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Profound head modifications in *Claviger testaceus* (Pselaphinae, Staphylinidae, Coleoptera) facilitate integration into communities of ants

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Abstract

Clavigeritae is a group of obligate myrmecophiles of the rove beetle subfamily Pselaphinae (Staphylinidae). Some are blind and wingless, and all are believed to depend on ant hosts through feeding by trophallaxis. Phylogenetic hypotheses suggest that their ancestors, as are most pselaphines today, were free-living predators. Morphological alterations required to transform such beetles into extreme myrmecophiles were poorly understood. By studying the cephalic morphology of *Claviger testaceus*, we demonstrate that profound changes in all mouthpart components took place during this process, with a highly unusual connection of the maxillae to the hypopharynx, and formation of a uniquely transformed labium with a vestigial prementum. The primary sensory function of the modified maxillary and labial palps is reduced, and the ventral mouthparts transformed into a licking/'sponging' device. Many muscles have been reduced, in relation to the coleopteran groundplan or other staphylinoids. The head capsule contains voluminous glands whose appeasement secretions are crucial for the beetle survival in ant colonies. The brain, in turn, has been shifted into the neck region. The prepharyngeal dilator is composed of an entire series of bundles. However, the pharynx does not show any peculiar adaptations to taking up liquid food. We demonstrate that far-reaching cephalic modifications characterize *C. testaceus*, and that the development of appeasement glands and adaptation of the mouthparts to trophallaxis determine the head architecture of this extreme myrmecophile.

KEYWORDS
3D reconstruction, cephalic anatomy, micro-CT, obligate myrmecophily, trophallaxis

1 | INTRODUCTION

An estimated 10,000 arthropod species exploit resources associated with ant communities (Elmes, 1996). Among the most successful myrmecophiles are some lineages within Pselaphinae, a subfamily of rove beetles (Staphylinidae). Pselaphines exceed 10,000 described species (Thayer (2005), and later publications), and have an evolutionary history at least as long as that of ants (Parker, 2016a; Barden, 2017: ca. 100 million years; Yin, Parker, Cai, Huang, & Li, 2018; Yin, Kurbatov, Cuccodoro, & Cai, 2019). They fulfil the synergistic ancestral preconditions for myrmecophily defined by Parker (2016b): predatory diet; microhabitats shared with ants; defensive morphology; a small body size; and an exposed abdomen with glandular structures. Pselaphines show also a great diversity of body forms and structures, reflecting their ecological plasticity (see Figures S1 and S2), which enabled them to reach remarkable abundance and species richness in terrestrial ecosystems. The supertribe Clavigeritae (Figure 1) of Pselaphinae comprises species that are all believed to be obligate

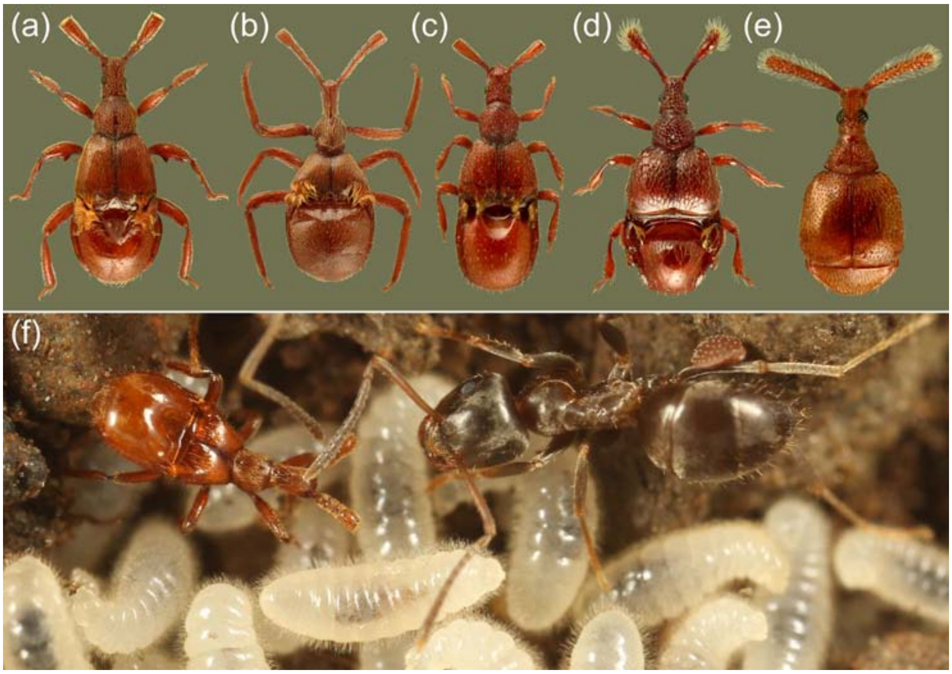

FIGURE 1 Diversity of extreme Clavigeritae myrmecophiles. (a) *Diartiger fossulatus* Sharp; (b) *Claviger apenninus* Baudi di Selve; (c) *Articerodes syriacus* (Saulcy); (d) *Cerylambus reticulatus* (Raffray); (e) *Disarthricerus integer* Raffray; and (f) *Claviger testaceus* Preyssler interacting with host *Lasius* sp. ant worker in larval chamber

myrmecophiles, and which developed the most intimate relationships with their hosts (Parker, 2016b). Some Clavigeritae are able to disperse actively; they have large eyes and long wings (Nomura, Sakchoowong, & Abd Ghani, 2008). Others, counted among the most extreme myrmecophiles, are blind, wingless, and helpless outside of ant colonies (Parker, 2016b). All clavigerite pselaphines share the 'myrmecophilous' groundplan (Figure 1a–f): mouthparts specialized to feed by trophallaxis (i.e., to take up liquid food regurgitated by ants); abdominal tergites IV–V fused and bearing specialized groups of setae (trichomes) associated with glands that secrete host appeasement compounds and trophallaxis stimulants; protective morphology that includes simplified and thickened antennae, partially reduced palps and tarsi; and a compound abdominal tergite that is heavily sclerotized to withstand the ant's grip whenever the beetle is carried by workers. The most thoroughly studied species is the European *C. testaceus* Preyssler (Figure 1f), well known to manipulate the host ant's behaviour to stimulate workers to regurgitate contents of their crops (Cammaerts, 1974, 1992). This species can be regarded as a model extreme myrmecophile.

Despite many scrupulous experiments and observations (Cammaerts, 1974, 1991, 1992, 1995, 1996, 1999), the modified mouthparts of *C. testaceus* were never studied in detail. Free-living pselaphine species are predatory on springtails or mites (e.g., Park, 1947; Engelmann, 1956; Schomann, Afflerbach, & Betz, 2008; see also Chandler (2001) for a summary), although scavenging on dead arthropods also seems possible (Figure S2e). They usually have elongate and dentate mandibles, long (often conspicuously so) maxillary palps and well-developed labial palps (e.g., Schomann et al., 2008; see also Figures S3 and S4). This is also the case in *Protopselaphus*, the sister group of Pselaphinae (Newton & Thayer, 1995), and is therefore clearly the plesiomorphic condition. Parker and Grimaldi ((2014), Figure 4a) place Clavigeritae among the 'higher Pselaphinae', as sister group of Arhytodini + Pselaphini, with these two tribes sister to the 'tyrine lineage'. Pselaphini and tyrines are predatory (e.g., Schomann et al., 2008), Arhytodini are poorly investigated and their biology is not known. Later Parker (2016a) resolved Clavigeritae nested within Pselaphitae. The consistent clustering of Clavigeritae with the predominantly predatory Pselaphitae suggests that predatory habits are the ancestral condition, and that trophallaxis is a specialized adaptation. This also clearly conforms with conditions observed in *Protopselaphus* (Newton & Thayer, 1995).

In order to elucidate how intimate relationships between obligate clavigerite myrmecophiles and their host ants evolved, it is essential to understand functional morphology of inquilines. Behavioural interactions with host ants, including transport (Cammaerts, 1999; Leschen, 1991) and feeding (Cammaerts, 1992, 1996; Park, 1932), and chemical camouflage (Akino, 2002) of Clavigeritae attracted some attention. However, although the appeasement glands of *Claviger* were discovered over a century ago (Krüger, 1910; Wasmann, 1903), and later studied in detail (Cammaerts, 1974), no other internal cephalic structures have been described and illustrated for Clavigeritae. Even the mouthparts, frequently illustrated in taxonomic studies related to many other pselaphines (mostly because of a great diagnostic value of often conspicuously long and elaborate maxillary palps), for Clavigeritae are usually illustrated in undissected specimens, with only their externally exposed components visible (e.g., Baňař & Hlaváč, 2014; Hlaváč & Nakládal, 2016; Nomura et al., 2008). In one case, dissected mouthparts were illustrated, but only as line drawings, and did not include the epi- and hypopharyngeal structures (Besuchet, 1991). Using modern techniques, including μ-CT, we provide the first insight into the architecture of musculature, cephalic central nervous system, glands, alimentary canal and skeletal structures of a pselaphine species, *C. testaceus*. Our study is focused on adaptations to trophallaxis, clearly a derived morphological, behavioural and physiological adaptation to life among ants. We compare mouthparts of *C. testaceus* with those of selected free-living pselaphines, and we identify structures whose function can be linked directly with trophallaxis, and in consequence, with myrmecophily. Because of an enormous morphological diversity, a large number of genera and tribes included in Pselaphinae, and the still poorly understood phylogeny, we do not attempt a detailed and formal reconstruction of the pselaphine morphological groundplan. Instead, we compare structures found in *C. testaceus* to the coleopteran groundplan (e.g., Beutel & Yavorskaya, 2019) and conditions found in less specialized staphylinoid beetles (e.g., Weide & Betz, 2009) including *Protopselaphus*, wherever relevant in the context of specialized feeding adaptations.

2 | MATERIALS AND METHODS

2.1 | Studied specimens

The species studied in detail is a blind obligate myrmecophile, *C. testaceus* Preyssler (Insecta: Coleoptera: Staphylinidae: Pselaphinae: Clavigeritae), an inquiline that is not naturally found outside ant colonies. Numerous beetles were collected near Prudnik ad Opole (SW Poland) in May 2019, in colonies of *Lasius* sp. found under stones by the first author. Specimens were preserved in FAE (10 ml 35% formalin, 5 ml glacial acetic acid, 85 ml absolute ethanol), and some in 75% ethanol. Dry-mounted specimens of the following Clavigeritae were examined by light microscopy, as examples of morphological diversity: *Articerodes syriacus* (Saulcy) (Israel), *Cerylambus reticulatus* (Raffray) (Vietnam), *Claviger apenninus* Baudi di Selve (Italy), *Claviger longicornis* Müller (Poland), *Diartiger fossulatus* Sharp (Japan), *Disarthricerus integer* Raffray (Malaysia), and several undetermined clavigerite species from South Africa, Madagascar, and New Caledonia (deposited in the Museum of Natural History, University of Wrocław, Poland, and in the private collection of the first author, Wrocław, Poland). Dissected mouthparts in transparent microscope slides of seven free-living Pselaphinae species were examined, illustrated and compared with exoskeletal structures found in *C. testaceus*: *Euplectus karstenii* (Reichenbach), *Trichonyx sulcicollis* (Reichenbach), *Brachygluta fossulata* (Reichenbach), *Bryaxis bulbifer* (Reichenbach), *Batrisodes venustus* (Reichenbach), *Tyrus mucronatus* (Panzer), and *Pselaphus heisei* Herbst (all collected in Poland; specimens deposited in the collection of the first author). Additionally, taxonomic literature was screened for illustrations of mouthparts of other Clavigeritae, to allow for more general conclusions.

