Two new genera of Caeninae (Ephemeroptera: Caenidae), with a cladistic analysis of the tribe Clypeocaenini

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Two new genera of Caeninae (Ephemeroptera: Caenidae), with a cladistic analysis of the tribe Clypeocaenini

Peter Malzacher & Arnold H. Staniczek

Abstract

The new genera *Mandelara* **n. gen.** (type species *M. immutata* **n. sp.**) from South Africa and *Papuaenis* **n. gen.** (type species *P. balkei* **n. sp.**) from Papua New Guinea are described. Both genera are included in the tribe Clypeocaenini that is defined by a bulged frons and more or less protruding clypeus. A cladistic analysis recovers the Clypeocaeninae as monophyletic. Caenini + Tasmanocoenini are revealed as sister group to Clypeocanini, together constituting the subfamily Caeninae. A larval key in provided to the genera of Clypeocaenini.

Keywords: Clypeocaenini, new genera, *Mandelara*, *Papuaenis*, phylogeny, South Africa, Papua New Guinea.

Zusammenfassung

Zwei neue Gattungen der Familie Caenidae werden beschrieben: *Mandelara* **n. gen.** (Typus-Art *M. immutata* **n. sp.**) von Südafrika und *Papuaenis* **n. gen.** (Typus-Art *P. balkei* **n. sp.**) von Papua Neuguinea. Beide Gattungen werden dem Tribus Clypeocaenini zugeordnet, dessen Gattungen sich durch ausgebeulte Frons und mehr oder weniger vortretenden Clypeus auszeichnen. Durch eine kladistische Analyse wird die Monophylie des Tribus abgesichert. Caenini + Tasmanocoenini bilden die Schwestergruppe der Clypeocaenini, alle zusammen die Unterfamilie Caeninae. Es wird ein Larvenschlüssel für die Gattungen der Clypeocaenini erstellt.

Contents

1 Introduction

Malzacher (2013) established the tribe Clypeocaenini within the subfamily Caeninae. The Clypeocaenini so far comprise the genera *Kalimaenis* (Oriental region, two species), *Amercaenis* (Nearctic region, two species), *Callistellina* (Madagascan region, one species), *Trichocaenis* (Madagascan region, one species), *Provonshara* (Ethiopian region, one species), *Clypeocaenis* (Ethiopian and Oriental regions, seven species), and *Barnardara* (Ethiopian region, one species) (Malzacher 2013, 2014). All these genera share two synapomorphic characters that define the Clypeocaenini as monophyletic taxon, namely (1) the reduction of filaments on abdominal gill III to 8 filaments at most, and (2) head bulged in lateral view, clypeus more or less protruding (Malzacher 2013).

In this contribution, two new monospecific genera, *Mandelara* n. gen. from South Africa and *Papuaenis* n. gen. from Papua-New Guinea, are described and placed within Clypeocaenini.

Malzacher (2013) presented a first phylogeny of Clypeocaenini and improved it with the description of *Provonshara* (Malzacher 2014). Both phylogenies were based on traditional Hennigian methodology. In this contribution, for the first time a cladistic analysis of Clypeocaenini is performed to check the monophyly of this taxon and to unravel its intergeneric relationships.

Acknowledgements

We are indebted to Michel Sartori and Jean-Luc Gattolliat (Musée cantonal de Zoologie, Lausanne) for providing material to this study, to Susanne Leidenroth and Milan Pallmann (Staatliches Museum für Naturkunde, Stuttgart) for making SEMs and microphotographs, and to Michel Sartori who kindly read the manuscript and provided valuable suggestions.

2 Material and methods

The investigated material is preserved in 75 % ethanol. *Mandelara* specimens were collected by a joint collection of the Musée Cantonal de Zoologie, Lausanne and Albany Museum,

Grahamstown in the Kruger National Park, South Africa, and that of *Papuaenis* by the Balke Expedition 2006 (Zoologische Staatssammlung München) in Papua New Guinea. The holotypes and paratypes are stored in the Musée cantonal de Zoologie, Lausanne.

Specimens used for SEM were dehydrated through a stepwise immersion in ethanol and then dried by critical point drying. The mounted material was coated with a 20 nm Au layer, examined and photographed with a Zeiss EVO LS 15 scanning electron microscope. Macrophotographs were taken with a Leica Z16APO Macroscope, processed with Leica Application Suite™ Version 3.1.0 to obtain combined photographs with extended depth of field. All digital photographs were subsequently enhanced by using PhotoFiltre 7.2.1.

For the cladistic analysis, 14 species of Caenidae were selected, *Neoephemera youngi* (Neoephemeridae) was chosen as outgroup (details see under section 5). Seven imaginal and 17 larval characters (see section 5) were chosen and treated as unordered character (except of character 15, which was treated as ordered). The data matrix (Tab. 1) was compiled in Nexus Data Editor (NDE) v.0.5.0 in ss file format converted into NEXUS file format (Maddison et al. 1997), and analysed using TNT v.1.1 (Tree analysis using Traditional Search) (Goloboff et al. 2008). For the Traditional Search, 99999 randomly seeded Wagner trees with 10000 replications were computed with 100 trees to save per replication. All trees found were kept. A strict consensus tree was calculated using all trees. The matrix was resampled with Jacknife 36 % removal probability, number of replicates 10000, output results as frequency differences (GC). Consensus tree and Jacknife yielded identical trees. Synapomorphies common to all calculated trees were delineated.

