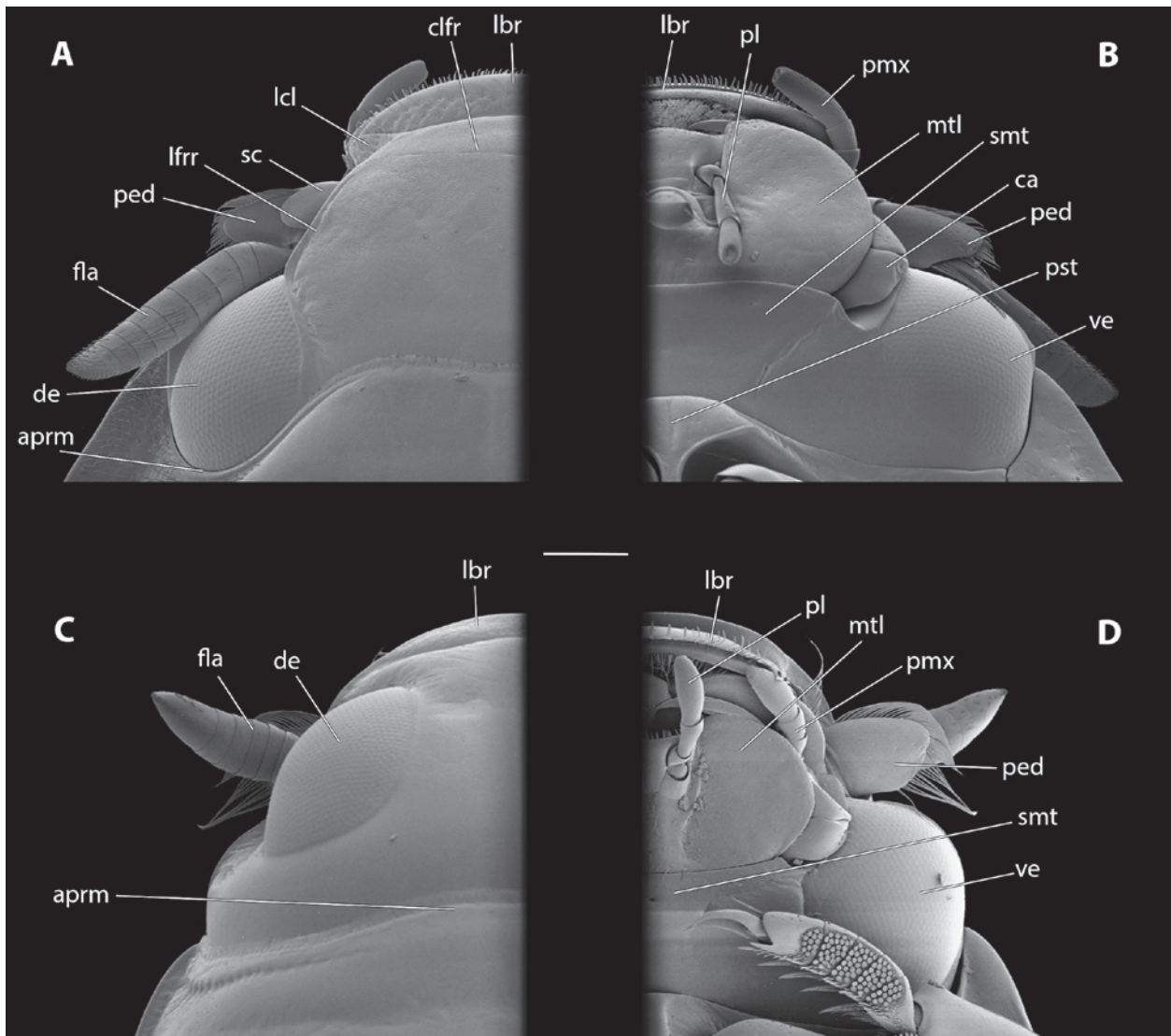
Fig. 3. *Heterogyrus milloti* and *Gyrinus substriatus,* head, SEM micrographs. **A**: *H. milloti* dorsal view; **B**: *H. milloti* ventral view. **C**: *G. substriatus* dorsal view; **D**: *G. substriatus* ventral view. — ***Abbreviations***: aprm – anterior pronotal margin, ca – cardo, clfr – clypeofrontal ridge, de – dorsal eye, fla – flagellum, lbr – labrum, lcl – lateral clypeal portion, lfrr – lateral frontal ridge, mtl – mental lobe, ped – pedicellus, pl – palpus labialis, pmx – palpus maxillaris, pst – prosternum, sc – scapus, smt – submentum, ve – ventral eye. — Scale bar 0.2 mm.

semitransparent areas is present paramedially close to the labral base. A dense regular row of short setae is inserted in a furrow along the anterior labral edge. A second and third row of long setae are present anteroventrally. The ventral side of the labrum is flat and sclerotized but mainly formed by endocuticle. Internally loosely arranged fat body tissue is present.

Musculature. Intrinsic (M. labroepipharyngalis, M.7 = Olb5) and extrinsic (M. frontolabralis, M.8 = Olb1; M. frontoepipharyngalis, M.9 = Olb2) labral muscles are absent.

3.4. Antennae
Figs. 1, 2A, 3A,B, 6A,B, 8C

The 11-segmented antenna is inserted in the ventral part of the triangular lateral frontal groove, above the dorsal mandibular articulation. The scapus is large and cup-

shaped, with a dorsal rounded lobe partly covering the basal part of the pedicellus, and shows a distinct basal constriction and a comparatively small, elongated articulatory piece. It rests on a narrow triangular genal projection with a sharp anterior edge, which separates it from the dorsal maxillary surface. The large parabolic or shield-shaped pedicellus is broadly inserted into the wide distal calyx of the scapus. It bears a dense row of long sensorial setae on its sharp lateral edge. The Johnston's organ is well developed (Fig. 9D). Flagellomere 1 is much smaller than the two basal segments and calyx-shaped, with a distinctly narrowed and curved basal part that articulates with a posterobasal orifice of the pedicellus. The entire 9-segmented flagellum is compact but still fairly elongate. The short, cylindrical segments fit very closely together, without externally visible articulatory membranes. Antennomeres 5 – 10 have a distinctly reticulate surface structure and are slightly decreasing in size towards the antennal apex. Setae of variable length

are inserted on the ventral side of antennomeres 6 (one pair) to 10, increasing in number on the distal segments. The terminal antennomere 11 is cone-shaped and densely covered with short setae.

Musculature (Figs. 8C, 10B). M. tentorioscapalis anterior (**M.1** = 0an1): (O) dorsolaterally on posterior tentorial arm; (I) anteroventrally on base of scapus; (F) depressor and rotator of the antenna. — M. tentorioscapalis posterior (**M.2** = 0an2): (O) anterolaterally on gular ridges and dorsal surface of laminatentoria; (I) posteriorly on the base of the scapus; (F) retractor and rotator of the antenna. — M. tentorioscapalis lateralis/medialis (**M.3/4** = 0an3/4): (O) basal part of anterior tentorial arm and mesally on the laminatentoria; (I) dorsally on the base of the scapus; (F) levator of the antenna. — M. scapopedicellaris lateralis (**M.5** = 0an6): (O) posterodorsal wall of the scapus, narrow at the origin; (I) dorsally on the base of the pedicellus; (F) depressor of the pedicellus and flagellum. — M. scapopedicellaris medialis (**M.6** = 0an7), a strongly developed cone-shaped muscle: (O) extensive area on mesal wall of the enlarged distal part of the scapus; (I) dorsally on the base of the pedicellus; (F) levator of the pedicellus and flagellum.

3.5. Mandibles

Figs. 4, 6C,D, 7, 9A–D

The slightly asymmetric mandibles are short and stout and almost completely concealed between the labrum and the ventral mouthparts in their resting position. They are articulated in a typical dicondylic manner. A strongly developed condyle on the ventral mandibular base fits into a corresponding socket of the head capsule, and a deep dorsal mandibular socket fits with a condyle of the head capsule. The two mandibular incisivi are widely separated from each other, the mesal one forming the tip of an extensive triangular projection. The lateral apex is rather pointed on the right mandible but blunt on the left one. The anterior cutting edge connecting the two incisivi is nearly straight on the left mandible, but irregular on the right one, and ending with a more pronounced mesal tooth. The shortened mesal edge proximad the triangular projection is set with a row of very short and fine setae. A mola and prostheca are lacking. The mesal edge of the basal part of the mandibles is short. The comparatively long external mandibular margin is strongly rounded and bears a flat lamella ventrally. The cuticle of the distal half of the lateral edge is perforated by thin channels, and also the anterior edge of the triangular projection.

Musculature (Figs. 6C,D, 9, 10). M. craniomandibularis internus (**M.11** = 0md1), the largest muscle of the head: (O) extensive areas of the posterior half of the head capsule, interocular bridge and circumocular ridges, ventrolateral, posterolateral and posterodorsal regions; (I) mesal mandibular base with a strongly developed, sclerotized tendon; (F) adductor of the mandible. — M. craniomandibularis externus (**M.12** = 0md3): (O) ventrolaterally on the posterior head capsule (I) lateral mandibular

base with a sclerotized tendon; (F) abductor. — M. hypopharyngo-mandibularis (**M.13** = 0md4): present but very thin, composed of three thin fibres accompanied by a nerve, (O) anterior tentorial arm; (I) dorsomesal inner surface of the mandible, very close to the basal margin.

3.6. Maxillae

Figs. 3B, 5

The maxillae are inserted in a shallow maxillary groove delimited by the lateral margin of the mentum and the anteriormost submentum, and a rounded edge on the genal region, which is anterolaterally adjacent to the anteroventral margin of the ventral subunit of the compound eye. It is almost completely covered by large mental lobes. Only the lateral parts of the cardo and basistipes and the palp are exposed. The transverse cardo is well-developed. An obtuse rounded angle separates the posterior margin from the oblique lateral edge, which bears several short setae. At the basal articulation with the head capsule the cardo is divided into two projections for attachment of the cardinal extensor and flexor, respectively (M. craniocardinalis, M. tentoriocardinalis). The latter is more distinct and rounded, and separated from the nearly straight posterior cardinal margin by a distinct notch. The basistipes and mediostipes are connected with the slightly rounded anterolateral cardinal edge, the former slightly overlapped by it. The basistipes is shaped like a narrow triangle in ventral view, with a rounded anterior tip. It is adjacent with the small palpifer, which is scarcely visible in ventral view between palpomere 1 and a separate sclerite covering the base of the galea and palp. The nearly straight mesal edge of the basistipes is connected with the mediostipes, which is completely fused with the strongly sclerotized lacinia, both forming the largest part of the maxilla. The mesal edge of the lacinia bears a fairly short ventral row of thin setae and dorsally a distinctly longer row of very strong, long setae, some of them distinctly curved. The hook-shaped distal part is tapering and rounded at its apex. The palp-like galea is slender, 2-segmented and inserted between the basal part of the rounded lateral edge of the lacinia and the basal palpomere. The small sclerite covering the base of the proximal galeomere is inserted in a lateral emargination of the lacinia and posteriorly adjacent with the anterior part of the basistipes. Palpomere 1 is inserted between this sclerite and the small palpifer on the dorsal side of the maxilla. It is slightly longer than wide and bears a short spine anteriorly at its distal edge. Palpomere 2 is about 1.5 times as long and slightly widening distally, palpomere 3 times as long as 2 and cylindrical. The terminal palpomere 4 is almost 3 times as long as 2 and 3, with a slightly convex posterior edge, and a nearly truncate apex with a membranous sensory field.