2.2 | μ-CT and microtome sections

Beetles were transferred to acetone and then dried in a critical point dryer (Emitech K850, Quorum Technologies Ltd., Ashford, UK). μ-CT scans were made at the Max Planck Institute für Menschheitsgeschichte (Jena, Germany) using a SkyScan 2211 (Bruker, Knotich, Belgium), with the following parameters: 70 kV voltage, 300 μA current, 3.600 ms exposure time, rotation step 0.150, frame averaging on, random movement off, and filter assembly open. Projections were reconstructed by NRecon (Bruker) into JPG files with a voxel size of 0.68 μm. Amira 6.1.1 (Thermo Fisher Scientific, Waltham, MA) and VG studio Max 2.0.5 (Volume Graphics, Heidelberg, Germany) were used for three-dimensional reconstructions and volume rendering. For microtome sectioning, one specimen of *Claviger* was embedded in araldite CY 212 (Agar Scientific, Stansted/Essex, UK). Sections were cut at 1 μm intervals using a microtome HM 360 (Microm, Walldorf, Germany) equipped with a diamond knife, and stained with toluidine blue and pyronin G (Waldeck GmbH and Co.KG/Division Chroma, Münster, Germany). The sections are stored in the collection of the Phyletisches Museum, Jena, Germany. The μ-CT dataset is archived at the same institution and available upon request.

2.3 | Light microscopy

Specimens were observed under a Nikon SMZ1500 stereomicroscope. Dissected mouthparts, cleared briefly in 10% aqueous solution of sodium hydroxide, dehydrated in isopropanol and mounted in Canada balsam, were observed with a Nikon Eclipse *Ni* compound microscope. Photographs were taken with a Nikon Coolpix 4500 camera (habitus images; as stacks processed with Helicon Focus v. 6.8.0 (HeliconSoft Ltd.)), transparent structures were photographed with a Nikon D7500 camera mounted respectively on the stereo- and compound microscope. A Nikon Eclipse *Ni* compound microscope was also used to observe an undissected head of a freshly killed *C. testaceus* in a droplet of water, in dark field. Living pselaphines were photographed with a Canon 7D Mark II camera with a MP-E 65 mm lens, and an Olympus C-750UZ digital camera with a Raynox MSN-202 close-up lens; photographs are from archives of the first author.

2.4 | Scanning electron microscopy

Beetles were transferred from 75% to 99% ethanol for 15 min and air-dried. Some of them were macerated for 20–60 min in a warm 10% aqueous solution of NaOH, thoroughly washed in distilled water and dissected; isolated mouthparts were dehydrated in 99% ethanol and air-dried. Five beetles were dissected in order to make sure that the observed fusion of the maxillae with the hypopharynx is not an artefact. Entire beetles and dissected parts were mounted on scanning electron microscopy (SEM) stubs with carbon tabs, sputter-coated (Leica EM ACE600) with 20 nm of carbon and examined using a Helios Nanolab 450HP scanning electron microscope (FEI, Hillsboro, OR). Images were processed using CorelDraw Graphic Suite 2017; the following adjustments were made: overall brightness and contrast enhanced; background manually replaced with black; selected structures highlighted with manually applied colour.

2.5 | Terminology and measurements

Cephalic muscles were designated following the terminology of von Kéler (1963), with the exception of Mm. compressores epipharyngis (Mm. III). For this muscle, we followed Belkaceme (1991). Muscles are also homologized according to Wipfler, Machida, Müller, and Beutel (2011), with homolog abbreviations added in parentheses after the designation of von Kéler (1963); for example, M44 - M. clypeobuccalis (0bu1). Muscles not mentioned in the morphological description are lacking. The length of the head capsule is measured in dorsal view, from the anterior clypeal margin to the posterior margin of vertex; width of head is maximum width of the anterior (exposed) part.

3 | RESULTS

3.1 | External head structures of *C. testaceus*

The head capsule (Figure 2a–c) is about 0.4 mm long and the maximum width is 0.25 mm. The coloration is light brown. The head is prognathous and appears cork-shaped in dorsal view (Figure 2b), truncated anteriorly with scarcely protruding mouthparts; it is distinctly bulging on the ventral side, but the main part is almost cylindrical; a nearly hemispherical neck region is present posteriorly. Any traces of ecdysial sutures are lacking on the dorsal side; a clypeofrontal strengthening ridge is not recognizable externally; gular sutures are largely reduced and sutures delimiting the submentum are also missing. Dorsal foveae and ocelli are absent. The compound eyes are completely reduced, without recognizable external or internal traces. The clypeal region is almost vertically sloping between the anterior edges of the large antennal fossae, which are enclosed by a distinct smooth bead except for the anterolateral margin; a slightly concave oblique smooth area anterolaterad the antennal fossa is continuous with the anterolateral clypeal region; the anterior margin of the clypeus is a sharp edge, separating it very distinctly from the labrum. A broad and short anteromedian emargination accommodates the prelabium (i.e., mentum and vestigial prementum) on the ventral side of the head (Figure 2c); a distinct smooth bead is present along its margin and continues anterolaterally. Dorsally and laterally the neck region is separated from the main cephalic part by a sharp occipital crest, which is obliterated ventrolaterally. The head capsule is slightly constricted laterally anterior to the crest; paired ventral foveae are present in this cephalic region; they appear like invagination sites laterally and are nearly confluent anteromedially. The main part of the head bears a relatively regular vestiture of medium-length bifurcate setae (ca. 30 μm) (Figure 2d); the length and density increases at the postoccipital crest; the surface between the setae appears shiny. The

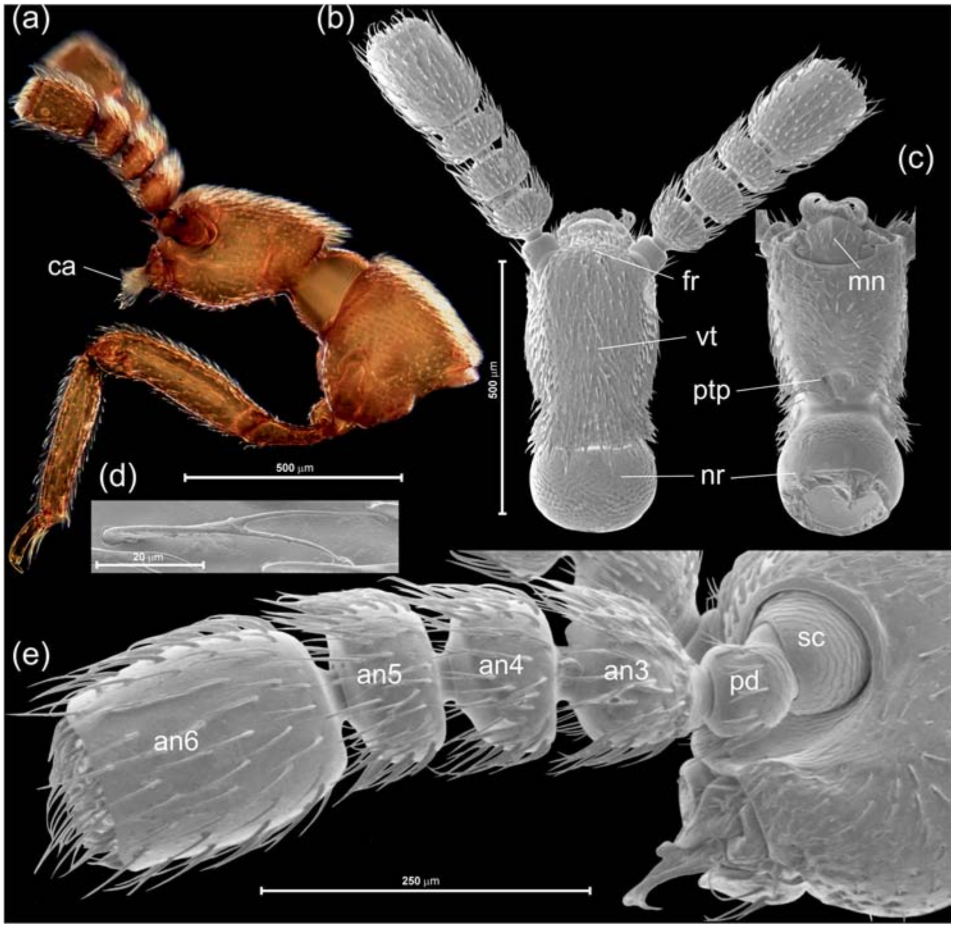

FIGURE 2 Cephalic morphology of *C. testaceus*, black field light micrograph (a) and scanning electron microscopy (SEM) images (b–e). (a) Head and prothorax in lateral view (preparation in water, showing projecting capillary mouthpart structures); (b) head in dorsal view; (c) head in ventral view; (d) bifurcate setae on vertex; (e) left antenna in lateral view. an3–6, antennomere 3–6; ca, capillary apparatus; fr, frons; mn, mentum; nr, neck region; pd, pedicel; ptp, posterior tentorial pit; sc, scape; vt, vertex

posteroventral and posterolateral regions of the main cephalic part are smooth and glabrous; the hemispherical 'neck' region lacks setae; most parts of its surface display a distinct reticulate pattern, but it is smooth posteroventrally. Short fissure-shaped vestiges of gular sutures are recognizable ventrolaterally at the posterior cephalic margin.

3.2 | Internal skeletal structures

The tentorium (Figure 5a) is distinctly reduced, with only a pair of stick-like arms arising in the posterior contracted area of the head capsule; the areas of origin of these structures comprising the posterior and dorsal arms are almost adjacent to each other on the ventral side, but they diverge anteriorly to insert on the dorsolateral head region, thus forming a V-shaped structure; the dorsal arms are apically fused with the dorsal wall of the head capsule; the areas of fusion are visible as shallow dorsolateral depressions (reduced 'vertexal foveae' of Chandler (2001)). The ventral posterior tentorial pits (gular foveae of Chandler (2001)) appear as a medially fused invagination site. Anterior tentorial grooves, anterior arms, the tentorial bridge and gular ridges are absent. The circum-antennal ridges are strongly developed and form round and deep antennal fossae. Circumocular ridges are absent.