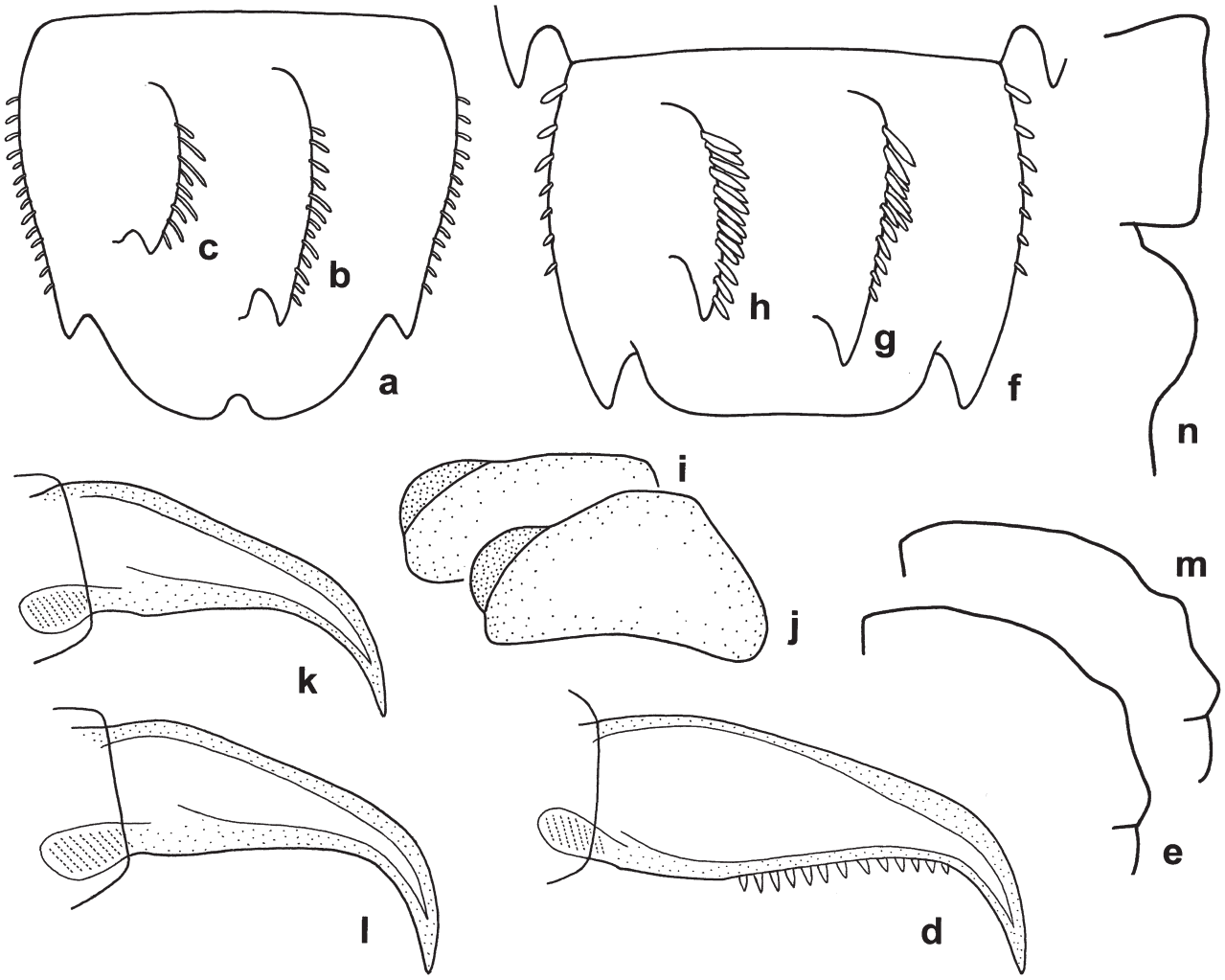


Fig. 1. *Mandelara immutata* n. sp., larva (a–e), *Papuaenis balkei* n. sp., larva (f–n). – **a**, **f**. Sternum IX. **b**, **g**. Marginal setation of segment VII. **c**, **h**. Marginal setation of segment V. **d**, **l**. Hind claw. **e**, **m**. Outline of head, lateral view. **i**. Coxal process of mid leg. **j**. Coxal process of hind leg. **k**. Fore claw. **n**. Lateral outline of pronotum and anterior part of mesonotum.

3 Systematic account

3.1 Genus *Mandelara* **n. gen.**

Type species: *Mandelara immutata* n. sp. by present designation.

Etymology

The genus is dedicated to NELSON MANDELA (1918–2013), the late president of the Republic of South Africa.