Musculature (Figs. 6E,F, 7A, 9, 10). M. craniocardinalis externus (**M.15** = 0mx1), a fan-shaped muscle: (O) ventrolaterally on the head capsule, between the ventral margin of the ventral eye and the gular ridges; (I) lat-
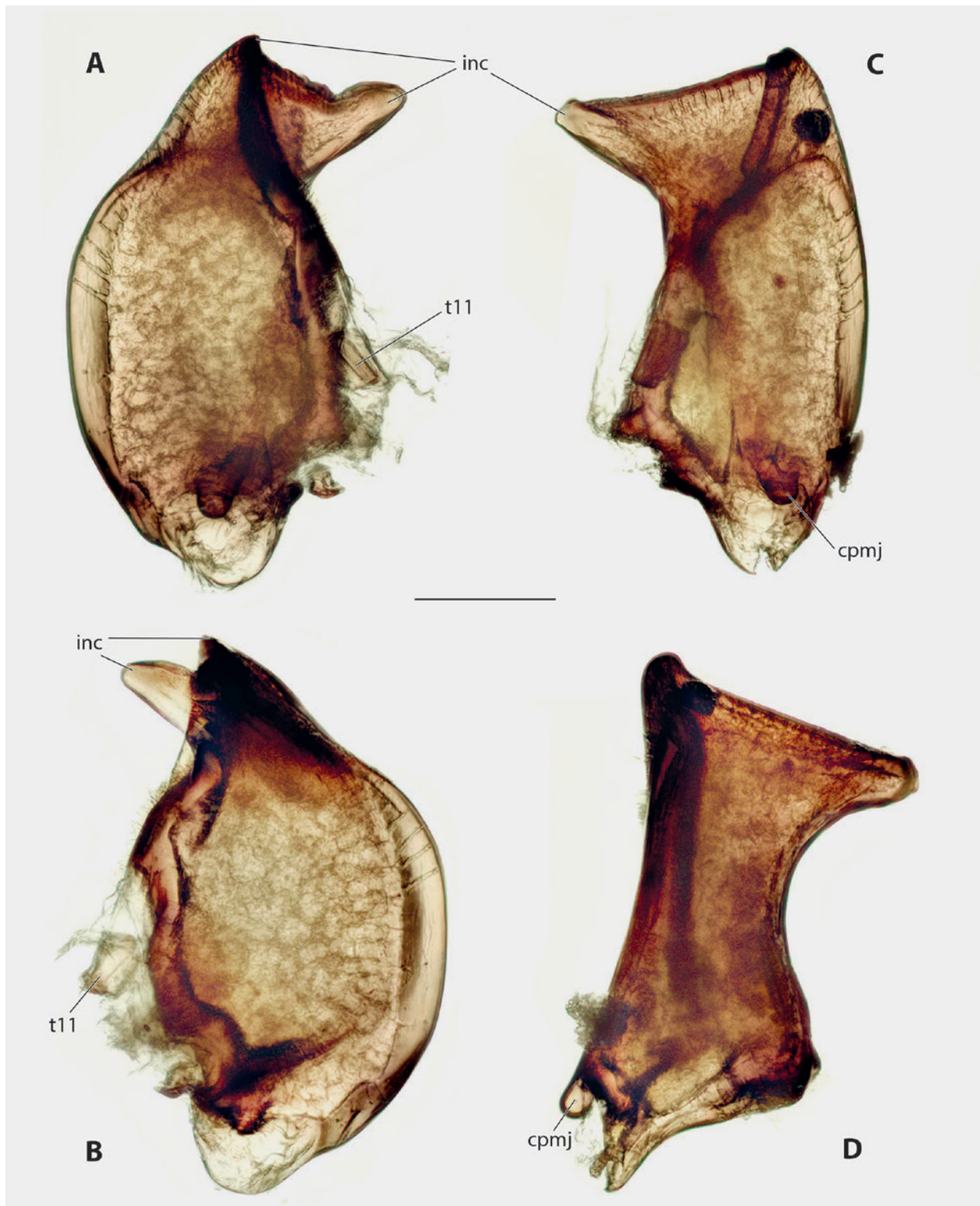
Fig. 4. *Heterogyrus milloti*, mandibles, LM micrographs (anterior is top). **A**: ventral view, right mandible; **B**: dorsal view, right mandible; **C**: ventral view, left mandible; **D**: dorsal view, left mandible. — ***Abbreviations***: cpmj – condyle of primary mandibular joint, inc – incisivus, t11 – tendon of M. craniomandibularis internus (M.11). — Scale bar 0.2 mm.

eral branch of the internal cardinal process, with a short sclerotized tendon; (F) extensor of the cardo (inserts laterad the cardinal articulation pivot). — M. tentoriocardinalis (**M.17** = 0mx3), fan-shaped: (O) lateral surface of the gular ridge; (I) mesal branch of the internal cardinal process; (F) flexor of the cardo, adductor of the maxilla,

antagonistic to M.15. — M. tentoriostipitalis (**M.18a,b,c** = 0mx4, 5), a strongly developed muscle with a triple origin: (O) posterior margin of gula, mid-gular apodeme, and mesal surface of gular ridge; (I) ventral membrane between the cardo and the stipital base; (F) stipital adductor and retractor. — M. craniolacinialis (**M.19** =
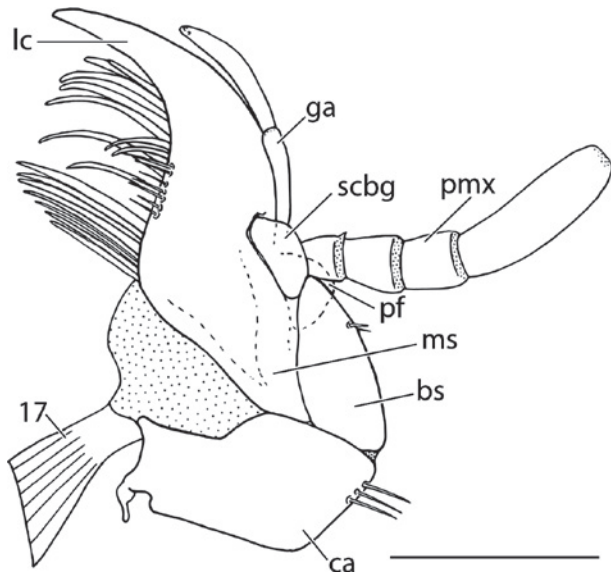
Fig. 5. *Heterogyrus milloti*, maxilla, line drawing, ventral view. — ***Abbreviations***: bs – basistipes, ca – cardo, ga – galea, lc – lacinia, ms – mediostipes, pf – palpifer, pmx – palpus maxillaris, scbg – sclerite covering base of galea, 17 – M. tentoriocardinalis. — Scale bar 0.2 mm.

0mx2): (O) ventrally on the head capsule, ventrolaterad M.15; (I) membrane at the base of the lacinia, with a slender tendon; (F) adductor and retractor of the lacinia. — M. stipitopalpalis internus (**M.23** = 0mx10): (O) ventrally on the stipital surface; (I) mesally on the base of the palpifer; (F) depressor of palpifer and palp. — M. palpopalpalis primus (**M.24** = 0mx12): (O) lateral wall of palpifer; (I) lateral base of palpomere 1. The distal palp muscles (Mm. palpopalpalis tertius and quartus, **Mm.26, 27** = 0mx13, 14) could not be identified on the available μCT scans and microtome sections.

3.7. Labium
Figs. 1A, 2A, 3B, 9

The submentum is firmly connected with the head capsule and posteriorly completely fused with the gula. Its lateral margin anterior to the posterior tentorial grooves is delimited a faintly impressed oblique line. Its anterior margin is slightly rounded laterally but nearly straight in the middle region. The large, plate-like mentum is connected with it by a membranous fold that is not visible externally as the posterior margin of the mentum is covered by the anterior submental edge. The rounded lateral lobes of the mentum are large, with their anterior margin projecting beyond the anterior premental margin.

The median emargination enclosed by them contains the prementum. The mesal edges are nearly straight and a broadly rounded median projection is present proximally. A distinct bead is present along the entire margin of the emargination. The prementum is well-developed and approximately quadrangular, with a slightly convex anterior margin. Its base is covered by transverse membranous seam, which is medially covered by the median projection of the emargination of the mentum. The small palpifers are distinctly recognizable as individual structures, only incompletely fused with the premental surface. The lateral borders are clearly visible. Their anterior edge is slightly concave and a faintly impressed transverse line is present between them. An internal apodeme is present but firmly connected with the lateral premental wall. A ligula is not developed and vestiges of paraglossae not recognizable. The short basal palpomere is curved downwards and slightly narrowing towards its distal edge, which is interrupted by a short, narrow slit dorsally. Palpomere 2 is more than twice as long, slender basally and widening distally. The terminal palpomere is similar to the apical maxillary palpomere but slightly shorter.

Musculature (Figs. 7A, 9). M. submentopraementalis (**M.28** = 0la8): (O) medially on the submentum, anterior to the mid-gular apodeme; (I) medially on the posterior edge of the prementum; (F) retractor of the prementum. — M. tentoriopraementalis inferior (**M.29** = 0la5): (O) two separate areas of origin, submentum laterad M.28 and anterior edge of mid-gular apodeme; (I) apodeme of palpiger. — M. praementopalpalis externus (**M.34** = 0la14): (O) posteriorly on internal apodeme of palpiger; (I) base of palpomere 1; (F) moves the labial palp.

3.8. Epipharynx and hypopharynx
Figs. 6A, 7A, 8A, 9

The anterior epipharynx is continuous with the flat, ventral labral surface. It is sclerotized, with a smooth surface. Paired sclerotized concavities fit with the upper surface of the mandibles. The concavities are divided by a strongly developed, sclerotized median bulge. The cuticle of the lateral walls of this structure is dorsally connected with the cuticle of the clypeus. On its posterior part it bears a sharp median keel and over a short distance also smaller lateral keels. These structures interact with the mesal mandibular apex. The cuticle of the epipharynx is semimembranous posterior to the median bulge. Anterolaterally this epipharyngeal region forms

→ **Fig. 6.** *Heterogyrus milloti*, head, 3D reconstructions, volume renders. **A**,**B**: cephalic nervous system and digestive tract in (**A**) dorsal view and (**B**) ventral view; **C**,**D**: mandibles and mandibular muscles in (**C**) dorsal view and (**D**) ventral view; **E**,**F**: maxillae and maxillary muscles in (**E**) dorsal view and (**F**) ventral view. — ***Abbreviations***: ata – anterior tentorial arm, bst – basistipes, ca – cardo, cor – circumocular ridge, ga – galea, gur – gular ridge, lbr – labrum, lc – lacinia, ltent – laminatentoria, md – mandible, mga – midgular apodeme, ol – optic lobe, ped – pedicellus, pmx – palpus maxillaris, pph – prepharynx, sc – scapus, soes – suboesophageal ganglion. Musculature: 11 – M. craniomandibulars internus, 12 – M. craniomandibulars externus, 13 – M. tentoriohypopharyngalis, 15 – M. craniocardinalis, 17 – M. tentoriocardinalis, 18 – M. tentoriostipitalis, 19 – M. craniolacinialis.
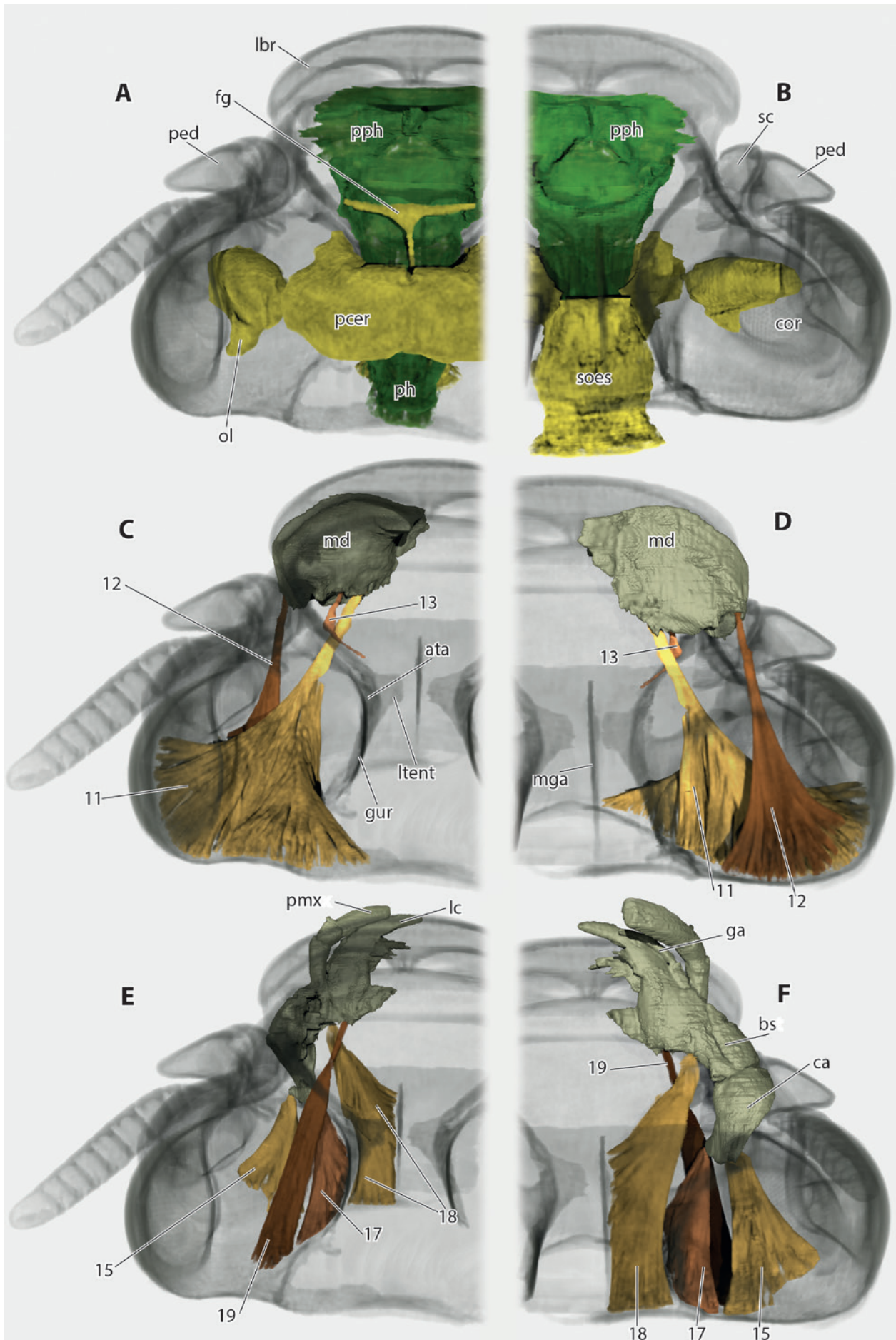
flattened, pad-like structures. In the middle region of the epipharynx a median concavity is enclosed by large lateral bulges, with a semimembranous cuticular structure, but reinforced by longitudinal sclerotized bars laterally. Epipharyngeal sensorial lobes or appendages are not developed and microtrichia are almost completely missing. The anterior hypopharynx is a transparent, ramp-like structure dorsally connected with the prelabium and continuous with the upper premental surface. Elongate-triangular lateral appendages are present, resembling those described for *Spanglerogyrus* (BEUTEL 1989a) and *Gyrinus* (HONOMICHL 1975). In its middle region the hypopharynx is strongly convex, slightly asymmetric, sclerotized, and roughly fitting with the median concavity of the middle epipharyngeal region. This part of the hypopharynx interacts with the mandibles.