3.3 | Antennae

The club-shaped antennae (Figure 2a,b,e) comprise only six compact and wide segments; articulatory membranes are not visible. With about 0.35 mm, the antennae are subequal to the total head length. The dorsomesal part of the oblique antennal foramen is visible in dorsal view, and also a small part of the scapus, which is short and hemispherical and inserted into the foramen in a ball-and-socket manner; the surface structure is scaly; setae are lacking. The pedicellus has a curved basal part and a more or less cylindrical distal part, which is about as wide as long; the surface of the basal portion is scaly; a ring of double setae is present at the border between both regions. The four flagellomeres are distinctly enlarged, with a smooth and fairly narrow basal peduncle and a strongly widened distal part. Flagellomeres 1–3 are distinctly wider than long; a vestiture of simple setae is present on the outer surface whereas the nearly flat apical surface is glabrous; the setae are slightly longer than those of the head capsule. The cylindrical apical antennomere is slightly shorter than the three preceding ones combined; it also bears a regular vestiture of setae; the truncate apex is densely covered with setae.

Musculature (Figure 5b,c) (for a list of all cephalic muscles of *C. testaceus* and their functions, see Table 1; for comparison to previously studied species of Staphylinoidea see Table S1): M1 - M. tentorioscapalis anterior (0an1), origin (O): large area of the lower tentorial region, insertion (I): anteroventral margin of the scapus; M2 - M. tentorioscapalis posterior (0an2), O: anterodorsal wall of the head capsule, directly in front of the tentorium, I: posterodorsal margin of the scapus; M4 - M. tentorioscapalis medialis (0an4), O: upper region of the tentorium and anterodorsal wall of the head capsule, in front of the tentorium, laterad m2, I: medioventrally on the basal scapal margin; M5 - M. scapopedicellaris lateralis (0an6), O: mesal wall of the scapus, I: dorsal margin of the pedicellar base; M6 - M. scapopedicellaris medialis (0an7), O: lateral wall of the scapus, I: ventrally on the median margin of the pedicellar base.

3.4 | Labrum

The labrum (Figure 3a–c), which is distinctly separated from the clypeus, is only about one-third as wide as the strongly pronounced anterior clypeal edge; viewed from above it appears transverse and short, with rounded anterolateral edges; however, the anterior edge is strongly extended ventrad, thus forming a large shield-like structure, slightly concave and covered with fairly large-scale-like surface structures. Setae are absent from the strongly sclerotized dorsal surface, the anterior edge, and the shield-like part, which is only visible in frontal view; however, a pair of very strongly developed setae are inserted on the ventral side in an anterolateral notch. Long apodeme-like tormae with apical muscle discs originate from the posterolateral labral edges.

Musculature (Figure 5h): M7 - M. labroepipharyngalis (0lb5); O: dorsal wall of the labrum, I: anteriormost area of the epipharynx.

3.5 | Mandibles

A heavily sclerotized shield-like structure formed by the mandibles (Figures 3a–c and 4c,d) and labrum covers the ventral mouthparts. The mandibles are characterized by a weakly developed apical region and a distinctly simplified molar area; only a single small and blunt apical tooth is present, followed by a simple, slightly rounded subapical edge. The ventral surface is almost entirely flat; the small ventral part of the molar area is mesally delimited by a rounded furrow; a dense fringe of medium-length microtrichia (ca. 20 μm) is present along the mesal edge; the surface of the ventral side is entirely smooth and glabrous. The dorsal part of the molar area is a slightly concave field densely covered with medium-length microtrichia. A somewhat irregular prominent elevation of the lateral dorsal surface is anterolaterally followed by a deep concavity with several very stout setae of about 25 μm length. The dorsal mandibular base is deeply emarginated. A distinct rounded process of the internal face of the lateralmost clypeal region overlaps with the mandibular base laterally. The adductor and abductor tendons are well developed and attached very close to the mesal and lateral edges of the mandibular base, respectively.

Musculature (Figure 5d,e): M11 - M. craniomandibularis internus (0md1), O: middle region of the lateral wall of the head capsule, anterior to the tentorium, I: close to the mesal mandibular base with the adductor tendon; M12 - M. craniomandibularis externus (0md3), O: ventral area of the head capsule, ventrad the origin of m11 and laterad the ventral tentorial base; I: with the abductor tendon on the

TABLE 1 Cephalic muscles of *C. testaceus*

Muscle	Symbol	Function
M. tentorioscapalis anterior	M1 (0an1)	Depressor and rotator of antenna
M. tentorioscapalis posterior	M2 (0an2)	Levator, retractor and rotator of antenna
M. tentorioscapalis medialis	M4 (0an4)	Depressor of antenna
M. scapopedicellaris lateralis	M5 (0an6)	Extensor and levator of flagellum
M. scapopedicellaris medialis	M6 (0an7)	Flexor and depressor of flagellum
M. labroepipharyngalis	M7 (0lb5)	Levator of epipharynx
M. craniomandibularis internus	M11 (0md1)	Adductor of mandible
M. craniomandibularis externus	M12 (0md3)	Abductor of mandible
M. hypopharyngomandibularis	M13 (0md4)	Proprioreceptor
M. craniocardinalis externus	M15 (0mx1)	Extensor of cardo
M. tentoriocardinalis	M17 (0mx3)	Flexor of cardo and entire maxilla
M. tentoriostipitalis	M18 (0mx4/0mx5)	Adductor of stipes and lacinia
M. craniolacinialis	M19? (0mx2)	Adductor of stipes and lacinia, retractor of maxilla
M. tentoriopraementalis superior	M30 (0la6)	Retractor of prementum
M. frontohypopharyngalis	M41 (0hy1)	Levator of hypopharynx, dilator of anatomical mouth opening
M. clypeopalatalis	M43 (0ci1)	Dilator of prepharynx
M. clypeobuccalis	M44 (0bu1)	Dilator of posteriormost prepharynx and anatomical mouth
M. frontobuccalis anterior	M45 (0bu2)	Dorsal dilator of anatomical mouth and anterior pharynx
M. frontobuccalis posterior	M46 (0bu3)	Dorsal dilator of middle section of pharynx
M. tentoriobuccalis anterior	M48 (0bu5)	Ventral dilator of prepharynx
M. tentoriobuccalis posterior	M50 (0bu6)	Ventral dilator of anterior pharynx
Mm. compressores epipharyngis	MmIII	Compressors of epipharynx

lateral mandibular base; M13 - M. hypopharyngomandibularis (0md4), very thin, O: ventral base of the tentorium, I: mesal area of the mandibular base, close to the adductor tendon.

3.6 | Maxillae

The maxillary groove (= fossa maxillaris; see, e.g., Dressler & Beutel, 2010; Beutel & Yavorskaya, 2019) is lacking; the small maxillae (Figures 3a–c and 4e) are inserted between the lateral base of the mentum and the lateral edge of the anteromedian emargination of the ventral wall of the head capsule. The well-developed cardo is roughly semicircular and bears a distinct internal process with a lateral and a mesal branch; setae are absent. The stipes is small and a subdivision is not recognizable; a conspicuous protuberance close to the lateral base bears a seta; an additional single seta is inserted proximad the base of the galea. The small palp (Figure 4e,f) appears undivided and S-shaped, with a wide-meshed reticulate surface structure, a strongly developed apical appendage, and three long apical digitform sensilla; a transverse sclerite possibly representing a palpifer is recognizable in dorsal view; an additional elongate and curved structure is present mesad this sclerite. The lacinia is a relatively large, roughly triangular structure and very distinctly separated from the stipes; along its mesal margin, it bears a very dense fringe of long flexible setae of about 70 μm length; the lacinia is ventrally connected to the hypopharynx (Figure 4h). The galea is connected to the distal stipital edge; it is also well-developed, elongate and quadrangular, and equipped will a dense fringe of long setae along its distal margin.

Musculature (Figure 5f,g): M15 - M. craniocardinalis externus (0mx1), O: anterolateral area of the ventral capsule; I: lateral branch of the cardinal process; M17 - M. tentoriocardinalis (0mx3), O: anterolateral area of the ventral head capsule, in front of the ventral tentorial base; I: mesal branch of the cardinal process; M18 - M. tentoriostipitalis (0mx4/0mx5), O: ventral side of basal tentorial part; I: basal area of stipes; M19? - M. craniolacinialis (0mx2), O: ventral wall of head capsule, in front of the ventral tentorial base; I: likely on a membrane attached to the lacinial base, but precise insertion site not recognizable on available data sets.

3.7 | Labium

The submentum is completely fused with the ventral wall of the head capsule. The large, plate-like mentum (Figures 3c and 4g) is inserted