Differential Diagnosis

Mandelara n. gen. can be characterised and distinguished from all other genera of Caenidae by the following combination of characters:

L a r v a . Great parts of cuticle covered with shield- or funnel-shaped microtrichia (Figs. 6, 7). – Thorax broadened (Fig. 2). – Head without ocellar tubercles. – Outline of head with bulges in lateral view (Fig. 1e). – Clypeus scarcely protruding anteriorly, without setation (Fig. 3). – Maxillar and labial palps three-segmented. – Fore tibia and fore tarsus without filtering setae. – Mid and hind tibia ventrally without long bristles. – Operculate gill ventrally with an irregular row of simple, scale-shaped microtrichia, basal half of row also with clusters of spines and numerous single spines (Fig. 5). – Row not reaching hind margin of gill (Fig. 4). – Nearly all filaments of gill III 1- or 2-branched, only 1(–3) filaments with 3 or more branches. – Lateral spines of abdomen not bent dorsally. – Posterior part of sternum IX rounded, not shortened, hind margin medially with a small semicircular indentation (Fig. 1a). – Abdominal terga VII–IX without mediolongitudinal ridge. – Hind margin of sternum IX without a bipointed process with concave margin between the points.

On the differences to other genera of the Clypeocaenini see the larval key below (section 4).

Mandelara immutata **n. sp.**
(Figs. 1a–e, 2–8)

H o l o t y p e , ♀ larva (microslide): South Africa, Riv. Crocodile/Riv. Incomati, KNP near Malelane, 31°37′04″ E, 25°23′57″ S, 17.V.2003.

P a r a t y p e : 1 larva, same dates as holotype.

Etymology

The species epithet is chosen because of the lack of unambiguous autapomorphies.

L a r v a

Measurements and colouration

Subadult female larva, body length 3.3 mm, length of cerci 2.5 mm.

Colouration of cuticle: Intense yellowish-brown, legs and abdomen lighter.

Epidermal pigmentation: Head in front of lateral ocelli dark brownish-grey, with a blackish line between the bases of antenna. Abdominal terga I, II and VII–IX with broad transverse brownish bands; operculate gills brownish, most intense in anterolateral part.

Morphology

Cuticle: Large parts of dorsal and ventral side covered with laminar, shield- or funnel-shaped microtrichia (Figs. 6, 7) (often hardly to be seen in light microscope). Cuticle of abdomen and operculate gill denticulated. Denticles of different length and shape and mostly covered by the laminar microtrichia (Fig. 6).

Head: Outline of head bulged in lateral view (Fig. 1e). Clypeus scarcely protruding anteriorly; genae moderately bulging out (Fig. 3). Mandibles dorso-laterally with bristles of different length. Maxillary palp relatively short and broad; segment 3 of labial palp as long as segment 2 (Fig. 3b).

Thorax: Sides of pronotum straight and parallel (Fig. 3a). Legs relatively short and stocky. Transverse row on fore femur consisting of 8–12 spatulate bristles of moderate length. Coxal processes forming inconspicuous ridges. Mid and hind legs with simple bristles of different length more or less blunt, on femora and tibiae nearly all bristles marginally. Fore tarsus with an inner row of about 10–12 strong simple bristles. Mid tarsus with inner row of 10–12 simple bristles and short outer row of 0–4 simple bristles, the apical one unipinnate. Hind tarsus with inner row of 10–12 simple bristles, the apical ones long and slender, and an outer row of about 10 small and simple bristles, the apical 4 unipinnate. Claws of all legs slightly bowed, with about 12 denticles (Fig. 1d).

Abdomen: Posterolateral processes short (Fig. 2). Lateral margins with short spatulate or blunt bristles (Fig. 1a–c). Posteromedian process of tergum II long, triangular and pointed, slightly bowed in lateral view. Hind margin of tergum VII with spatulate bristles (Fig. 8a), VIII–X with strong denticles apically more or less rounded (Fig. 8b, c), tergum VIII with a few additional bristles, clearly shorter than those from tergum VII (Fig. 8b). Basal half of tergum VII densely covered with long bipinnate bristles (Fig. 7). Hind margin of sternum IX with a semicircular indentation, dorsal shagreen not discernible (Fig. 1a). Gill I nearly half as long as operculate gill. Lateral and hind margins of operculate gill with short spatulate and pinnate bristles (Figs. 4, 6), inner margin with thin and pointed ones. Medial ridge slightly keeled, with 3–4 short and strong bristles at the base. On ventral side of operculate gill an irregular row of microtrichia running in a greater distance from lateral and hind margin, ending far from the latter (Fig. 4). The row consists of simple scales of different size and shape, in the basal part of the row also with clusters and a broad band of single spines (Fig. 5a, b). Gills III(–V) with only 1 filament with 3 branches; all the