A very short preoral chamber is enclosed by the posterior epipharynx and hypopharynx and the membrane adjacent with the mandibular bases. In this region the flat semimembranous epipharyngeal surface is enclosed by lateral folds. The corresponding hypopharyngeal surface is also flat. An internal thickening serves as attachment area of the strongly developed hypopharyngeal retractor, M. tentoriobuccalis anterior (M.48), and is posteriorly continuous with a strongly developed tendon. Two layers of a very strongly developed transverse muscle are present in this region and between them a thin layer of longitudinal muscles. The edges of the posteriormost parts of the epi- and hypopharynx are fused, thus forming a prepharyngeal tube, shaped like a transverse crescent in cross section, with distinctly upturned lateral edges. The posterior hypopharynx is reinforced by a distinct median sclerotization.

Musculature (Figs. 7B, 8A). M. frontohypopharyngalis (**M.41** = 0hy1), a well-developed bipartite muscle: (O) frons, two nearly adjacent areas at the level of the anterior margin of the dorsal eye, laterad M.45; (I) apically on dorsolateral edge of prepharynx, immediately anterad the anatomical mouth; (F) elevator of the anatomical mouth. — M. clypeopalatalis (**M.43** = 0ci1), a strongly developed muscle with many parallel fibers: (O) laterally on the clypeus; (I) lateral bulges of middle region of epipharynx, laterad the median concavity; (F) dilator of the preoral cavity; M. clypeobuccalis (**M.44** = 0bu1), an oblique, nearly horizontal muscle, composed of numerous very thin fibres which penetrate the strongly developed transverse epipharyngeal muscles: (O) broad area on anterior clypeus, between M.43; (I) immediately anterior to the anatomical mouth; (F) levator of the poste-
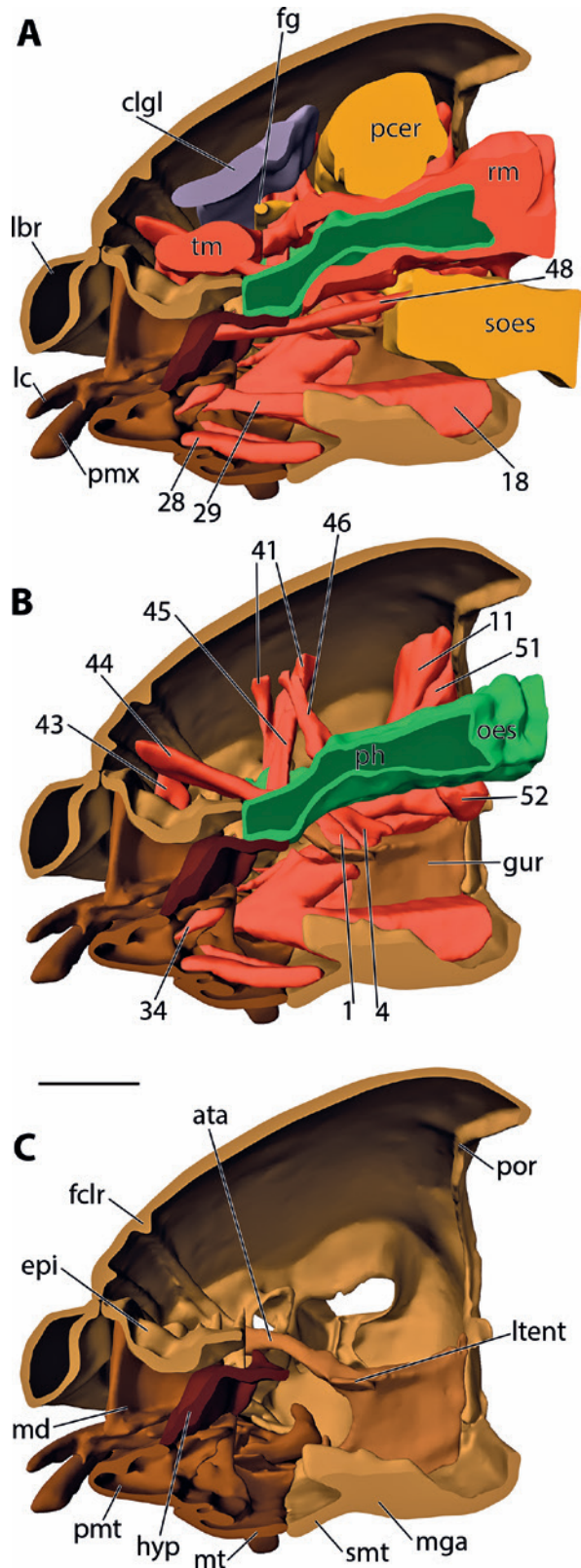
Fig. 7. *Heterogyrus milloti*, head, 3D reconstructions, surface renders, sagittal section, mesal view (anterior is left). **A**: with all internal structures; **B**: nervous system, several muscles, and clypeofrontal gland removed; **C**: exo- and endoskeleton only. — *Abbreviations*: ata – anterior tentorial arm; clgl – clypeal gland, epi – epipharynx, fclr – frontoclypeal ridge, fg – frontal ganglion, gur – gular ridge, hyp – hypopharynx, lbr – labrum, lc – lacinia, ltent – laminatentoria, md – mandible, mga – midgular apodeme, mt – mentum, oes – oesophagus, pcer – protocerebrum, ph – pharynx, pmx – palpus maxillaris, pmt – prementum, por – postoccipital ridge, smt – submentum, soes – suboesophageal ganglion, tm – transverse muscle. Musculature: 1, 4 – Mm. tentorioscapales anterior, medialis, 11 – M. craniomandibularis internus, 18 – M. tentoriostipitalis, 28 – M. submentopraementalis, 29 – M. tentoriopraementalis inferior, 34 – M. praementopalpalis externus, 41 – M. frontohypopharyngalis, 43 – M. cypeopalatalis, 44 – M. clypeobuccalis, 45 – M. frontobuccalis anterior, 46 – M. frontobuccalis posterior, 48 – M. tentoriobuccalis anterior, 51 – M. verticopharyngalis, 52 – M. tentoriopharyngalis. — Scale bar 0.2 mm.
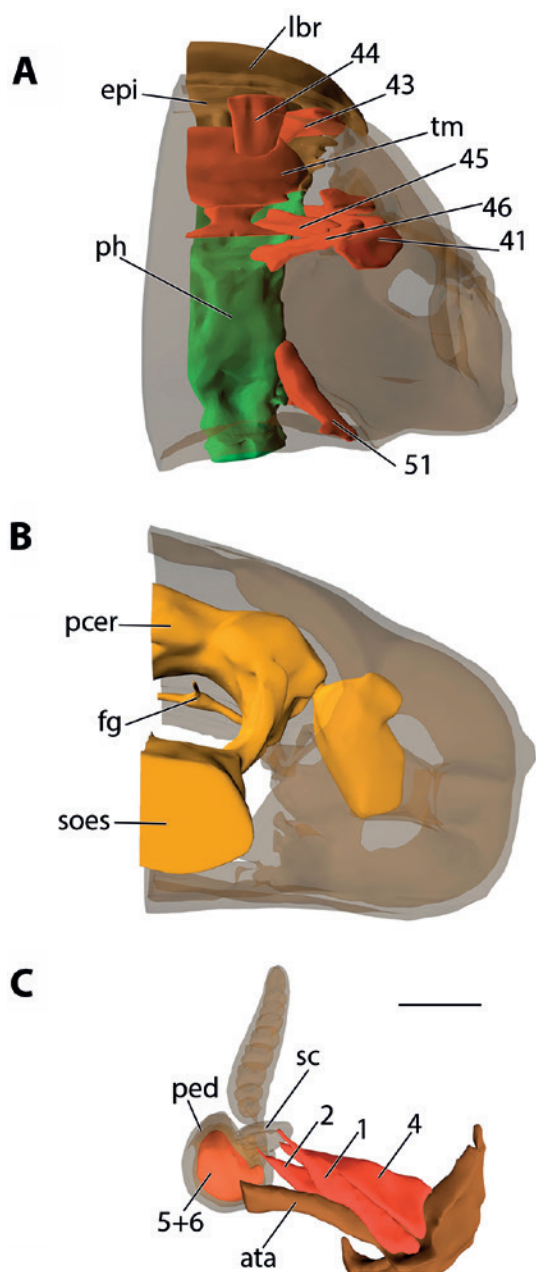
Fig. 8. *Heterogyrus milloti*, selected head structures, 3D reconstructions, surface renders. **A**: cephalic digestive tract, ring muscle layer removed, dorsal view (anterior is top; left head side largely omitted); **B**: brain with divided optic lobes and suboesophageal ganglion, posterior view, head capsule semitransparent (left head side omitted); **C**: right antenna, antennal muscles and tentorium, dorsal view (anterior is left). — *Abbreviations*: epi – epipharynx, lbr – labrum, pcer – protocerebrum, ped – pedicellus, ph – pharynx, sc – scapus, soes – suboesophageal ganglion, tm – transverse muscle. Musculature: 1, 2, 4 – Mm. tentorioscapales anterior, posterior, medialis, 5, 6 – M. scapopedicellaris lateralis, medialis, 41 – M. frontohypopharyngalis, 43 – M. clypeopalatalis, 44 – M. clypeobuccalis, 45 – M. frontobuccalis anterior, 46 – M. frontobuccalis posterior, 51 – M. verticopharyngalis. — Scale bar 0.2 mm.

rior epipharynx, dilator of the prepharynx and anatomical mouth. Mm. compressores epipharyngis (**M.67**, not covered in the nomenclature of WIPFLER et al. 2011): a very strongly developed transverse muscle is present above the insertion of M.44.