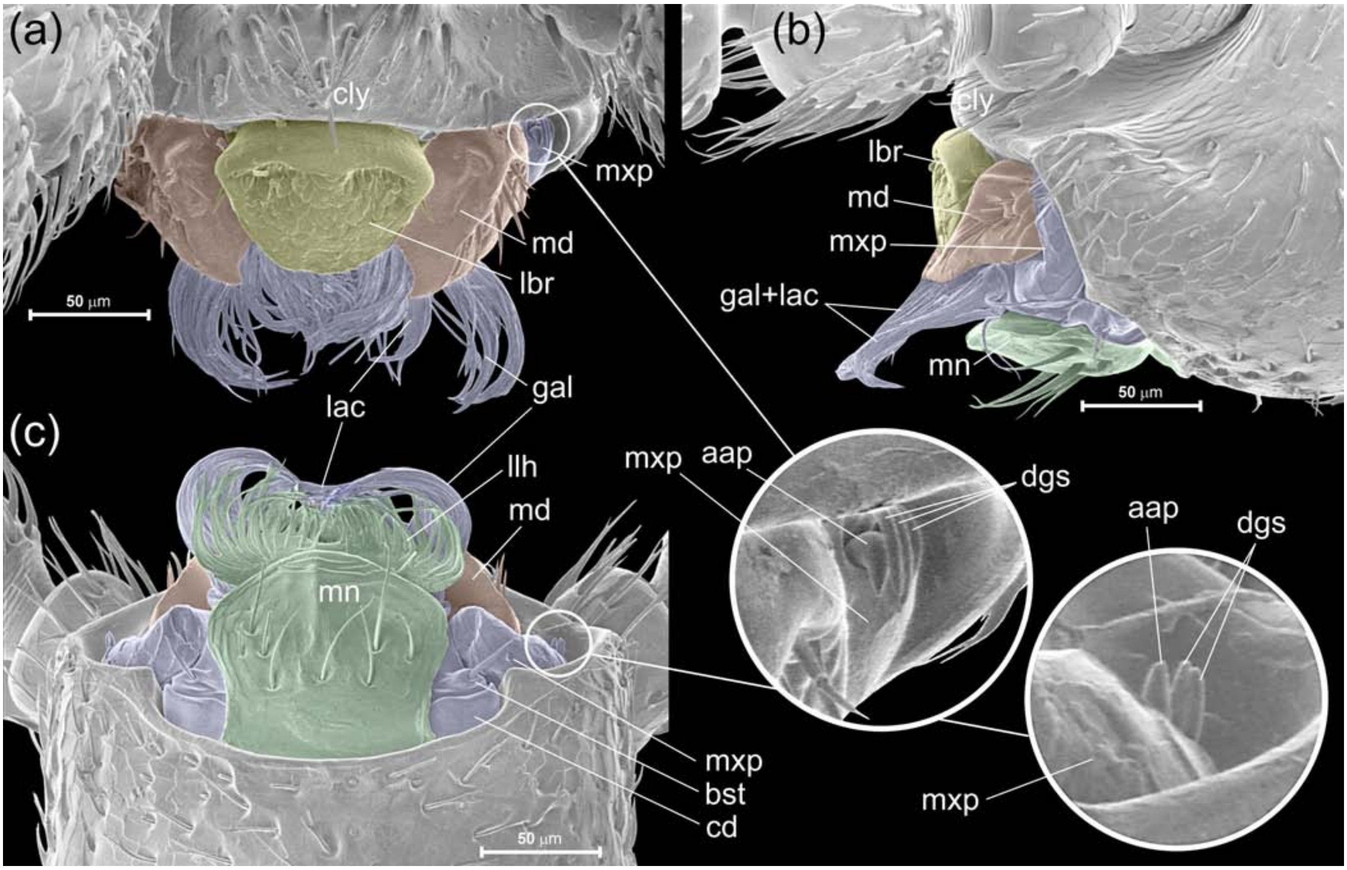

FIGURE 3 Cephalic morphology of *C. testaceus*, scanning electron microscopy (SEM) images of undissected mouthparts in anterodorsal (a), lateral (b), and ventral (c) view. aap, apical appendage; bst, basistipes; cd, cardo; cly, clypeus; dgs, digitiform sensilla; gal, galea; lac, lacinia; lbr, labrum; llh, lateral lobes of hypopharynx; md, mandible; mn, mentum; mxp, maxillary palp

into the anteromedian emargination and internally connected with the submental region by a membranous fold; it is slightly diverging anteriorly, with rounded anterolateral margins; the anterior margin is distinctly convex. The largely concealed prementum is vestigial and lacks labial palps.

Musculature (Figure 5e,h): M30 - M. tentoriopraementalis superior (0la6), O: median area of the vestigial submentum, I: parasagittally on the dorsal region of the prelabium (Figure 5h,e) (precise site not recognizable with available data).

3.8 | Epipharynx and hypopharynx

The anteriormost epipharynx (Figure 4a,b), that is, the ventral side of the labrum, is sclerotized, smooth and glabrous; two distinct transverse ridges are present laterally, one of them long and one short; a spindle-shaped distinctly delimited median region bears a pair of sensorial papillae, each of them with a distinct pore. The middle epipharyngeal region, which forms the roof of the laterally open cibarium, is anteriorly delimited by a semicircular sclerotized bead; it is flat, semimembranous, and bears a regular and dense vestiture of short microtrichia (ca. 5 μm); a distinct median bulge or process with longer microtrichia is absent; posteriorly this epipharyngeal section is delimited by a posteriorly directed semicircular sclerotized bar, between the basal region of the tormae; the sclerotized area is slender laterally; the broader median part bears three deep pits with setae inserted in them; a median group of longer microtrichia (ca. 8 μm) is present at the posterior margin. The posterior epipharynx is laterally fused with the posterior hypopharynx thus forming the roof of an elongate prepharyngeal tube. The anterior hypopharynx (Figure 4h) forms a structural and functional unit with the prelabium; its anteromedian region forms a pair of lateral hypopharyngeal lobes densely set with long trichia.

Musculature (Figure 5h): M41 - M. frontohypopharyngalis (0hy1), O: posterodorsal area of the head capsule, anterior to the neck region, I: laterad the anatomical mouth; M43 - M. clypeopalatalis (0ci1), two subcomponents, M43a with two bundles, M43b with a long series of bundles, O: on the anterodorsal wall of the head capsule (M43a) and on the frontal area including the median region between the antennal sockets (M43b), I: dorsal wall of middle epipharyngeal region (M43a) and dorsal wall of prepharyngeal tube (M43b); M44 - M. clypeobuccalis (0bu1), three closely adjacent bundles immediately anterior to the anatomical mouth, between insertion sites of M41, O: posterior area of the frontal region, I: dorsally on the prepharynx immediately anterad the anatomical mouth.

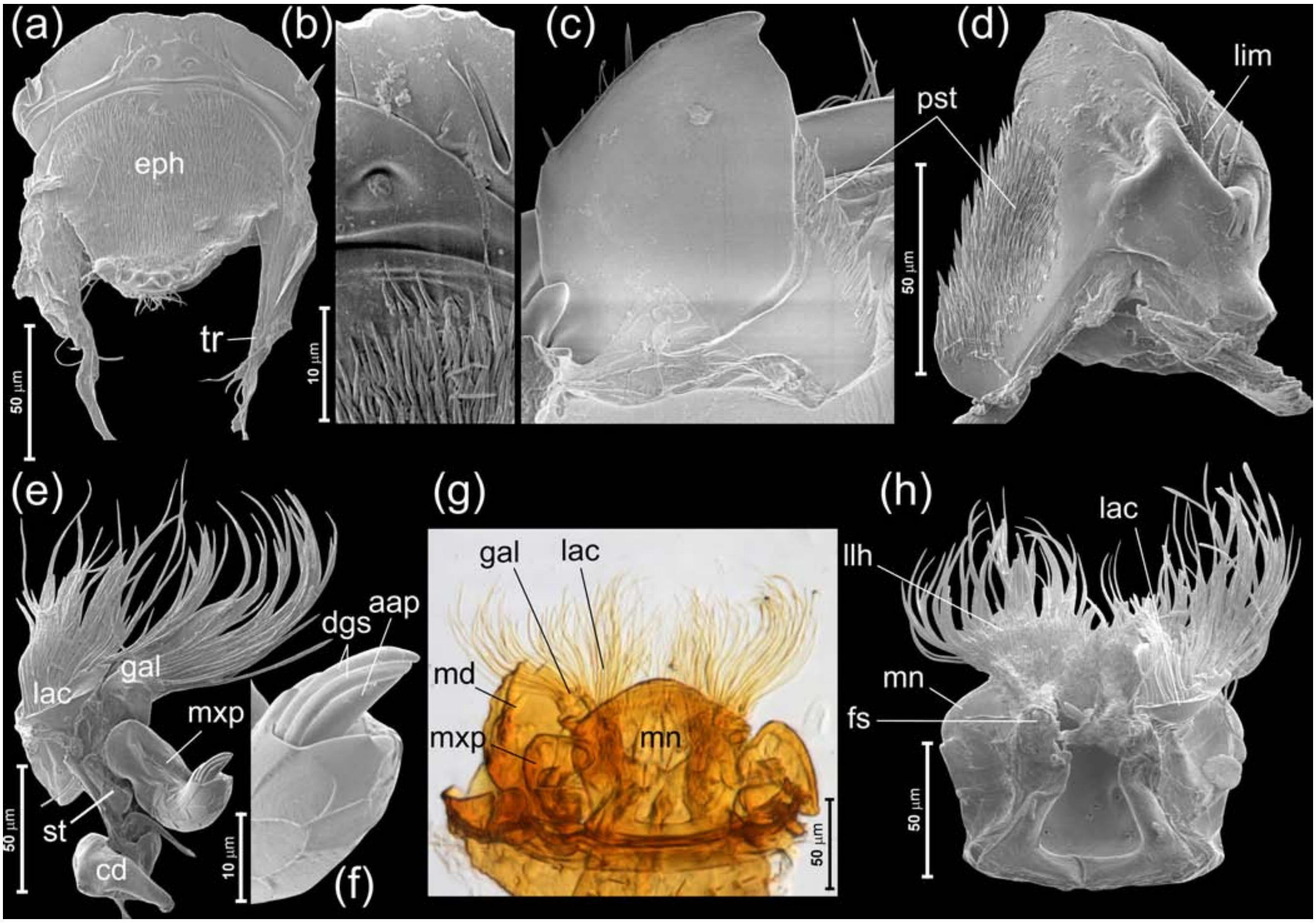

FIGURE 4 Cephalic morphology of *C. testaceus*, scanning electron microscopy (SEM) images (a–f,h) and light micrograph (g) of dissected mouthparts. (a) Labrum in ventral view; (b) anterior sublateral area of epipharynx; right mandible in ventral (c) and dorsal (d) views; (e) left maxilla in dorsal view; (f) apex of left maxillary palp in ventral view; labium, mandible and maxilla in ventral view; (g) labio-maxillary complex and right mandible in ventral view; (h) labio-maxillary complex in ventral view, with right maxilla torn off. aap, apical appendage; cd, cardo; dgs, digitoform sensilla; eph, epipharyngeal microtrichial field; fs, fusion site of lacinia; gal, galea; lac, lacinia; lim, lateral impression; llh, lateral lobe of hypopharynx; mn, mentum; mxp, maxillary palp; pst, microtrichial field on prostheca; st, stipes; tr, torma

3.9 | Prepharynx and pharynx

The anatomical mouth, that is, the border between the prepharynx and the pharynx (e.g., Beutel, Friedrich, Yang, & Ge, 2013), is marked by the position of the frontal ganglion. An elongate, closed prepharyngeal tube is formed by lateral fusion of the posterior epipharynx and hypopharynx. In cross section, this prepharynx appears heart-shaped; it is ventrally rounded and sclerotized, and a median invagination (for muscular insertion) is present on the flexible dorsal side. The pharynx (Figure 5h) is mostly enclosed by the cerebrum and suboesophageal complex, and posteriorly distinctly narrowed. The pharyngeal lumen appears round to oval in cross section. Longitudinal pharyngeal folds are recognizable but indistinct.

Musculature (Figure 5h): M45 - M. frontobuccalis anterior (0bu2), vertically oriented, O: middle region of the dorsal wall of the head capsule, I: dorsal pharyngeal wall; M46 - M. frontobuccalis posterior (0bu3), O: middle region of the vertex, posterior to M45 and laterad M41, I: dorsal pharyngeal wall; M48 - M. tentoriobuccalis anterior (0bu5) (for a discussion of the homology of this muscle and M. tentoriohypopharyngalis (M42), see Beutel, Kristensen, and Pohl (2009)), O: ventral tentorial base, I: ventral prepharyngeal wall; M50 - M. tentoriobuccalis posterior (0bu6), vertically oriented, O: ventrally on the middle region of the head capsule, I: ventral wall of the precerebral pharynx; MmIII-Mm. compressores epipharyngis, numerous transverse bundles on the posterior epipharynx.