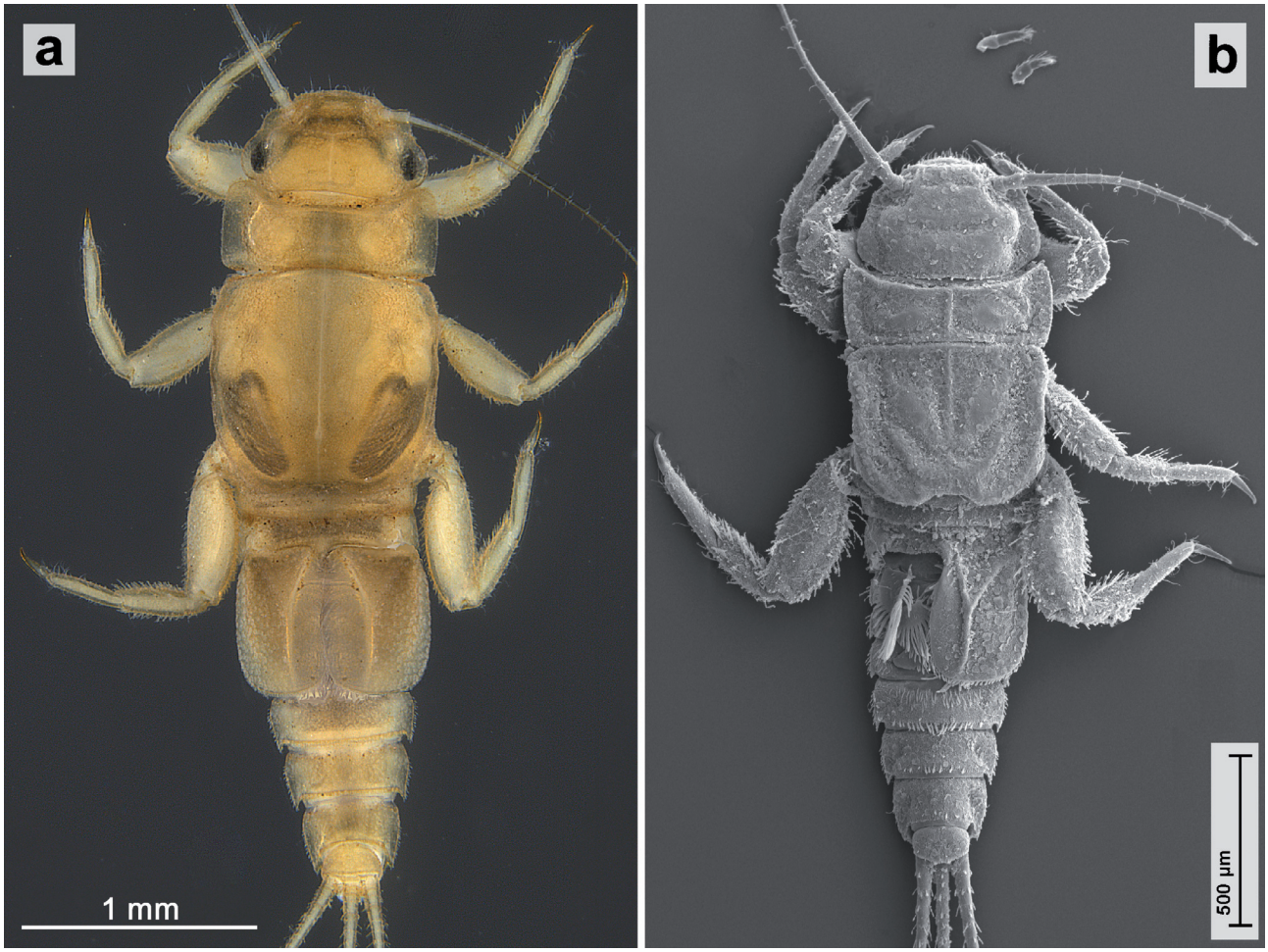


Fig. 2. *Mandelara immutata* n. sp., larva, light microscope (a), young larva, SEM (b).

other filaments (about 30) simple or two-branched. Basal segments of cerci with apically rounded bristles.

Imaginal stages unknown.

3.2 Genus *Papuaenis* **n. gen.**

Type species: *Papuaenis balkei* n. sp. by present designation.

Etymology

The genus name is a combination of P a p u a (New Guinea) where the genus was found, and C a e n i s .

Differential Diagnosis

Papuaenis n. gen. can be characterised and distinguished from all other genera of Caenidae by the following combination of characters:

L a r v a . Large parts of cuticle densely covered with shield-shaped microtrichia (Figs. 10, 15, 20). – With broad spatulate and frayed, sometimes tuft-shaped bristles of different length, arranged marginally on femora, tibiae, operculate gills etc. (Fig. 21). – Different types of frayed and comb-shaped bristles particularly on maxilla, labrum and fore tibia (Fig. 16). – Thorax broadened (Fig. 9). – Head without ocellar tubercles. – Outline of head with bulges in lateral view (Fig. 1m). – Clypeus slightly protruding anteriorly, without setation (Fig. 10). – Maxillary and labial palps three-segmented. – Fore tibia and fore tarsus without filtering setae. – Mid and hind tibia ventrally without numerous long bristles. – Gill I only about 0.2 the length of operculate gill. – Operculate gill ventrally with a row of simple scale-shaped microtrichia of different shape and size (Fig. 19). – Row not reaching hind margin of gill (Fig. 17). – Nearly all filaments of gill III 1- or 2-branched, only 1(–3) filaments with 3 or more branches. – Lateral spines of abdomen not bent dorsally. – Abdominal terga VII–IX with mediolongitudinal ridge (Fig. 9). – Posterior part of sternum IX shortened and cut (Fig. 1f), without a bi-pointed process with concave margin between the points.