## 3.9. Pharynx

Figs. 7A,B, 8A, 9, 10

Shaped like a flattened U in cross section at the anatomical mouth opening (Figs. 9F, 10A), then nearly quadrangular, with a relatively wide lumen (Fig. 10B–D). Dorsal, lateral and ventrolateral longitudinal folds for muscle attachment are low in the precerebral pharyngeal region, but more distinct in the postcerebral section where Mm. verticopharyngalis (M.51) and tentoriopharyngalis (M.52) insert (Figs. 9, 10).

Musculature (Figs. 7B, 8A, 9, 10). M. frontobuccalis anterior (**M.45** = 0bu2): (O) paramedially on the anterior frons, mesad M.41; (I) laterally on the anatomical mouth, immediately behind the frontal ganglion, very close to the attachment of M.41; (F) dilator of the precerebral pharynx. — M. frontobuccalis posterior (**M.46** = 0bu3), composed of several parallel bundles: (O) frons, directly anterior to the anterior protocerebral margin; (I) dorsolaterally on the posterior precerebral pharynx; (F) dilator of the precerebral pharynx; together with M.50. — M. tentoriobuccalis anterior (**M.48** = 0bu5) (often misinterpreted as M. tentoriohypopharyngalis [M.42]; e.g., DRESSLER & BEUTEL 2010), a strongly developed unpaired muscle (Fig. 9E): (O) on extremely thin, paired tendons posteriorly attached to gular ridges; (I) on the median thickening of the posterior hypopharynx; (F) retractor and depressor of the hypopharynx, dilator of the preoral cavity. The muscle always stretches posteriorly between the tritocerebral commissure and the suboesophageal ganglion (Fig. 5E) and anteriorly between the mesal margins of the laminatentoria (Fig. 5D). — M. verticopharyngalis (**M.51** = 0ph1), well developed: (O) dorsolaterally on postoccipital ridge; (I) dorsolaterally on the postcerebral pharynx, opposite to M.52; (F) dilator of the postcerebral pharynx, together with M.52. — M. tentoriopharyngalis (**M.52** = 0ph2), relatively short but well developed: (O) posterior edge of gular ridge; (I) ventrolaterally on the postcerebral pharynx, opposite to M.51; (F) dilator of postcerebral pharynx, together with M.51. — A ring muscle layer encloses the entire pharynx.

3.10. Cephalic central nervous system

Figs. 6A,B, 7A, 8B

The brain (cerebrum) is moderately sized in relation to the head size and located slightly posterior to the middle region of the head, between the anterior parts of the dorsal compound eyes. The protocerebrum including the large optic lobes appears dumbbell-shaped. The central body is clearly defined in the central protocerebral region and the optic neuropils are also distinct. The optic lobes are divided distally (Fig. 8B), with a smaller portion connected with the dorsal ocular subunit and a larger one to the ventral eye, both passing through the relatively small openings of the extensive internal circumocular ridges. The tritocerebral halves are continuous with relatively short and broad circumoesophageal connectives and connected
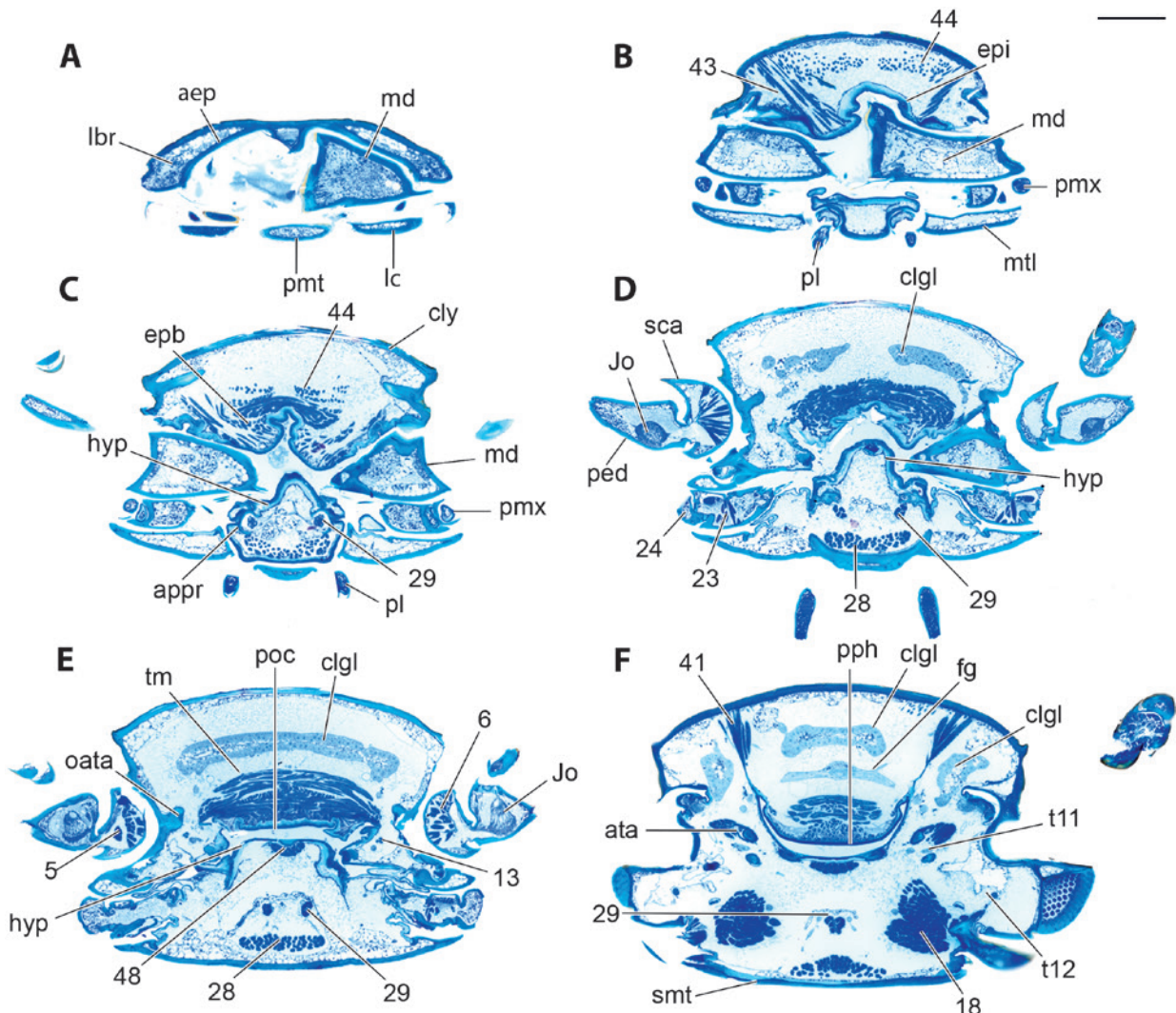
Fig. 9. *Heterogyrus milloti*, LM micrographs of transverse histological sections through anterior part of head (from anterior to posterior). **A**: anterior clypeal region; **B**: intermediate clypeal region; **C**: posterior clypeal region at level of secondary mandibular joint (left side); **D**: anterior frontal region; **E**: prepharyngeal region; **F**: anatomical mouth region, showing insertion of M. frontohypopharyngalis (41) and frontal ganglion (fg). — ***Abbreviations***: aep – anterior epipharynx, appr – premental apodeme, ata – anterior tentorial arm, clgl – clypeofrontal glands, cly – clypeus, epb – epipharyngeal bulge, epi – epipharynx, fg – frontal ganglion, hyp – hypopharynx, Jo – Johnston's organ, lc – lacinia, lbr – labrum, md – mandible, mtl – mental lobe, mx – maxilla, oata – origin of anterior tentorial arm, ped – pedicellus, pl – palpus labialis, pmx – palpus maxillaris, poc – preoral cavity, pph – prepharynx, pmt – prementum, sc – scapus, tm – transverse muscle, t11 – tendon of M.11, t12 – tendon of M.12. Musculature: 13 – M. tentoriohypopharyngalis, 18 – M. tentoriostipitalis, 23 – M. stipitopalpalis internus, 24 – M. palpopalpalis maxillae primus, 28 – M. submentopraementalis, 29 – M. tentoriopraementalis inferior, 41 – M. frontohypopharyngalis, 43 – M. clypeopalatalis, 44 – M. clypeobuccalis, 48 – M. tentoriobuccalis anterior. — Scale bar 0.2 mm.

with each other by a very distinct tritocerebral commissure below M. tentoriobuccalis anterior (M.48). The large suboesophageal complex is enclosed by the gular ridges (Fig. 10D,E). It reaches the laminatentoria anteriorly and extends into the anterior prothorax posteriorly, where it appears almost fused with the prothoracic ganglia.

3.11. Stomatogastric nervous system
Figs. 6A, 7A, 8B, 10E,F

The well-developed transverse frontal ganglion has a triangular shape in dorsal view, with an obtuse posterior angle. The nervus recurrens originates posteromedially

from the frontal ganglion and the frontal connectives at its lateral edges.

3.12. Glands
Fig. 7A, 9D–F

A large, transverse and flattened structure is present in the clypeofrontal region, with a thick gland-like epithelium. Ducts are recognizable internally and some very limited lumina. A duct connecting it directly with the lumen of the mandible or with the exterior could not be found on the available section series. Glands in the labral and mandibular lumen and salivary glands are absent.
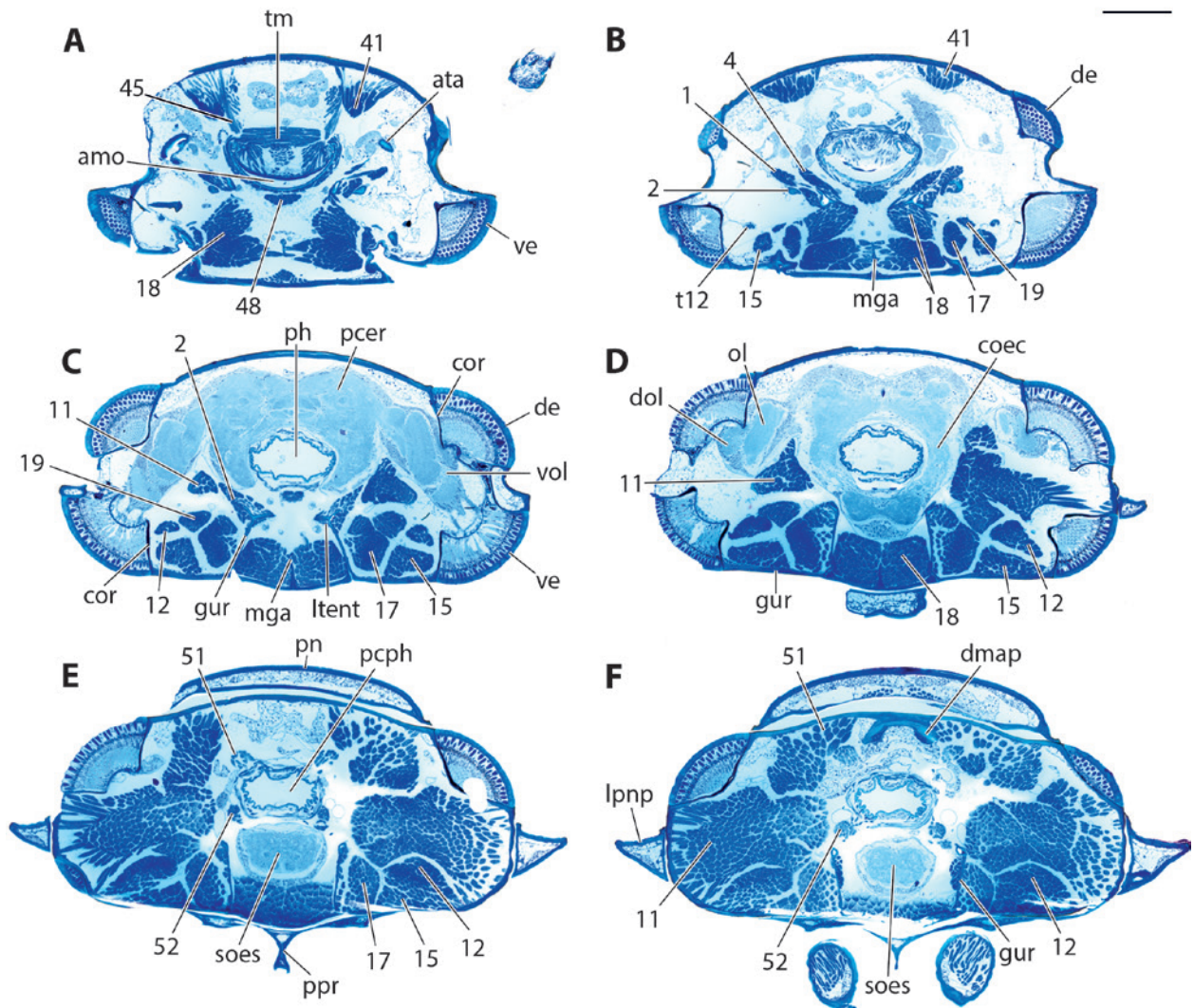
Fig. 10. *Heterogyrus milloti*, LM micrographs of transverse histological sections through posterior part of head (from anterior to posterior). **A**: anteriormost pharyngeal region, anterior part of ventral compound eye; **B**: middle pharyngeal region, anterior part of dorsal compound eye; **C**: posterior frontal region; **D**: occipital region; **E**: postoccipital region; **F**: region of foramen occipitale. — ***Abbreviations***: amo – anatomical mouth opening, ata – anterior tentorial arm, coec – circumoesophageal connective, cor – circumocular ridge, de – dorsal ocular subunit (dorsal eye), dmap – dorsomedian apodeme of postoccipital ridge, dol – dorsal subunit of optic lobe, gur – gular ridge, ltent – laminatentoria, lpnp – lateral pronotal projection, md – mandible, mga – midgular apodeme, mx – maxilla, ol – optic lobe, pcer – protocerebrum, pcph – postcerebral pharynx, ph – pharynx, pn – pronotum, ppr – prosternal process, soes – suboesophageal ganglion, tm – transverse muscle, t12 – tendon of M. 12, ve – ventral ocular subunit (ventral eye), vol – ventral subunit of optic lobe. Musculature: 1, 2, 4 – Mm. tentorioscapales anterior, posterior, medialis, 11 – M. craniomandibularis internus, 12 – M. craniomandibularis externus, 15 – M. craniocardinalis, 17 – M. tentoriocardinalis, 18 – M. tentoriostipitalis, 19 – M. craniolacinialis, 41 – M. frontohypopharyngalis, 45 – M. frontobuccalis anterior, 48 – M. tentoriobuccalis anterior, 51 – M. verticopharyngalis, 52 – M. tentoriopharyngalis. — Scale bar 0.2 mm.