3.10 | Nervous system

The brain (Figure 6a,b) is of medium size compared to the entire volume of the head and located in the posterodorsal cephalic area, occupying a large portion of the neck region; it is constricted in the area of the occipital crest; anteriorly it almost reaches the tentorial arms. The ventral side of the suboesophageal complex (Figure 6a,b) is very close to the ventral wall of the head capsule; the posterior part is distinctly enlarged and protrudes from the cephalic lumen. The protocerebrum lacks optic neuropils,

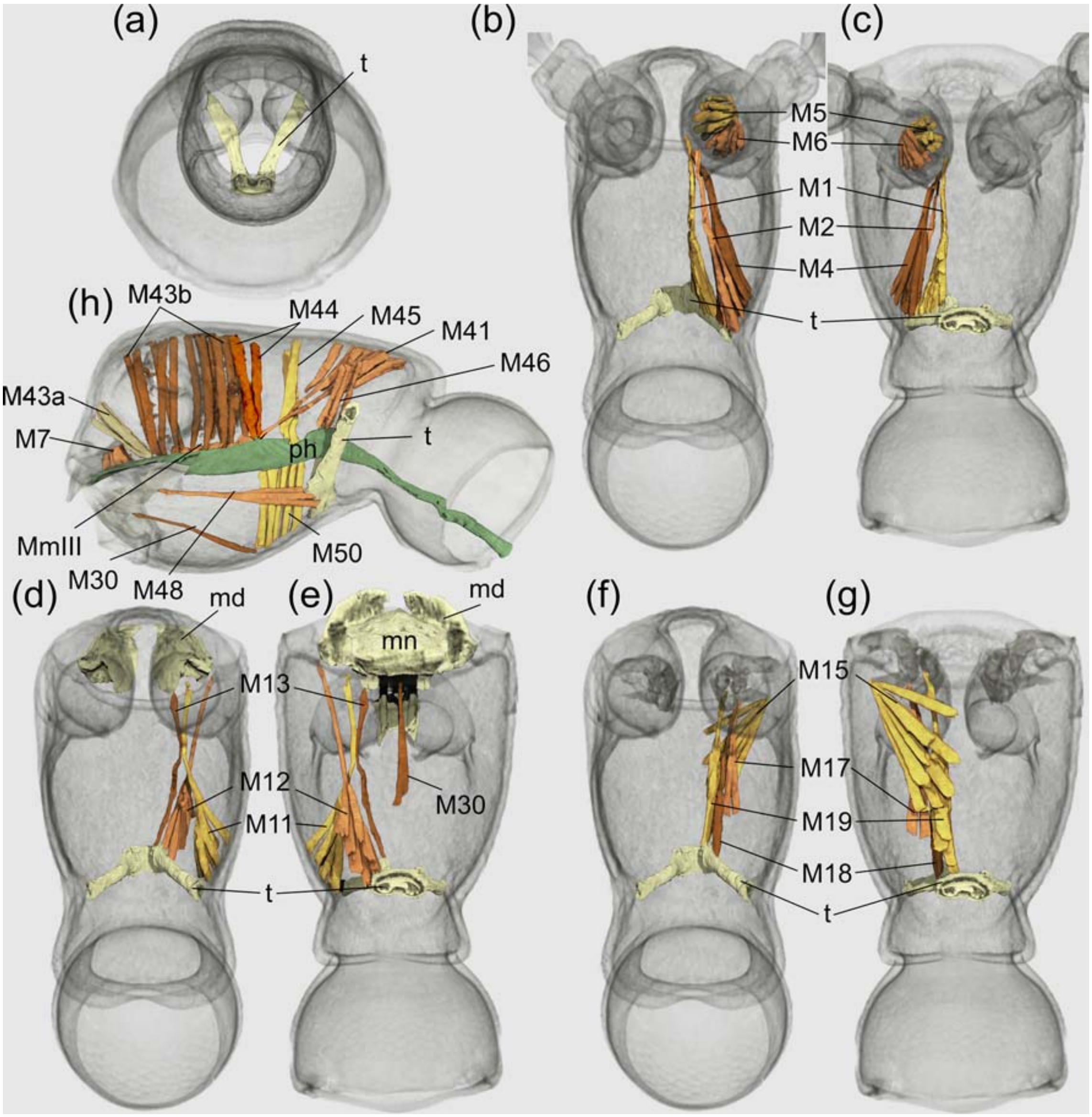

FIGURE 5 Cephalic morphology of *C. testaceus*, microcomputed tomography (μ-CT) reconstructions of tentorium in caudal view (a); antennal muscles in dorsal (b) and ventral (c) views; mandibular muscles in dorsal view (d); mandibular and labial muscles in ventral view (e); maxillary muscles in dorsal (f) and ventral (g) view; epi- and hypopharyngeal musculature, and muscles associated with cephalic section of alimentary tract in lateral view (h). M1, M. tentorioscapalis anterior; M2, M. tentorioscapalis posterior; M4, M. tentorioscapalis medialis; M5, M. scapopedicellaris lateralis; M6, M. scapopedicellaris medialis; M7, M. labroepipharyngalis; M11, M. craniomandibularis internus; M12, M. craniomandibularis externus; M13, M. hypopharyngomandibularis; M15, M. craniocardinalis externus; M17, M. tentoriocardinalis; M18; M. tentoriostipitalis; M19,? M. craniolacinialis; M30, M. tentoriopraementalis superior; M30, M. tentoriopraementalis superior; M41, M. frontohypopharyngalis; M43, M. clypeopalatalis; M44, M. clypeobuccalis; M45, M. frontobuccalis anterior; M46, M. frontobuccalis posterior; M48, M. tentoriobuccalis anterior; M50, M. tentoriobuccalis posterior; md, mandible; MmIII, Mm. compressores epipharyngis; mn, mentum; t, tentorium

whereas the deutocerebral antennal nerves are well developed. The frontal ganglion is located in the middle region of the head; it is posteriorly connected to the brain by the frontal connectives.

3.11 | Glands

Tubular glands (Figure 6c,d; and schematically in Figure 6e) are strongly developed in the anterior region of the head and occupy a considerable portion of this area. Several pairs extend from the middle cephalic region in front of the tentorial arms to the base of the maxillae and hypopharynx. Connecting ducts open in a pair of anterior labral impressions and in the lateral impression on each mandible (Figure 6e).

4 | DISCUSSION

It was long known that individuals of *C. testaceus* accept food directly from ant workers, and that regurgitation is triggered by a

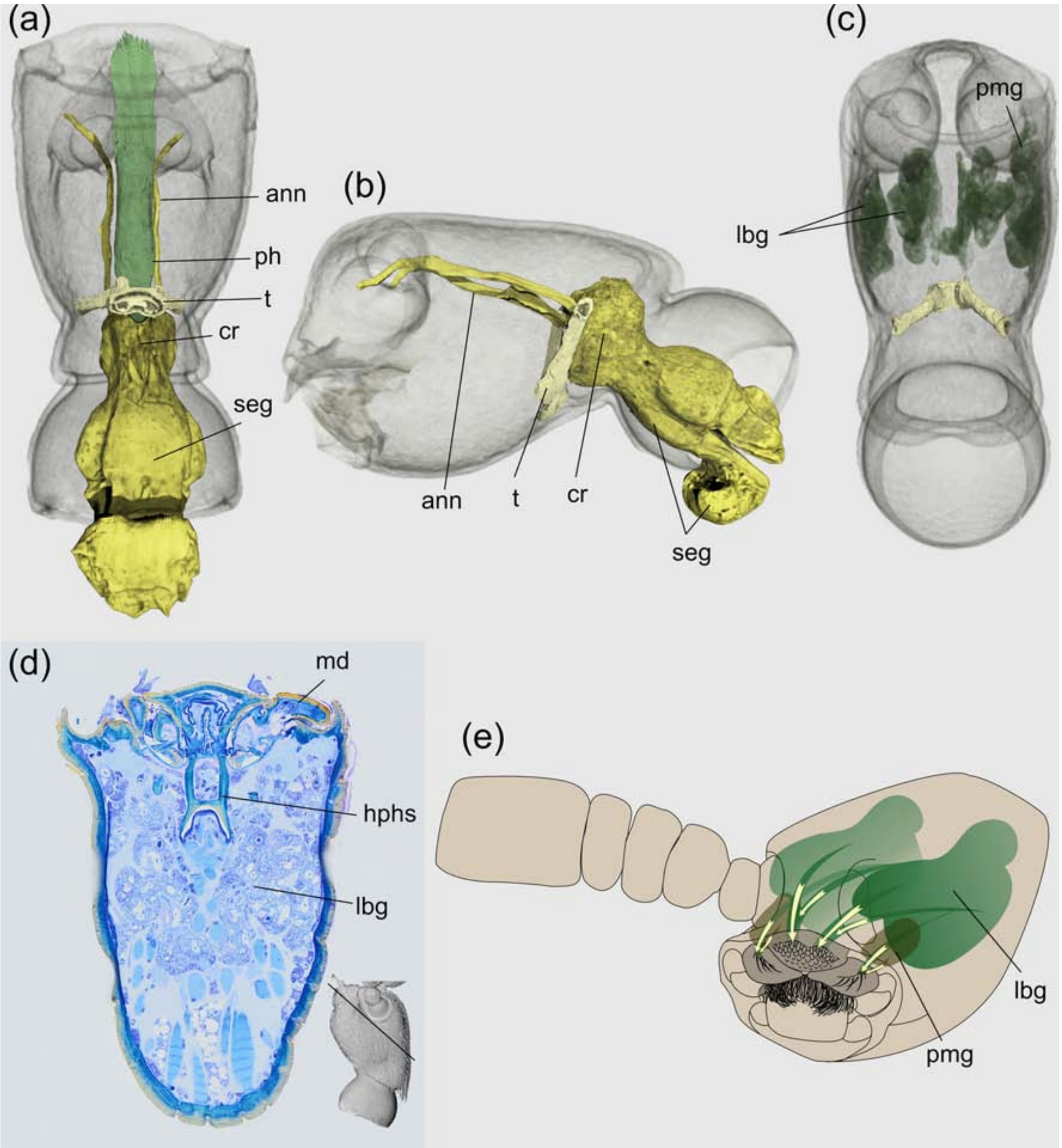

FIGURE 6 Cephalic morphology of *C. testaceus*, microcomputed tomography (μ-CT) reconstructions of central nervous system and pharynx (a,b), and cephalic glands (c–e); histological section of head (inlet showing the plane of section) (d); schematic arrangement of appeasement glands and their connecting ducts, combined from our results and Cammaerts (1974) (e). ann, antennal nerve; cr, cerebrum (brain); hphs, hypopharyngeal suspensorium; lbg, clusters of labral glands; md, mandible; ph, pharynx; pmg, clusters of postmandibular glands; seg, suboesophageal ganglia; t, tentorium

physical contact between beetles and ants, made possible by secretions produced by cephalic and abdominal glands of the beetles (Cammaerts, 1991, 1992, 1995, 1996). Many small beetles evolved special protective mechanisms to reduce contacts with aggressive predators, for instance size reduction of the head (Corylophidae: Polilov and Beutel (2010)), or modifications that allow to conceal the head on the ventral side of the body or to retract it into the prothorax (Clambidae: (Anton, Yavorskaya, & Beutel, 2016); some Leiodidae: (Park, Leschen, & Ahn, 2014); some Scydmaeninae: (Jałoszyński, 2013); and Histeridae, including myrmecophiles, e.g., Parker, 2016b). In contrast, the elongate head of *Claviger* is exposed to contacts with ants. All pselaphines and *Protopselaphus* (Newton & Thayer, 1995) have exposed heads, except for a short part of the neck region that is retracted into the prothorax. Among Pselaphini, which include taxa phylogenetically close to Clavigeritae, the head is distinctly elongate (examples are shown in Figure S1b,c). *Claviger* clearly uses variations of the subfamily groundplan, with many modifications to facilitate trophallaxis.