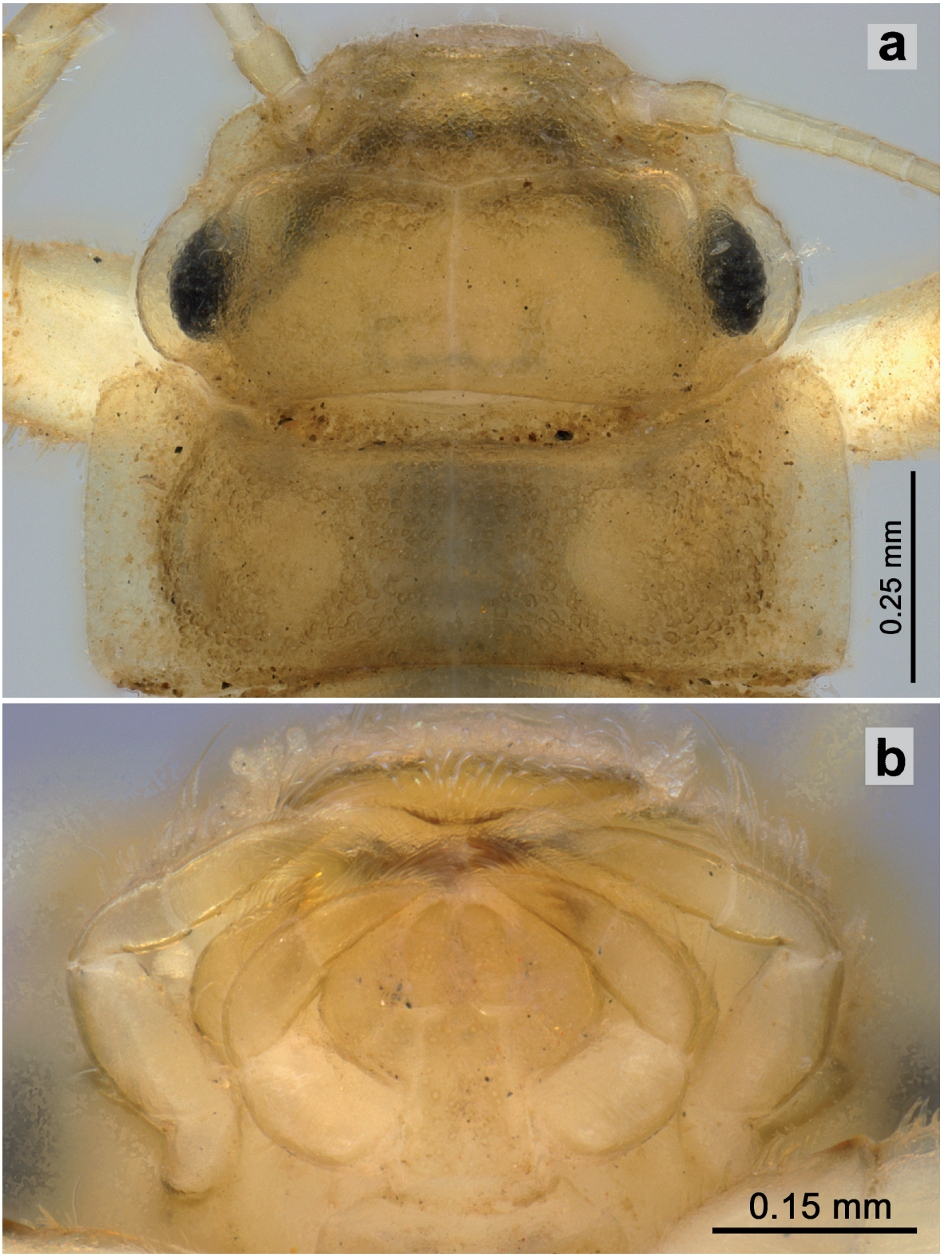
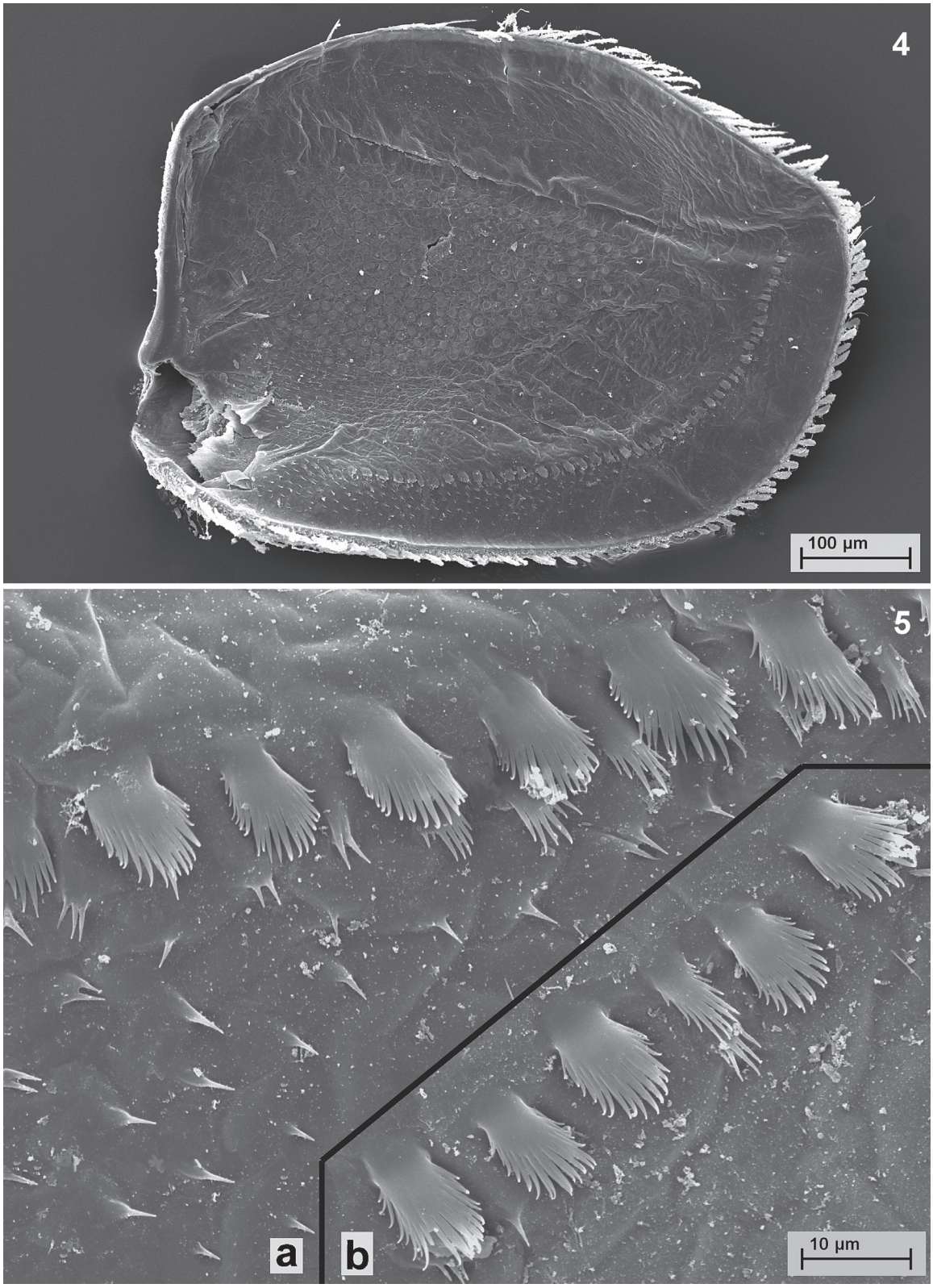