## 4. Characters of the adult head

A character state matrix in Winclada format is provided as Electronic Supplement File 2.

1. Contact between anterior pronotal margin and upper surface of head: (0) surfaces distinctly separated; (1) surfaces almost merging. The anterior pronotal margin and the dorsal surface of the head appear almost fused in the monospecific ancestral genus *Spanglerogyrus* (Beutel 1989a), in *Gyrinus* (Fig. 2B) and in *Heterogyrus* (Figs. 1A, 2A). This is likely a groundplan apomorphy of Gyrinidae, but this condition is very insufficiently documented in the

family. The character is unknown in other groups of Adephaga (Beutel 1986; Belkaceme 1991; Dressler & Beutel 2010).

2. Shape of clypeal region: (0) evenly sloping, head wedge-shaped; (1) clypeal region almost vertical. A nearly vertical clypeal region is likely an autapomorphy of *Spanglerogyrus* (Fig. 11; Beutel 1989a: figs. 3, 4).

3. Tactile setae on head capsule: (0) present; (1) absent. Absent in *Heterogyrus* like in all genera of Gyrininae, but present in *Spanglerogyrus* (Fig. 11; Beutel 1989a: figs. 1, 3). The absence is likely a synapomorphy of *Heterogyrus* and Gyrininae. Tactile setae are present in terrestrial groups of Adepha-
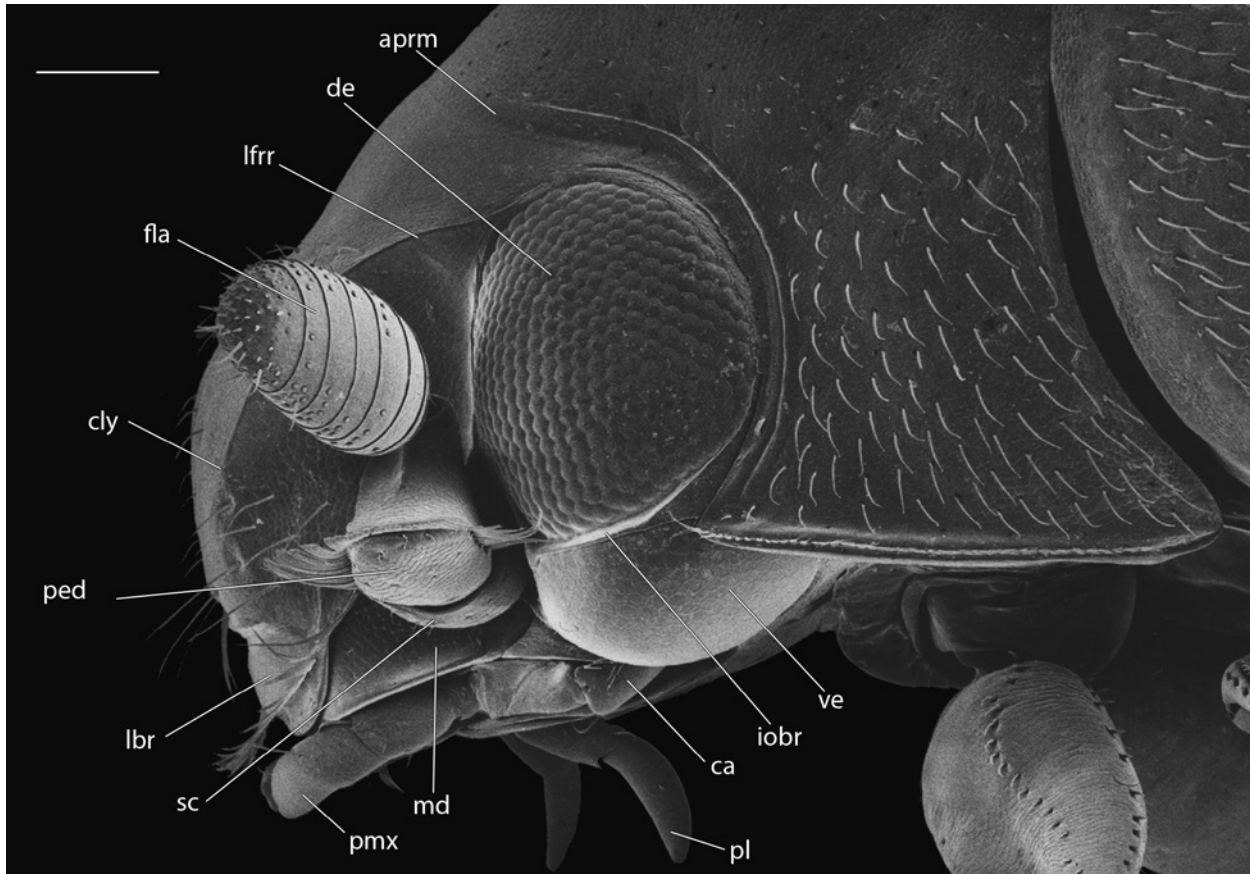
Fig. 11. *Spanglerogyrus albiventris*, SEM micrograph, head and prothorax, lateral view (anterior is left). — ***Abbreviations***: aprm – anterior pronotal margin, ca – cardo, cly – clypeus, de – dorsal eye, fla – flagellum, iobr – interocular bridge, lbr – labrum, lfrr – lateral frontal ridge, md – mandible, ped – pedicellus, pl – palpus labialis, pmx – palpus maxillaris, sc – scapus, ve – ventral eye. — Scale bar 0.2 mm.

ga (DRESSLER & BEUTEL 2010) and very likely in the groundplan of the suborder.

4. Dense field of setae on frontal region: (0) absent; (1) present. Absent in *Heterogyrus* and all genera of Gyrininae (Figs. 1, 2). The specifically arranged setae of *Spanglerogyrus* (BEUTEL 1989a: figs. 1, 3) are probably an autapomorphy of the genus (BEUTEL 1989a: figs. 1, 3).

5. Lateral frontal ridge: (0) joining supraocular bead along dorsal margin of dorsal eye; (1) not joining bead along dorsal margin of dorsal eye. The lateral frontal ridge is continuous with the bead along the dorsal margin of the dorsal eye in *Spanglerogyrus* (Fig. 11; BEUTEL 1989a: figs. 1, 3) and *Heterogyrus* (Fig. 2A). This is possibly a groundplan apomorphy of Gyrinidae. The absence of this connection, probably linked with the dorsal shift of the dorsal ocular subunit, is a possible synapomorphy of Gyrinini (Fig. 2B), Enhydrini and Orectochilini (Gyrininae). In *Gyrinus* the frontal ridge joins the ventral margin of the ventral ocular subunit and is continuous with the interocular suture (HONOMICHL 1975: figs. 3, 4).

6. Division of compound eyes: (0) absent; (1) upper and lower portion divided by narrow stripe; (2) interocular bridge less than half as wide as dorsoventral diameter of ventral ocular subunit; (3) interocular bridge about as wide as dorsoventral diameter of ventral

ocular subunit. The compound eyes are subdivided by a narrow stripe in *Spanglerogyrus* (Fig. 11). This is likely a groundplan apomorphy of Gyrinidae (BEUTEL 1989a). A moderately wide interocular bridge as it is found in *Heterogyrus* (Fig. 2A; GUSTAFSON et al. 2017) is probably a derived groundplan feature of Gyrinidae excluding *Spanglerogyrus*, and a distinctly widened bridge (HATCH 1925b, 1926; HONOMICHL 1975) a potential synapomorphy of Gyrinini (Fig. 2A), Enhydrini and Orectochilini (Gyrininae).

7. Flagellar groove of interocular bridge: (0) absent; (1) present. The bridge separating the ocular subunits in Gyrininae bears a distinct longitudinal groove for reception of the antennal flagellum on its dorsal surface (Fig. 2). This is likely a synapomorphy of *Heterogyrus* and Gyrininae.

8. Field of sensilla on flagellar groove: (0) absent; (1) present. A field of sensorial setae is present on the anterior region of the flagellar groove of *Heterogyrus* (Fig. 2A). This is likely an autapomorphy, as this condition has not been observed in other genera (HATCH 1925b, 1926; HONOMICHL 1975; BEUTEL 1989a).

9. Size of upper subunit of compound eye: (0) as large as ventral subunit; (1) distinctly smaller. Both subunits are approximately equally sized in *Heterogyrus* and *Spanglerogyrus* (Figs. 1, 2A, 11), where-

as the dorsal ocular subunit is distinctly smaller in Gyrininae (Fig. 2B).

10. Position of dorsal ocular subunit: (0) not shifted onto dorsal surface of head capsule; (1) shifted onto dorsal surface of head capsule. The dorsal ocular subunit is shifted onto the dorsal surface of the head in Gyrininae (Fig. 2B). This apomorphic feature is apparently correlated with the broadened interocular bridge.

11. Contact of posterior margin of dorsal eye with anterolateral pronotal edge: (0) present; (1) absent. The hind margin of the dorsal ocular subunit is directly in contact with the anterolateral pronotal margin in *Heterogyrus* and *Spanglerogyrus* (BEUTEL 1989a) (Figs. 2A, 11). This is likely a groundplan feature of Gyrinidae, and the distinct separation a possible autapomorphy of Gyrininae (Fig. 2B).

12. Separate lateral clypeal portion: (0) absent; (1) present. A small lateral portion is distinctly separated from the main part of the clypeus of *Heterogyrus* and the remaining gyrinid genera including *Spanglerogyrus* (HATCH 1925b, 1926; HONOMICHL 1975; BEUTEL 1989a) (Figs. 1A, 2, 11). This is likely an autapomorphy of the family.