Our results demonstrate that the mouthparts of *Claviger* are well adapted to frequent contacts with the sharp mandibles of ants, as there are no projecting components that could be accidentally damaged during feeding. This is in clear contrast to most other Pselaphinae, which have large, exposed mandibles, tetramerous and long maxillary palps (e.g., Figures S1c, S3, and S4), and dimerous labial palps inserted on a well-developed prementum (e.g., Figures S3b,d,g and S4b,d). The mouthparts of *Claviger* strongly deviate from those of free-living beetles (e.g., Antunes-Carvalho et al., 2017; Beutel & Yavorskaya, 2019; Newton & Thayer, 1995), and also from those of myrmecophilous non-Clavigeritae pselaphines. The labrum bears a pair of openings for the labral glands, whose secretion was previously found to attract ants and to trigger regurgitation (Cammaerts, 1974). The scale-like microsculpture likely facilitates spreading of the secretion onto the entire anterior labral surface by means of capillary forces. As the labral musculature consists of only internal bundles, the entire structure can be moved only passively. We interpret the shape and structure of the labrum as being well suited to present the glandular secretion to approaching ants and to transfer liquids into the oral cavity, by capillary forces created by the microtrichial field on the anterior epipharynx.

The mandibles form a functional complex with the labrum: together they form a heavily sclerotized shield that covers the more delicate ventral mouthparts and presumably protects them during the frequent contacts with ants' mandibles. The lateral mandibular impressions bear glandular openings and deliver appeasement secretions to ants (as demonstrated by Cammaerts, 1974, 1992). The microtrichial fields of the prostheca suggest that liquids can be also transferred into the oral cavity in this manner. The mandibles of *Claviger* are conspicuously short and their muscles weakly developed. Even the adductor (M11), typically the most voluminous and most powerful muscle of the head in Coleoptera (e.g., Antunes-Carvalho et al., 2017; Dressler & Beutel, 2010; Weide & Betz, 2009), is strikingly small in *Claviger* and composed of only few bundles. In predatory pselaphines, the long and often toothed mandibles (Figures S3a,c,e and S4a,c,e,g), when closed, strongly project from under the labrum and overlap or cross in the median line (e.g., Schomann et al., 2008). In *Claviger*, the labrum covers most of their mesal edges, even when the mandibles are extended. The primary function in beetles with sharp mesal mandibular edges, that is, cutting, is clearly obsolete in *Claviger*.

The maxillary palps are strongly transformed, each composed of one only segment, and resting permanently in a lateral concavity of the buccal region. In *Protopselaphus* (Newton & Thayer, 1995, figure 4) and predatory pselaphines, the long maxillary palps are four-segmented (Figures S3b,d,f and S4b,d,f,g), and in some species participate in catching springtails, as demonstrated by Schomann et al. (2008). They also show a tendency to extreme elongation, for instance in members of Pselaphini (Figures S1b,c and S4g). It is plausible that once the ancestor of *Claviger* shifted from predatory feeding to trophallaxis, involvement of the palps in catching prey was no longer required. It has been proposed that myrmecophilous habits promote shortening of the maxillary palps to prevent possible damage during contacts with aggressive ants (Parker, 2016b). Even though the palps of *Claviger* have become the most vestigial known among beetles, their sensory function has been at least partly preserved. The palps are recurved exposing the sensory apical appendage and digitiform sensilla, and when directed forward they are able to receive tactile or chemical stimuli. However, while the maxillary palps are long-range sense organs in free-living pselaphines, they have been transformed into short-range sensors in *Claviger*, strategically situated near the mandibular glandular impressions that are crucial for the trophallaxis. The maxilla is equipped with a set of only four muscles that adduct the cardo, stipes, and lacinia, and protract, adduct and retract the entire maxilla. Muscles of the endite lobes are missing, for instance M20, but their loss is common among many unrelated non-myrmecophilous beetles (Anton & Beutel, 2004, 2006, 2012; Antunes-Carvalho et al., 2017; Dressler & Beutel, 2010; Weide & Betz, 2009), and also all palp muscles. The function of the maxillae is integrated with that of the labrum and mandibles. The extremely long and dense distal setae of the lacinia and galea, projecting far beyond the mandibles, form a licking/'sponging' capillary apparatus, which is lacking in all beetles with predacious or fungivorous feeding habits (Beutel & Yavorskaya, 2019). A small ventral area of the lacinia is fused with the hypopharynx, a modification unknown in any other beetles (e.g., Beutel & Yavorskaya, 2019). Dissections of seven free-living pselaphine species (illustrated in Figures S3 and S4) revealed in each case that the lacinia is not fused or connected with the hypopharynx. This unique fusion in *Claviger* may align all the capillary elements in a certain arrangement, even when the maxillae are moved in relation to other structures, to ensure efficient uptake of liquids. Moreover, the site of fusion located on a relatively small and flexible membranous area creates an additional fulcrum. This presumably changes the lever system of sclerotized maxillary structures and modulates their movements. A mechanical modelling study may be required to address this interesting question.

The labium of *Claviger* is highly modified. The palps are absent and the prementum is vestigial, recognizable only by the presence of M30, which in beetles inserts on the prementum and functions as one of its retractors (e.g., Anton & Beutel, 2004; Weide & Betz, 2009). The only other muscle indirectly associated with the labium in *Claviger* is the hypopharyngeal levator and dilator of the anatomical mouth opening (M41). In contrast to this, three or four pairs of labial muscles are usually present in Coleoptera that have a fully developed labium (e.g., Anton & Beutel, 2004; Antunes-Carvalho et al., 2017; Weide & Betz, 2009), underlining the profoundness of the modifications in *Claviger*. The extremely long setae of the lateral lobes of the hypopharynx are probably suitable for taking up regurgitates. This setal complex of the mouthparts is best visible in Figure 2a; the flexible fringes of the maxillae and the lateral hypopharyngeal lobes project far beyond the mandibles. The mouthparts of the recently discovered early Eocene (50–52 Ma) *Protoclaviger*, assigned to stem-group Clavigeritae, look similar in lateral view (illustrated in Parker and Grimaldi (2014)), demonstrating that this morphological modification has a long evolutionary history.

Far-reaching modifications of the anterior section of the alimentary tract were described in some beetles that take up liquid food in large quantities. An example is the system of pharyngeal valves found in larvae of *Cephennium* (Staphylinidae: Scydmaeninae), which feed on liquefied tissues of mites. This innovation may play a role in synchronizing the transfer of food from each pharyngeal section into the next one (Jałoszyński & Beutel, 2012). In *Claviger*, we found only one peculiarity in this region: the prepharyngeal dilator M. clypeopalatalis is distinctly increased in size compared to other staphylinoid beetles (Weide & Betz, 2009, figure 1; Antunes-Carvalho et al., 2017); it is composed of an entire series of bundles. Aside from this, the prepharynx and pharynx do not show specializations facilitating the transport of liquid food. Modifications are also lacking in adults of *Cephennium*, which feed in the same manner as the larvae. This shows that an efficient transfer of liquids can be maintained without major changes to the anterior digestive tract (Jałoszyński & Beutel, 2012). Similarly, it seems that no changes of the pharyngeal complex were required in the ancestral lineage of Clavigeritae to adopt trophallaxis.

It appears that the reduced primary sensory functions of the palps of Clavigeritae are partly compensated by adaptations of the antennae. The antennae of Clavigeritae are always strongly modified, apically broadening, and with only three to six antennomeres (Parker, 2016b), somewhat similar to conditions occurring in the myrmecophilous paussines of Carabidae (e.g., Geiselhardt, Peschke, & Nagel, 2007). In contrast, filiform antennae with 11 antennomeres belong to the groundplan of crown-group Coleoptera and Pselaphinae (Beutel & Hörnschemeyer, 2008; Newton & Thayer, 1995). The scape and pedicel of *Claviger*, segments crucial for antennal movements, are the smallest. The scape is subglobose and only a small portion is exposed, whereas it is most commonly elongate and largely exposed in other pselaphines and also in *Protopselaphus* (Newton & Thayer, 1995, figure 6). The flagellomeres of *Claviger*, although reduced in number, are enlarged, which increases their sensory surface covered with sensilla trichodea. Additionally, the modified apex of the terminal segment bears a large sensory/glandular setose field enhancing the sensorial apparatus. Despite of the small size of the scapus and the reduced number of flagellomeres, the movability of the antennae is fully retained, with a well-developed set of three extrinsic muscles, and two additional intrinsic bundles moving the pedicel.

Since they live inside ant colonies, and specialized on trophallaxis, *Claviger* beetles do not need to detect escaping prey or test surrounding objects for their edibility, which are typical functions of maxillary and labial palps in beetles. As the conditions inside ant colonies promoted the far-reaching reduction of palps, it is likely that the eyeless *Claviger* mostly relies on the antennae to recognize ants as food donors, in order to stimulate the regurgitation at the right moment. The set of detectors (antennae), stimulators (glands), and food uptake devices (the capillary mouthpart complex) constitute the core of myrmecophilous adaptations of the head of *Claviger*.