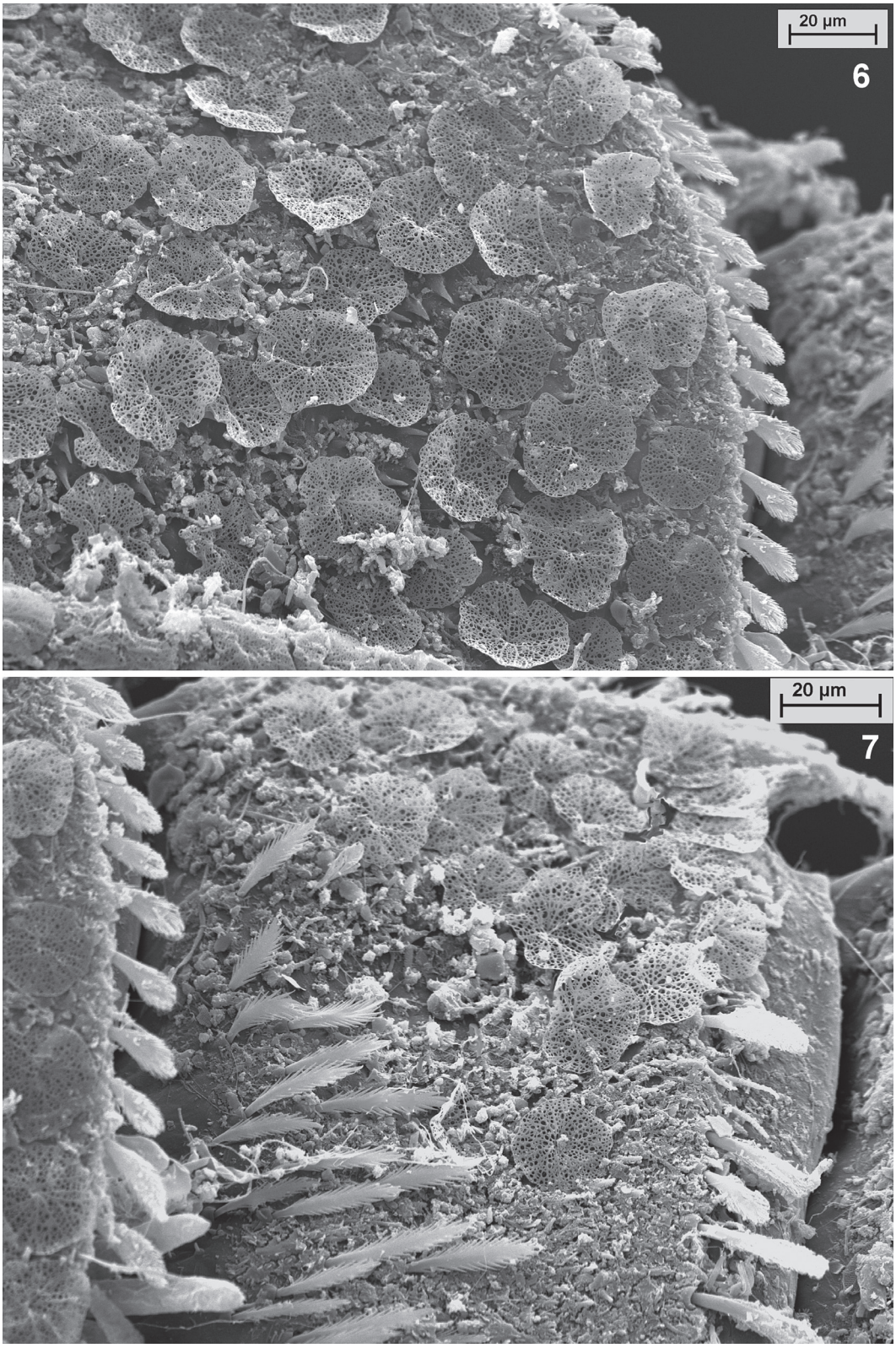