13. Field of setae on lateral clypeal portion and labral base: (0) absent; (1) present. Its presence in *Heterogyrus* is likely an autapomorphy (Fig. 2B). The lateral clypeal portion is smooth in *Gyrinus* (Fig. 2B) and few setae are present in *Spanglerogyrus* (HONOMICHL 1975; BEUTEL 1989a) (Fig. 11).

14. Midgular apodeme: (0) absent; (1) present. Well-developed in *Heterogyrus* (Figs. 6E,F, 7C, 10) like in other gyrinid genera (HATCH 1925b, 1926; BEUTEL 1989a). This structure possibly belongs to the groundplan of Adephaga but it varies strongly within the suborder (e.g., Carabidae; see e.g., DRESSLER & BEUTEL 2010).

15. Anterior tentorial arms: (0) present; (1) absent. Present in *Heterogyrus* (Fig. 7C), *Spanglerogyrus* (BEUTEL 1989a), Enhydrini and Orectochilini (HATCH 1925b, 1926; HONOMICHL 1975). Almost generally present in Adephaga (DRESSLER & BEUTEL 2010) but absent in *Gyrinus* and *Aulonogyrus* (HATCH 1925b, 1926; HONOMICHL 1975; BEUTEL 1989a).

16. Dorsal tentorial arm: (0) present; (1) absent. Absent in *Heterogyrus* (Fig. 7C) like in all other Gyrinidae (HATCH 1925b, 1926; BEUTEL 1989a). Very likely an autapomorphy of the family.

17. Tentorial bridge: (0) present; (1) vestigial, present as thin tendons; (2) absent. Present in *Spanglerogyrus* and Enhydrini, but absent as a distinctly developed transverse bar in *Heterogyrus* (Fig. 7C), Gyrinini and Orectochilini (HATCH 1925b, 1926; BEUTEL 1989a). The thin tendons connecting M. tentoriobuccalis anterior with the gular ridges in *Heterogyrus* are probably vestiges of the tentorial bridge.

18. Laminatentoria: (0) not fused medially; (1) fused medially. The laminatentoria are small but present in Gyrininae (absence is an overstatement in

DRESSLER & BEUTEL 2010). They are separate in *Heterogyrus* (Fig. 7C) and in all Gyrininae examined (HATCH 1925b, 1926). Their median fusion in *Spanglerogyrus albiventris* (BEUTEL 1989a) is likely an autapomorphy of this species.

19. Labral setae: (0) one row; (1) three rows. Three rows are present in *Heterogyrus* (Fig. 1B) and the other Gyrininae examined (HONOMICHL 1975; BEUTEL 1989a). This is apparently an autapomorphy of Gyrininae (BEUTEL 1989a).

20. M. labroepipharyngalis (M.7): (0) present; (1) absent. M. labroepipharyngalis is absent in *Heterogyrus* (Fig. 8A, 9) and in all other Gyrinidae examined (HONOMICHL 1975; BEUTEL 1989a). Labral muscles are probably generally absent in Adephaga (e.g. BEUTEL 1986; BELKACEME 1991; DRESSLER & BEUTEL 2010), but occur in different groups of Polyphaga (e.g. ANTON & BEUTEL 2004; ANTON et al. 2016).

21. Scapus and pedicellus: (0) cylindrical; (1) scapus distally cup-shaped, pedicellus with large auricular lobe with dense fringes of long mechanoreceptive setae. Highly modified in *Heterogyrus* like in all other Gyrinidae (HONOMICHL 1975; BEUTEL 1989a).

22. Flagellum: (0) filiform; (1) flagellomeres short and wide, broadly connected. A stout flagellum with broadly connected flagellomeres is present in all Gyrinidae (Figs. 1, 2; HATCH 1926; BEUTEL 1989a). It is quite long in *Heterogyrus* but this is only a gradual difference to the condition found in other gyrinid taxa. The length of the flagellum varies considerably within the family (e.g., HATCH 1926).

23. Number of flagellomeres: (0) nine; (1) less than nine. The number of nine free flagellomeres is certainly a groundplan feature of the family and of Adephaga (e.g., BEUTEL 1989a; DRESSLER & BEUTEL 2010). This number is found in *Heterogyrus* (Figs. 1, 2) and *Spanglerogyrus* (Fig. 11) and some other genera (HATCH 1925b, 1926; BEUTEL 1989a). Fusions occur in Gyrinini (7 free flagellomeres in *Gyrinus* and *Aulonogyrus*), Enhydrini (6 in *Enhydrus* and *Dineutus*, 8 in *Macrogyrus* and *Porrhorhynchus*) and Orectochilini (6 in *Orectogyrus*) (HATCH 1925b, 1926; BEUTEL 1989a).

24. Pubescence of flagellomeres: (0) present; (1) partly reduced but still distinct; (2) absent. A distinctly reduced but still distinct pubescence on flagellomeres, especially the apical one, is present in *Heterogyrus* and in *Spanglerogyrus* (BEUTEL 1989a) (Figs. 1A, 2A, 11). It is almost completely absent in the other Gyrinidae (e.g., HONOMICHL 1975; Fig. 2B).

25. Mesal side of mandibular base: (0) almost as long as total length of mandible, moderately long; (1) much shorter. Of normal length in *Spanglerogyrus* (BEUTEL 1989a: figs. 6–9). Distinctly shortened in *Heterogyrus* (Fig. 4) and Gyrininae (HATCH 1926; HONOMICHL 1975; BEUTEL 1989a).

26. Area between apical mandibular incisivi: (0) narrow; (1) wide triangular area. A very wide triangular area is present between the mandibular incisivi of

Heterogyrus (Fig. 4) and the genera of Gyrininae (HATCH 1926; HONOMICHL 1975; BEUTEL 1989a). In contrast to the interpretation in BEUTEL et al. (1989a), this unusual condition (see e.g., BEUTEL 1986; BELKACEME 1991; DRESSLER & BEUTEL 2010) is likely a synapomorphy of *Heterogyrus* and Gyrininae. This character is possibly correlated with the previous one.

27. M. tentoriomandibularis (M.13): (0) present; (1) absent. This muscle is present even though very thin in *Heterogyrus* (Fig. 6C, D), and also in *Spanglerogyrus* (BEUTEL 1989a) and *Gyrinus* (HONOMICHL 1975). The presence as a thin proprioreceptor is very likely a groundplan feature of Gyrinidae and Adephaga (e.g., BEUTEL 1989a). The very thin muscle was probably overlooked in some studies. It can only be identified with microtome sections or μCT data of very good quality.

28. Galea: (0) 2-segmented; (1) 1-segmented; (2) absent. A 2-segmented galea as it is present in *Spanglerogyrus* and *Heterogyrus* (Fig. 1B; MILLER & BERGSTEN 2012) is apparently a groundplan feature of Gyrinidae and Adephaga (e.g., DRESSLER & BEUTEL 2010). A 1-segmented galea as it is found in Gyrinini (HATCH 1926) is likely a derived groundplan feature of Gyrininae, and the absence of the galea a potential synapomorphy of Enhydrini and Orectochilini (HATCH 1926; BEUTEL 1989a).

29. Origin of M. tentoriostipitalis (M.18): (0) single area of origin on posterior tentorial arm; (1) double area of origin on posterior tentorial arm and gula; (2) triple origin on gula, posterior tentorial arm and mid-gular apodeme. A double area of origin is likely a derived groundplan feature of Adephaga (e.g., BEUTEL 1986; BELKACEME 1991; DRESSLER & BEUTEL 2010). An additional origin on the mid-gular apodeme as it is found in *Heterogyrus* (Figs. 6E,F, 9, 10) is possibly an autapomorphy of the genus.

30. M. stipitogalealis (M.21): (0) present; (1) absent. The muscle is present in *Spanglerogyrus* and also occurs in other groups of Adephaga (e.g., BAUER 1910; DRESSLER & BEUTEL 2010). It is absent in *Heterogyrus* and *Gyrinus* (HONOMICHL 1975) (unknown for other Gyrininae).

31. Submento-mental border: (0) distinct; (1) incomplete and indistinct. Usually distinct (Fig. 1B) but almost completely reduced in *Spanglerogyrus* (BEUTEL 1989a: fig. 2).

32. Lateral lobes of mentum: (0) absent; (1) not reaching anterior premental margin; (2) large, projecting beyond anterior premental margin. The lateral lobes of the mentum are distinctly enlarged in *Heterogyrus* (Fig. 1B) and the remaining Gyrininae (HATCH 1926: fig. 34; HONOMICHL 1975; BEUTEL 1989a). This is an autapomorphy of the subfamily. The structure in question was erroneously interpreted as submentum by HATCH (1926).

33. Palpiger: (0) not fused with prementum; (1) fused with prementum. The palpiger is free in *Spanglero-gyrus* but more or less completely fused with the prementum in *Heterogyrus* and Gyrininae (Fig. 1B; BEUTEL 1989a).

34. Internal apodeme of the palpiger: (0) present; (1) absent. The apodeme is distinctly developed in *Spanglerogyrus* and in other adephagan groups (BEUTEL 1986, 1989a; BELKACEME 1991). It is still recognizable as a separate internal element in *Heterogyrus* (Fig. 7C) but absent in the genera of Gyrininae, as for instance in *Gyrinus* (HONOMICHL 1975; BEUTEL 1989a).

35. M. tentoriopraementalis superior (M.30): (0) present; (1) absent. The muscle is absent in *Spanglerogyrus*, in *Heterogyrus* and probably in all genera of Gyrininae (HONOMICHL 1975; BEUTEL 1989a). The muscles interpreted as Mm. tentoriopraementales inferior and superior (Mm.29, 30) by HONOMICHL (1975) are in fact M. submentopraementalis and M. tentoriopraementalis inferior (Mm.28, 29).

36. Origin of M. praementopalpalis (M.34): (0) apodeme of palpiger; (1) surface of prementum. The origin on the apodeme of the palpiger is likely a derived groundplan feature of Adephaga. This condition is preserved in *Spanglerogyrus* (BEUTEL 1989a) and also in *Heterogyrus* (Fig. 7A,B). The origin on the inner surface of the prementum is possibly a synapomorphy of Gyrinini, Enhydrini and Orectochilini (HONOMICHL 1975; BEUTEL 1989a). However, precise anatomical data are still lacking for most genera.

37. M. clypeobuccalis (M.44): (0) moderately sized, not composed of numerous very thin fibres, not nearly horizontal; (1) large, composed of numerous very thin fibres, nearly horizontal. The muscle is large and nearly horizontal in *Heterogyrus* (Figs. 7A, 8B) and *Gyrinus* (HONOMICHL 1975). The numerous very thin fibres penetrate the very large transverse cibarial muscles. This is a potential synpomorphy of *Heterogyrus* and Gyrininae. An intermediate condition with a less oblique M.44 is found in *Spanglerogyrus* (BEUTEL 1989a: fig. 4).

38. Transverse muscles of cibarium (M.67): (0) moderately sized; (1) very strongly developed. The transverse cibarial muscles are moderately developed in most groups of Adephaga (e.g., BEUTEL 1986; BELKACEME 1991; DRESSLER & BEUTEL 2010). They are greatly enlarged in *Heterogyrus* (Figs. 7A, 8B) and *Gyrinus* (HONOMICHL 1975), and also in *Spanglerogyrus* (BEUTEL 1989a: figs. 4, 12). This likely is an autapomorphy of Gyrinidae.