Cammaerts (1974) found several separate pairs of exocrine glands inside the head of *Claviger*: mandibular, mandibulo-maxillary, external and internal labral, and postantennal clusters. The most important in stimulating trophallaxis are the labral glands that discharge onto the anterior surface of the labrum, and, to a lesser extent, the lateral concavities of the mandibles (Cammaerts, 1992). We found interconnected groups of tubular glands that almost completely fill the space between other organs in the anterior head region, making it difficult to define individual clusters. The general arrangement is similar to that found by Cammaerts (1974), with the largest volume occupied by the labral-postmandibular clusters, which discharge (via connecting ducts) their secretions onto the labrum and mandibles. No reservoirs were found that would allow secretions to be stored until needed and discharged in large quantities. This may explain the large size of the glands, enabling them to produce enough secretion to satisfy ants, and why the abdominal glands serve the same purpose, that is, to co-stimulate regurgitation, even though ants regurgitate onto the beetle's abdomen, as observed by Cammaerts (1992). An ant licking the abdominal trichomes, even if initially regurgitating onto a wrong place, remains interested long enough to eventually get in a close contact with the beetle's head and repeat the regurgitation into its mouth. We postulate that both the cephalic and abdominal glands are necessary to ensure sufficient production of the stimulants (which cannot be stored in large quantities), and that the labral and mandibular glandular openings direct the regurgitation into the beetle's mouth.

The glands in *Claviger* occupy a considerable space inside the head capsule. The brain is shifted posterior to the tentorium. A similar arrangement was found in males of some scydmaenine beetles, which have an anterodorsal cavity serving as a reservoir for secretions produced by a very large cephalic gland, and the brain shifted to the neck region (Jałoszyński, Hünefeld, & Beutel, 2012). In contrast, the brain of scydmaenines without cephalic glands is located in the pre-tentorial lumen of the head (Jałoszyński et al., 2012). The development of cephalic glands in *Claviger*, crucial for its integration into ant communities, may be the reason for the posterior shift of the brain. A similar shift in other beetles (and larvae) to the 'neck' or even into the prothorax, is usually associated with a small relative size of the head (e.g., Polilov and Beutel (2010)). However, the head of *Claviger* is large in relation to the prothorax and it is unlikely that miniaturization played an important role in the observed architecture and placement of the brain.

5 | CONCLUSIONS

In order to achieve a full integration with ant communities, pselaphines of certain lineages fundamentally changed their diet and considerably transformed their mouthparts to take up ants' regurgitate as food. This was not possible without an earlier development or re-programming of existing (epidermal?) glands to produce secretions that manipulate the ants' behaviour to an extreme extent, with hosts not only tolerating the beetles in their brood chambers, but also feeding them upon request. A reduction of most projecting components of the mouthparts, with their sensorial apparatus so important in the context of feeding in free-living beetles, suggests physical aggression from ants as a driving factor in the evolution of Clavigeritae. Evolution of mouthparts was determined by adopting to trophallaxis, which involved shortening and simplification of the mandibles, shortening of the maxillary palp, a hypertrophy of capillary structures, a unique connection between the lacinia and hypopharynx, and reduction of the prementum, labial palps, and different muscles. The development of conspicuously enlarged cephalic glands producing specific secretions made it possible to chemically manipulate the host ants' behaviour. Loss of optic neuropils and eyes, along with reduction of wings, sealed the fate of extreme myrmecophiles as dependent on ants not only in feeding but also for dispersal.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Paweł Jałoszyński designed the research, carried out SEM study, and drafted the manuscript to which Xiao-Zhu Luo and Rolf Georg Beutel contributed; Xiao-Zhu Luo and Rolf Georg Beutel conducted the μ-CT and 3D reconstructions.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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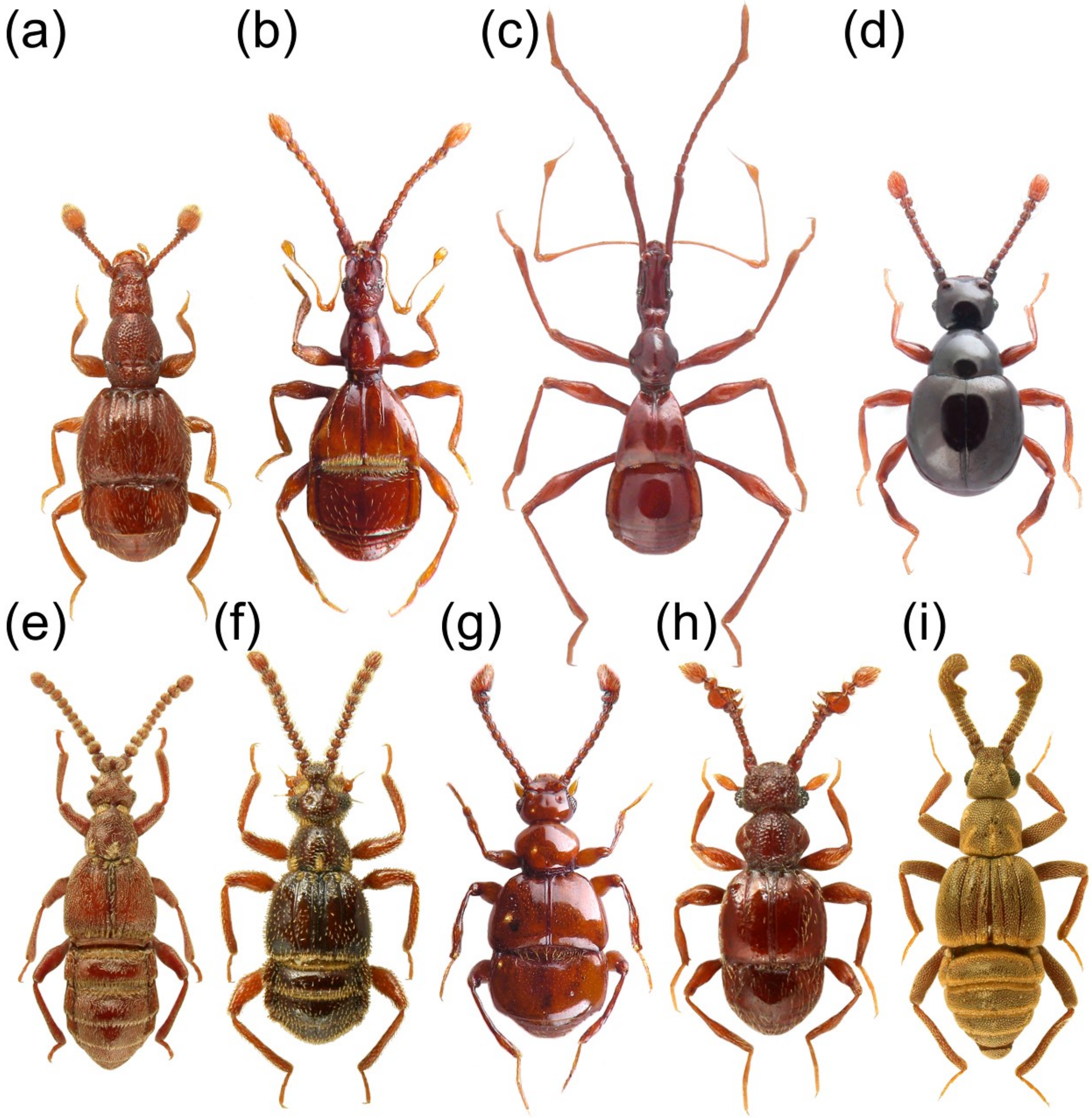

FIGURE S1 Diversity of non-Clavigeritae pselaphines; selected examples. (a) *Philoscotus longulus* Sawada (Japan); (b) *Pselaphogenius orientalis* Besuchet (Japan); (c) Pselaphini, genus indet. (New Caledonia); (d) *Eupines* sp. (New Caledonia); (e) *Chennium bituberculatum* Latreille (Ukraine); (f) *Centrotoma prodiga* Sharp (Japan); (g) *Batraxis splendida* Nomura (Jeannel); (h) *Trisinus galloisi* (Jeannel) (Japan); (i) *Apharinodes papageno* Nomura (Japan).

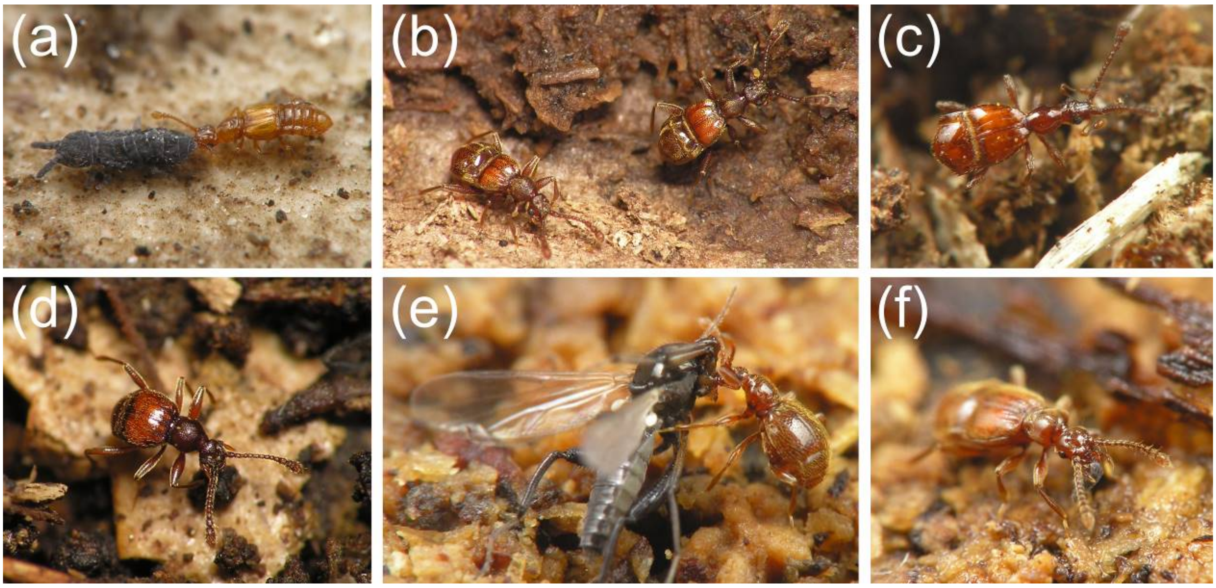

FIGURE S2 Diversity of non-Clavigeritae pselaphines; living beetles. (a) *Euplectus* sp. (Poland) attacking a springtail; (b) *Raphitreus speratus* (Sharp) (Japan); (c) *Pselaphus heisei* Herbst (Poland); (d) *Triomicrus* sp. (Japan); (e) *Briaxis* sp. (Poland) feeding on a dead fly; (f) *Trichonyx sulcicollis* (Reichenbach) (Poland), feeding on springtail.