Fig. 3. *Mandelara immutata* n. sp., larva, head and pronotum (a), mouthparts (b).



Figs. 4–5. *Mandelara immutata* n. sp., larva, operculate gill. – **4**. Ventral view. **5**. Microtrichia from ventral side, sector from median part of the band (a) and near posterolateral corner (b).



Figs. 6–7. *Mandelara immutata* n. sp., larva. – **6**. Operculate gill, microtrichia on dorsal side. **7**. Sector from tergum VII.

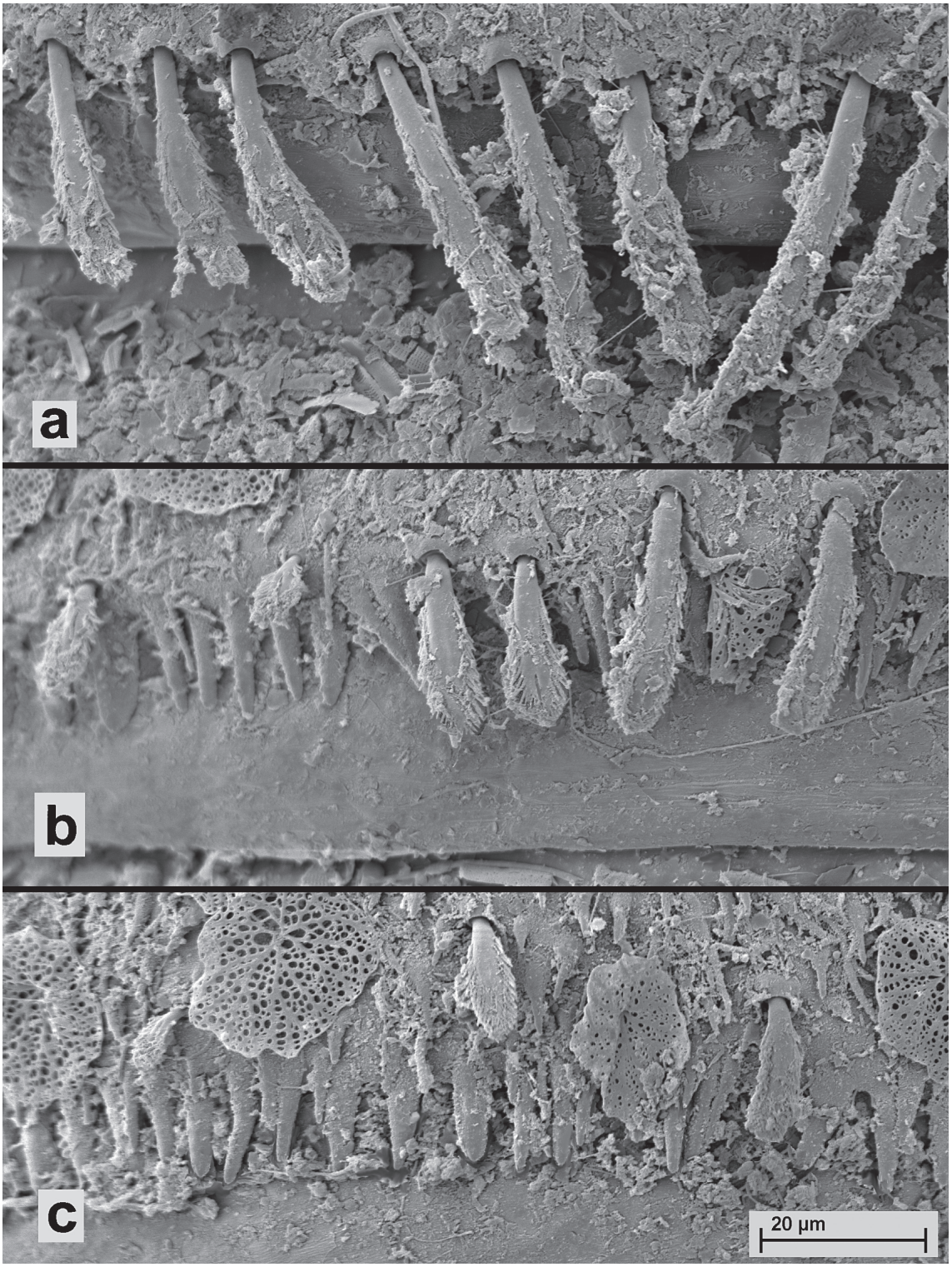


Fig. 8. *Mandelara immutata* n. sp., larva, sector from hind margin of tergum VII (a), VIII (b) and IX (c).

Egg. Epithemata very large, with single threads with tassel-shaped terminal knobs running along the meridians (Figs. 22, 23).

On the differences to other genera of the Clypeocaenini see the larval key below (section 4).