39. Lateral appendages of hypopharynx: (0) absent or vestigial; (1) distinctly developed elongate-triangular lateral appendages with setae. Well-developed lateral elongate-triangular appendages with setae are present in *Spanglerogyrus* (BEUTEL 1989a: fig. 13), and also in *Gyrinus* and *Heterogyrus* (BEUTEL 1989a). The phylogenetic interpretation is difficult in this case, as this character is insufficiently investigated in Gyrininae. The hypopharynx is also insufficiently
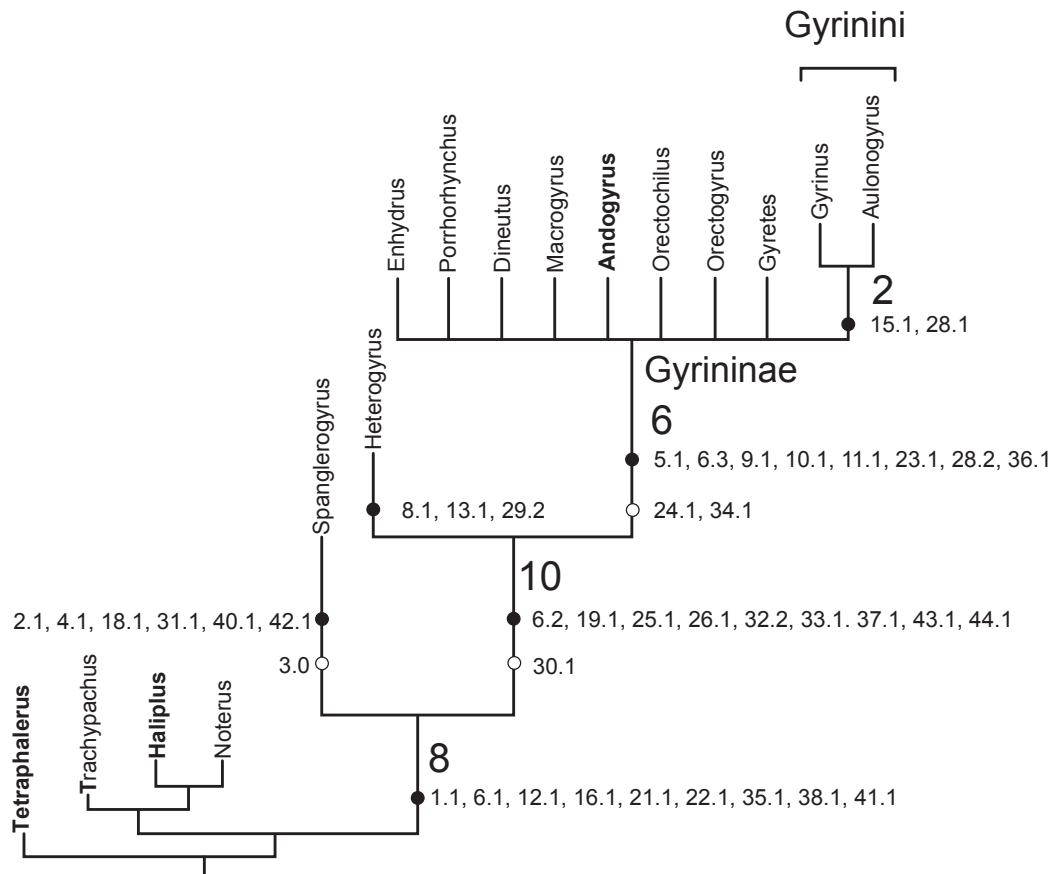
Fig. 12. Phylogeny of Gyrinidae, unambiguous cephalic apomorphies mapped on strict consensus tree, non-homoplasious transformations indicated by full circles, homoplasious changes by empty circles. **Gyrinidae**: 1.1. Tight connection of dorsal surface of head with anterior pronotal margin; 6.1. Compound eyes completely subdivided by a narrow interocular bridge; 12.1. Separate lateral clypeal portion present; 16.1. Dorsal tentorial arm absent; 21.1. Scapus cup-shaped, pedicellus with auricular lobe with fringes of mechanoreceptive setae; 22.1. Flagellum compact; 35.1. M. tentoriopraementalis superior (M.30) absent; 38.1. Transverse cibarial muscle (M.67) enlarged; 41.1. Clypeofrontal glands. *Spanglerogyrus*: 2.1. Steep clypeal region; 3.0. Tactile setae present (probably plesiomorphic in Gyrinidae, see character description in the text); 4.1. Regular field of setae on frontal region; 18.1. Fused laminatentoria; 31.1. Border between submentum and mentum indistinct; 40.1. M. tentoriobuccalis anterior (M.48) originates on laminatentorium; 42.1. Brain shifted to posterior head region. **Gyrinidae excl. *Spanglerogyrus***: 6.2. Interocular bridge widened but less than half as wide as dorsoventral diameter of ventral ocular subunit; 19.1. Three rows of setae on anterior edge and ventral side of labrum; 25.1. Shortened mesal mandibular edge; 26.1. Widely separated mandibular incisivi; 30.1. M. stipitogalealis (M.21) absent; 32.2. Lateral mental lobes distinctly enlarged; 33.1. Palpiger fused with prementum; 37.1. M. clypeobuccalis (M.44) large, composed of numerous very thin fibres, nearly horizontal; 43.1. Optic lobes divided into dorsal and ventral subunit; 44.1. Internal opening of circumocular ridge subdivided. **Gyrininae** (excl. *Heterogyrus*): 5.1. Lateral frontal ridge not joining bead along dorsal ocular margin; 6.3. Interocular bridge about as wide as dorsoventral diameter of ventral ocular subunit; 9.1. Dorsal subunit of eye reduced in size; 10.1. Dorsal subunit of eye shifted dorsad; 11.1. Dorsal subunit of eye separated from anterior pronotal margin; 24.1. Pubescence of flagellomeres absent; 28.2. Galea absent (but see character description in the text and discussion); 36.1. Origin of M. praementopalpalis (M.34) on surface of prementum. ***Heterogyrus***: 8.1. Field of sensilla on interocular antennal groove; 13.1. Field of sensilla on lateral clypeal portion and labral base; 29.2. Triple origin of M. tentoriostipitalis (M.18). **Gyrinini** (*Gyrinus* + *Aulonogyrus*): 15.1. Anterior tentorial arms absent; 28.1. Galea 1-segmented (but see character description in the text and discussion).

documented in other groups of Adephaga. Apparently different degrees of reduction of lateral appendages occur (DRESSLER & BEUTEL 2010: figs. 3D, 9C).

40. Origin of M. tentoriobuccalis anterior (M.48): (0) on tentorial bridge; (1) on laminatentoria; (2) attached to gular ridges with very thin, posteriorly diverging tendons. The highly unusual attachment on the gular ridges with very thin posteriorly diverging tendons is very likely an autapomorphy of *Heterogyrus*. The origin on the fused laminatentoria is an autapomorphy of *Spanglerogyrus* (BEUTEL 1989a).

41. Clypeofrontal glands: (0) absent; (1) present. A single flat gland-like structure with some small lumina

in its interior is present in the clypeofrontal region of *Heterogyrus* (Figs. 7A, 9). Similar structures are probably generally present in Gyrinidae (BEUTEL 1989a) and an autapomorphy of the family. However, the interpretation of this organ varies. It was hollow in a single sectioned specimen of *Spanglerogyrus* and consequently described as an air sac (BEUTEL 1989a), like structures of *Dineutus* with a similar placement (TONAPI 1978).

42. Position of the brain: (0) central region of the head; (1) posterior head region close to foramen occipitale. The posterior shift and relatively large size of the brain is likely an autapomorphy of *Spanglero-*

gyrus (BEUTEL 1989a). This condition is very likely correlated with a reduced head size.

43. Division of the optic lobes: (0) absent; (1) present. The optic lobes are subdivided into a dorsal and a ventral branch in *Heterogyrus* (Fig. 8C) and probably in all Gyrininae (HONOMICHL 1975). This is obviously correlated with the separate openings of the circumocular ridges, which are still united in *Spanglerogyrus* (BEUTEL 1989a: fig. 19). This is likely an autapomorphy of Gyrininae.

44. Internal opening of circumocular ridge: (0) not separated; (1) separate openings for dorsal and ventral ocular subunits. Separate openings in *Heterogyrus* and all examined Gyrininae (Fig. 7C) (HATCH 1926; HONOMICHL 1975).

5.   Results of the phylogenetic analyses

The analysis (16 terminal taxa, 4 outgroup terminals) yielded 12 minimum length trees of 55 steps with NONA (consistency index 0.87) and TNT. The monophyly of Gyrinidae (branch support [BS] 8), Gyrinidae excluding *Spanglerogyrus* (BS 10) and Gyrininae (BS 6) was strongly supported. Within Gyrininae only a clade *Gyrinus + Aulonogyrus* (Gyrinini) was consistently resolved (BS 2). The strict consensus tree with apomorphies mapped on it is shown in Fig. 12.

6.   Discussion

Not surprisingly, the results of the present study clearly confirm the monophyletic origin of Gyrinidae (Fig. 12). This was already supported by numerous derived features in earlier studies (HATCH 1925b, 1926; BEUTEL 1989a,b, 1990; MILLER & BERGSTEN 2012) and has never been seriously questioned. Some unique and complex autapomorphies are directly related to the surface swimming habits (e.g., BENDELE 1986), like the far-reaching modification of the antenna or the complete subdivision of the compound eyes (Figs. 2, 11). New or little known apomorphies are the very tight connection of the upper surface of the head capsule with the anterior pronotal edge (Figs. 1A, 2), the presence of a separate lateral clypeal portion (Figs. 2, 11), the loss of the dorsal tentorial arms (Fig. 7C), the complete absence of labral muscles, and the strong enlargement of the transverse muscles of the cibarium (M.67). Another apomorphy is the presence of glands in the clypeofrontal region (Figs. 7A, 9), previously also described as mandibular glands (HONOMICHL 1975: *Gyrinus*) or air sacs (TONAPI 1978: *Dineutus*; BEUTEL 1989a: *Spanglerogyrus*). The histological properties in *Heterogyrus* clearly show that this structure is formed by glandular tissue. It is accompanied by tracheae but they do not enter it, and the internal hollow space is very limited. As a connection with the mandibles could not be found in *Heterogyrus*, it is named clypeofrontal gland according to its placement in this cephalic region. A re-examination of this structure in well-preserved specimens of *Spanglerogyrus albiventris* is desirable. The function is presently unclear.

Like the monophyletic origin of the family, the monophyly of a clade *Heterogyrus* + Gyrininae (as defined in MILLER & BERGSTEN 2012) is strongly supported by cephalic features (branch support 8). In contrast to *Spanglerogyrus* (Fig. 11), doubtlessly the sistergroup of the remaining family (FOLKERTS 1979; BEUTEL 1989a,b, 1990), the compound eyes are not only divided by a thin interocular stripe of cuticle, but by a more or less wide interocular bridge (Fig. 2). The openings of the internal circumocular ridges, which are still connected in *Spanglerogyrus* (BEUTEL 1989a: fig. 19), are distinctly separated. Tactile setae are absent from the surface of the head capsule (Figs. 1, 2). The mandibles are modified, with a distinctly shortened mesal edge (BEUTEL 1989a) and a wide triangular area enclosed between the widely separated incisivi. Another convincing cephalic autapomorphy is the presence of three rows of setae on the labrum, instead of only one in *Spanglerogyrus*, and possibly the loss of a triangular field of microtrichia on the anteroventral labral surface (BEUTEL 1989a). An apomorphic character of the labium is the distinct enlargement of the rounded lateral mental lobes (Fig. 1B).

The data presented here clearly corroborate a sistergroup relationship between *Heterogyrus* and Gyrininae (branch support 10), as already discussed by MILLER & BERGSTEN (2012) as one of two possible options. In particular, *Heterogyrus* lacks a series of features related to the compound eyes that form a complex synapomorphy of Gyrinini, Enhydrini and Orectochilini. The bridge separating the dorsal and ventral ocular subunit is distinctly broader in species of Gyrininae than in *Heterogyrus* (Fig. 2). The dorsal eye is smaller than its ventral counterpart and shifted onto the dorsal side of the head. Moreover, in contrast to *Spanglerogyrus* and *Heterogyrus*, it is distinctly separated from the anterior pronotal margin. The lateral frontal ridge, which is continuous with the supraocular bead in *Spanglerogyrus* and *Heterogyrus*, is completely separated from the upper margin of the dorsal eye in Gyrininae (e.g., HATCH 1926; HONOMICHL 1975). Even though these characters are likely more or less closely correlated, it is plausible to assume that they have evolved only once as a complex innovation of Gyrininae. An independent character is the almost complete loss of the setation of the antennal flagellum, in contrast to *Spanglerogyrus* and *Heterogyrus*, where a relatively sparse but distinct vestiture is still present (Figs. 1, 2A, 11; BEUTEL 1989a: figs. 1–3, 21). Another argument for a placement of *Heterogyrus* as the second branch in the family is the presence of two galeomeres, in contrast to one or none in Gyrininae. Additional apomorphies of Gyrininae not shared by *Heterogyrus* are the reduction

of the apodeme of the palpiger (premental apodeme) and the origin of the labial palp muscles from the inner surface of the prementum.