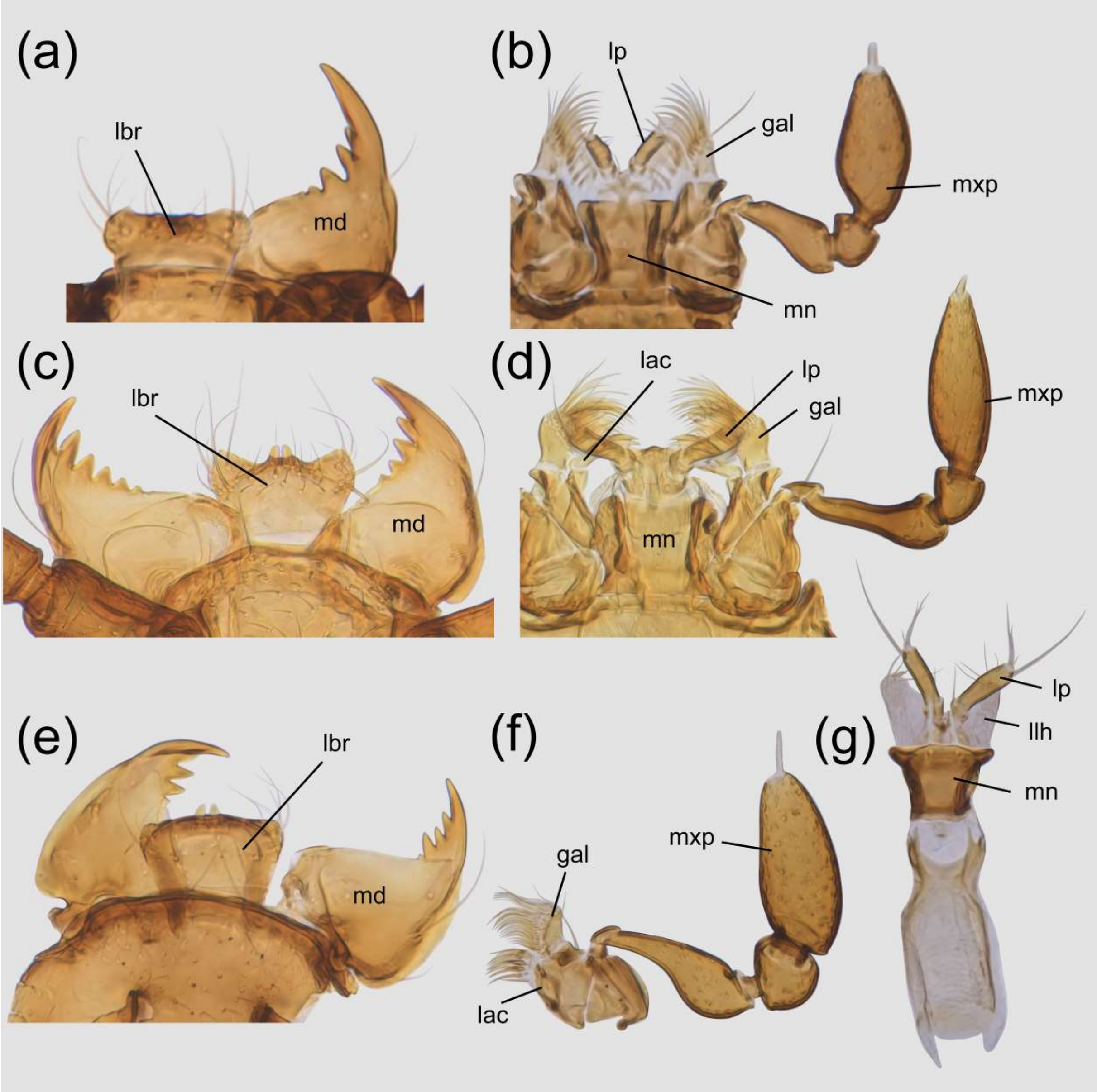

FIGURE S3 Examples of dissected mouthparts in free-living European pselaphines. (a, b) *Euplectus karstenii* (Reichenbach); (c, d) *Trichonyx sulcicollis* (Reichenbach); (e–g) *Brachygluta fossulata* (Reichenbach). Abbreviations: gal, galea; lac, lacinia; lbr, labrum; llh, lateral lobe of hypopharynx; lp, labial palp; md, mandible; mn, mentum; mxp, maxillary palp.

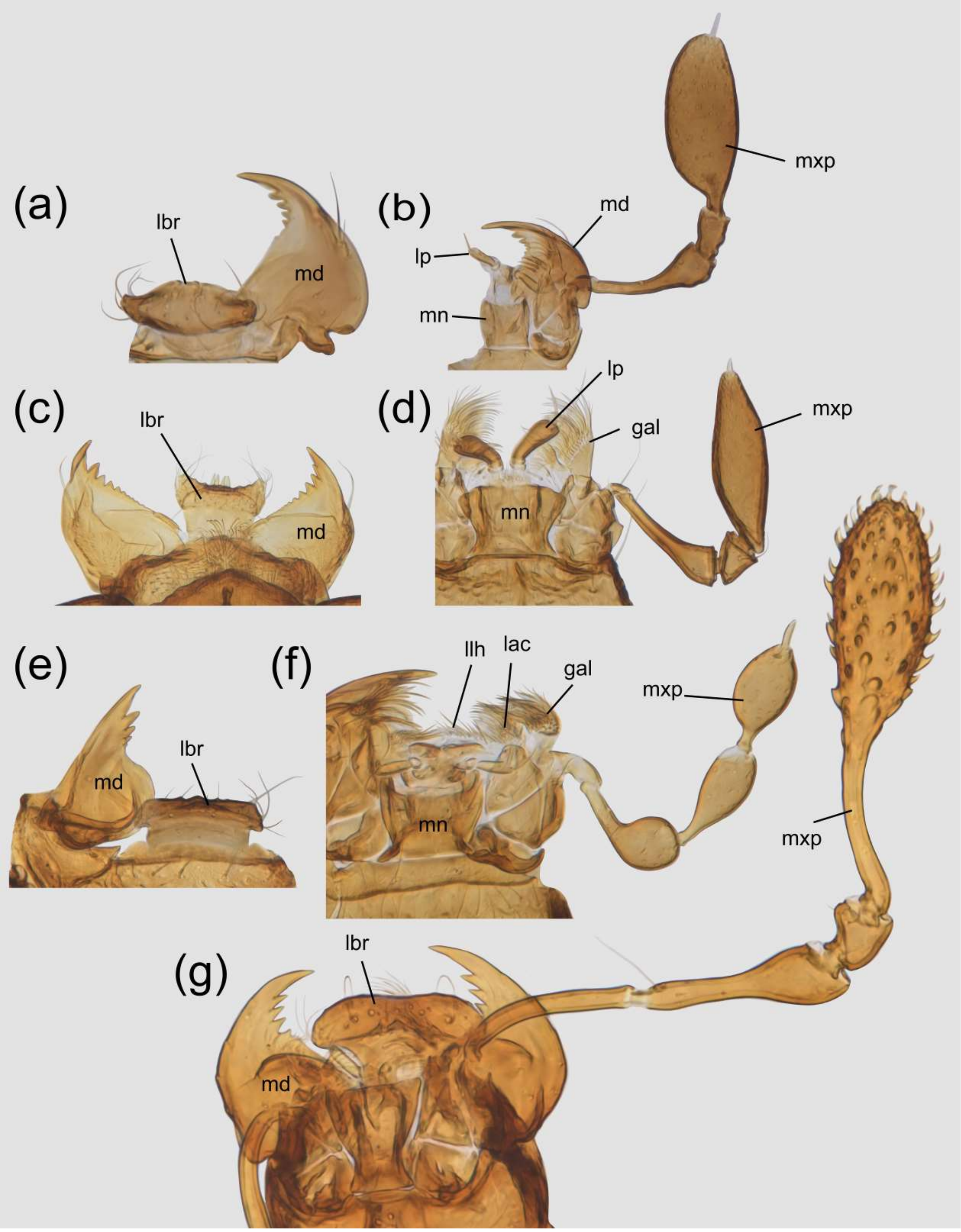

FIGURE S4 Examples of dissected mouthparts in free-living European pselaphines. (a, b) *Bryaxis bulbifer* (Reichenbach); (c, d) *Batrisodes venustus* (Reichenbach); (e–f) *Tyrus mucronatus* (Panzer); (g) *Pselaphus heisei* Herbst. Abbreviations: gal, galea; lac, lacinia; lbr, labrum; llh, lateral lobe of hypopharynx; lp, labial palp; md, mandible; mn, mentum; mxp, maxillary palp.

Table S1 Head musculature comparison of several species of Staphylinidae and one species of Leiodidae (Staphylinoidea). Homology is mainly based on Kéler (1963). Notes of superscirpts: a, U1 is only recorded in Weide and Betz (2009); b, Mx is homologized by Jäch et al. 2000; c, Muscles which were identified as M48 in Weide and Betz (2009) are M42.

Muscle	*Omalium rivulare* (Paykull, 1789)	*Lesteva longoelytrata* (Goeze, 1777)	*Atheta laticollis* (Stephens 1832)	*Tachyporus chrysomelinus* Linnaeus 1758	*Catops ventricosus* (Weise, 1877)	*Claviger testaceus* Preyssler, 1790
M1	+	+	+	+	+	+
M2	+	+	+	+	+	+
M3	-	-	-	-	-	-
M4	+	+	+	+	+	+
U1[a]	-	-	+	-	-	-
M7	+	+	+	+	+	+
M8	-	-	-	-	-	-
M9	+	+	+	+	-	-
M10	-	-	-	-	-	-
M11	+	+	+	+	+	+
M12	+	+	+	+	+	+
M13	-	-	-	-	+	+
M14	-	-	-	-	-	-
M15	+	+	+	+	+	+
M16	-	-	-	-	-	-
M17	+	+	+	+	+	+
M18	+	+	+	+	+	+
M19	+	+	+	+	+	+?
Mx[b]	+	+	-	-	+	-
M28	+	+	+	+	+	-
M29	+	+	+	+	+	-
M30	+	+	+	+	+	+
M31	-	-	-	-	-	-
M32	-	-	-	-	-	-
M33	-	-	-	-	-	-
M34	+	+	+	+	+	-
M37	-	-	-	-	-	-
M38	-	-	-	-	-	-
M39	-	-	-	-	-	-
M40	-	-	-	-	-	-
M41	+	+	+	+	+	+
M42[c]	-	-	-	-	+	-
M43	+	+	+	+	+	+
M44	+	+	+	+	+	+

M45	+	+	+	+	+	+
M46	+	+	+	+	+	+
M47	-	+	-	-	-	-
M48[c]	+	+	-	+	+	+
M49	-	+	-	-	-	-
M50	+	+	+	+	+	+