Despite of its basal placement in Gyrinidae, *Spanglerogyrus* has evolved some apomorphic features of the head. This includes the shortening of the head with a steep clypeal surface (Fig. 11) (Beutel 1989a), a partly reduced submento-mental suture, fused laminatentoria, the origin of M. tentoriobuccalis anterior (M.48) from this structure, and the transverse field of regularly arranged setae on the frons. The large size of the brain in relation to the size of the head is probably an effect of miniaturization (Beutel 1989a; Beutel & Haas 1998). *Heterogyrus* is probably very close to the groundplan of Gyrinidae excluding *Spanglerogyrus* in its cephalic features. A potential autapomorphy is the attachment of M. tentoriobuccalis anterior to the gular ridges by tendons. These very thin structures are likely vestiges of a tentorial bridge, which is completely missing in Gyrinini and Orectochilini (Hatch 1925b, 1926). Another potential apomorphy of *Heterogyrus* is the field of setae on the antennal groove between the ocular subunits. This feature has not been described for other genera of Gyrinidae thus far.

The results presented here, i.e. the monophyly of Gyrinidae, Gyrinidae excl. *Spanglerogyrus*, and Gyrininae (excl. *Heterogyrus*) are in agreement with earlier studies based on morphological characters (e.g., Beutel 1989a,b, 1990) and a recent study based on molecular and morphological data (Miller & Bergsten 2012 [preferred topology]). However, as already pointed out by Beutel (1989a), characters of the adult head do not provide unambiguous evidence for the relationships between the tribes of Gyrininae. Only few cephalic features tentatively indicate relationships within this subfamily. The complete loss of the tentorial bridge is a shared derived feature of Gyrinini and Orectochilini, and the absence of the anterior tentorial arm a synapomorphy of *Gyrinus* and *Aulonogyrus* (Gyrinini). The loss of the galea is a potential synapomorphy of Orectochilini and Enhydrini, which also share a number of larval features (Beutel & Roughley 1994). It is evident that a broader spectrum of characters is necessary for a clarification of this issue, including extensive molecular data.

7.   Conclusions

Even though phylogenetic conclusions should be based on features of more than one body part and of different life stages, the head with its complex external and internal structures provides a rich character set for provisionally placing *Heterogyrus*. The cephalic characters clearly suggest that this Malagasy genus is the second branch in the phylogenetic tree of Gyrinidae, the sistergroup of a clade comprising the three tribes Gyrinini, Enhydrini and Orectochilini.

9.   References

Anton E., Beutel R.G. 2004. On the head morphology and systematic position of *Helophorus* (Coleoptera: Hydrophiloidea: Helophoridae). – Zoologischer Anzeiger **242**: 313–346.

Anton E., Yavorskaya M.I., Beutel R.G. 2016. The head morphology of adults of Clambidae (Coleoptera: Polyphaga: Scirtoidea). – Journal of Morphology **277**(5): 615–633.

Arndt E., Beutel R.G., Will K. 2016. 7.10. Carabidae Latreille, 1802. Pp. 162–190 in: Beutel R.G., Leschen R.A.B. (vol. eds), Handbook of Zoology, Vol. IV Arthropoda: Insecta. Part 38. Coleoptera, Vol. 1: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga (partim)). – Walter De Gruyter, Berlin, New York.

Baca S.M., Alana A., Gustafson G.T., Short A.E.Z. 2017. Ultraconserved elements show utility in phylogenetic inference of Adephaga (Coleoptera) and suggest paraphyly of 'Hydradephaga'. – Systematic Entomology 2017; DOI: 10.1111/syen.12244.

Balke M., Hendrich L. 2016. 7.1. Dytiscidae Leach, 1815. Pp. 118–140 in: Beutel R.G., Leschen R.A.B. (vol. eds), Handbook of Zoology, Vol. IV Arthropoda: Insecta. Part 38. Coleoptera, Vol. 1: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga (partim)). – Walter De Gruyter, Berlin, New York.

Bauer A. 1910. Die Muskulatur von *Dytiscus marginalis*. – Zeitschrift für wissenschaftliche Zoologie **95**: 594–646.

Belkaceme T. 1991. Skelet und Muskulatur des Kopfes und Thorax von *Noterus laevis* Sturm. Ein Beitrag zur Morphologie und Phylogenie der Noteridae (Coleoptera: Adephaga). – Stuttgarter Beiträge zur Naturkunde (A) **462**: 1–94.

Bendele H., 1986. Mechanosensory cues control chasing behaviour of whirligig beetles (Coleoptera, Gyrinidae). – Journal of Comparative Physiology A **158**(3): 405–411.

Beutel R.G. 1986. Skelet und Muskulatur des Kopfes und Thorax von *Hygrobia tarda* (Herbst). Ein Beitrag zur Klärung der phylogenetischen Beziehungen der Hydradephaga (Insecta: Coleoptera). – Stuttgarter Beiträge zur Naturkunde (A) **388**: 1–54.

Beutel R.G. 1989a. The head of *Spanglerogyrus albiventris* Folkerts (Coleoptera: Gyrinidae). Contribution towards clarification of the phylogeny of Gyrinidae and Adephaga. – Zoologische Jahrbücher für Anatomie **118**: 431–461.

Beutel R.G. 1989b. The prothorax of *Spanglerogyrus albiventris* Folkerts, 1979 (Coleoptera, Gyrinidae). Contribution towards clarification of the phylogeny of Gyrinidae. – Entomologica Basiliensia **13**: 151–173.

BEUTEL R.G. 1990. Phylogenetic analysis of the family Gyrinidae (Coleoptera) based on meso- and metathoracic characters. – Quaestiones Entomologicae **26**: 163–191.

BEUTEL R.G. 1993. Phylogenetic analysis of Adephaga (Coleoptera) based on characters of the larval head. – Systematic Entomology **18**: 127–147.

BEUTEL R.G., HAAS A. 1996. Phylogenetic analysis of larval and adult characters of Adephaga (Coleoptera) using cladistic computer programs. – Entomologica Scandinavica **27**: 197–205.

BEUTEL R.G., HAAS A. 1998. Larval head morphology of *Hydroscapha natans* LeConte, 1874 (Coleoptera, Myxophaga, Hydroscaphidae) with special reference to miniaturization. – Zoomorphology **18**: 103–116.

BEUTEL R.G., ROUGHLEY R.E. 1988. On the systematic position of the family Gyrinidae (Coleoptera: Adephaga). – Zeitschrift für Zoologische Systematik und Evolutionsforschung **26**: 380–400.

BEUTEL R.G., ROUGHLEY R.E. 1994. Phylogenetic analysis of Gyrinidae based on characters of the larval head (Coleoptera: Adephaga). – Entomologica Scandinavica **24**: 459–468.

BEUTEL R.G., ROUGHLEY R.E. 2016. 7.1. Gyrinidae. Pp. 80–88 in BEUTEL R.G., LESCHEN R.A.B. (vol. eds), Handbook of Zoology, Vol. IV Arthropoda: Insecta. Part 38. Coleoptera, Vol. 1: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga (partim)). – Walter De Gruyter, Berlin, New York.

BEUTEL R.G., WANG B., TAN J.-J., GE S.-Q., REN D., YANG X.-K. 2013. On the phylogeny and evolution of Mesozoic and extant lineages of Adephaga (Coleoptera, Insecta). – Cladistics **29**(2): 147–165.

BRINK P. 1955. A revision of the Gyrinidae (Coleoptera) of the Ethiopian region. I. – Lunds Universitets Årsskrift N.F. 2, **51**(2): 1–141.

DRESSLER C., BEUTEL R.G. 2010. The morphology and evolution of the adult head of Adephaga (Insecta, Coleoptera). – Arthropod Systematics and Phylogeny **68**: 239–287.

FOLKERTS G.W. 1979. *Spanglerogyrus albiventris*, a primitive new genus and species of Gyrinidae (Coleoptera) from Alabama. – The Coleopterists Bulletin **33**(1): 1–8.

GOLOBOFF P. 1995. NONA Version 1.5. Tucuman: Fundación e Instituto Miguel Lillo.

GUSTAFSON G.T., BERGSTEN J., RANARILALATIANA T., RANDRIAMIHAJA J.H., MILLER K. 2017. The morphology and behavior of the endemic Malagasy whirligig beetle *Heterogyrus milloti* Legros, 1953 (Coleoptera: Gyrinidae: Heterogyrinae). – The Coleopterists Bulletin **71**(2): 315–328.

HATCH M.H. 1925a. An outline of the ecology of Gyrinidae. – Bulletin of the Brooklyn Entomological Society **20**: 101–114.

HATCH M.H.1925b. The phylogeny and phylogenetical tendencies of Gyrinidae. – Papers of the Michigan Academy of Science, Arts and Letters **5**: 429–467.

HATCH M.H. 1926. The morphology of Gyrinidae. – Papers of the Michigan Academy of Science, Arts and Letters **7**: 311–350.

HONOMICHL K. 1975. Beitrag zur Morphologie des Kopfes der Imago von *Gyrinus substriatus* Stephens, 1829 (Coleoptera, Insecta). – Zoologische Jahrbücher für Anatomie **94**: 218–295.

KÉLER S. V. 1963. Entomologisches Wörterbuch. – Berlin: Akademie Verlag.

LARSÉN O. 1954. Die Flugorgane der Gyriniden (Coleoptera). – Opuscula Entomologica **19**: 5–17.

LARSÉN O. 1966. On the morphology and function of locomotor organs of the Gyrinidae and other Coleoptera. – Opuscula Entomologica (Suppl.) **30**: 1–241.

LEGROS C. 1953. Un gyrinide nouveau de Madagascar (Coleoptera). – Naturaliste Malgache **5**(1): 63–67.

MCKENNA D.D., WILD A.L., KANDA K., BELLAMY C.L., BEUTEL R.G., CATERINO M.S., FARNUM C.W., HAWKS D.C., IVIE M.A., JAMESON M.L., LESCHEN R.A.B., MARVALDI A.E., MCHUGH J.V., NEWTON A.F., ROBERTSON J.A., THAYER M.K., WHITING M.F., LAWRENCE J.F., ŚLIPIŃSKI A., MADDISON D.R., FARRELL B.D. 2015. The beetle tree of life reveals Coleoptera survived end Permian mass extinction to diversify during the Cretaceous terrestrial revolution. – Systematic Entomology **40**(4): 835–880.

MILLER K.B., BERGSTEN J. 2012. Phylogeny and classification of whirligig beetles (Coleoptera: Gyrinidae): relaxed-clock model outperforms parsimony and time-free Bayesian analyses. – Systematic Entomology **37**(4): 706–746.

NACHTIGALL W. 1961. Funktionelle Morphologie, Kinematik und Hydromechanik des Ruderapparates von *Gyrinus*. – Zeitschrift für Vergleichende Physiologie **45**: 193–226.

OMER-COOPER J. 1934. Notes on the Gyrinidae. – Odbitka z Archiwum Hydrobiologii o Rybactwa **8**: 1–26.

SHULL V.L., VOGLER A.P., BAKER M.D., MADDISON D.R., HAMMOND P.M. 2001. Sequence alignment of 18S ribosomal RNA and the basal relationships of adephagan beetles: evidence for monophyly of aquatic families and the placement of Trachypachidae. – Systematic Biology **50**: 945–969.

TONAPI G.T. 1978. Some adaptive features in the respiratory system of *Dineutes indicus* Aubé (Coleoptera, Gyrinidae). – Zoologica Scripta **6**(2): 107–112.

WIPFLER B., MACHIDA R., MÜLLER B., BEUTEL R.G. 2011. On the head morphology of Grylloblattodea (Insecta) and the systematic position of the order – with a new nomenclature for the head muscles of Neoptera. – Systematic Entomology **36**: 241–266.

Electronic Supplement Files

at http://www.senckenberg.de/arthropod-systematics

File 1: beutel&al-heterogyrushead-asp2017-electronicsupplement1.pdf – Interactive PDF of the head of *Heterogyrus milloti*. Click on the image to activate it.

File 2: beutel&al-heterogyrushead-asp2017-electronicsupplement2.winc – Character matrix and optimizations.