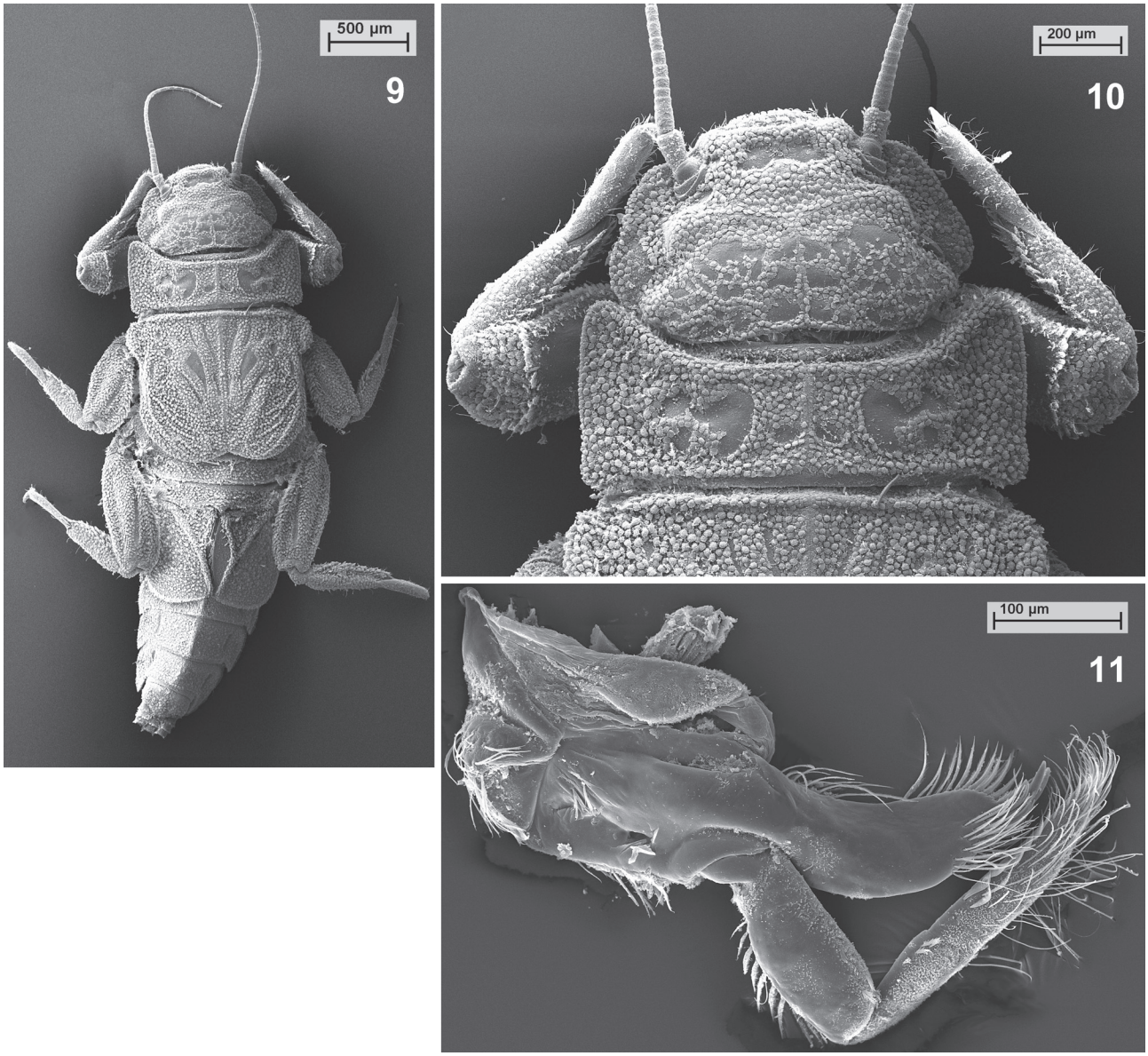
Papuaenis balkei **n. sp.**
(Figs. 1f–n, 9–24)

Holotype, ♂ larva (microslide): PNG 102, Papua New Guinea, Marobe, Wagau, Herzog Mts., 146°48′068″ E, 06°51′067″ S, 19.XI.2006, Balke & Kinibel leg.

Paratypes: PNG 87, Papua New Guinea, Eastern Highlands, Marawaka, Ande, 145°49′807″ E, 07°01′697″ S, 09.XI.2006, 1 ♀ larva, Balke & Kinibel leg. – PNG 90, Papua New Guinea, Gulf, Marawaka, Mala, 145°44′467″ E, 07°05′664″ S, 11.XI.2006, 2 ♀♀ larvae, Balke & Kinibel leg. – PNG 119, Papua New Guinea, Madang, Keki, Adalbert Mts., 145°24′437″ E, 04°43′058″ S, 29.XI.2006, 1 ♀ larva, Binatang Boys.

Etymology

The species is dedicated to the collector and leader of the Papua New Guinea expedition 2006, Michael Balke (Zoologische Staatssammlung München).



Figs. 9–11. *Papuaenis balkei* n. sp., larva. – **9**. Habitus. **10**. Head and pronotum. **11**. Maxilla.

Larva

Measurements and colouration

Subadult male larva, body length 3.5 mm, length of cerci 1.5 mm; subadult female larva, body length 4.2 mm, length of cerci 2.2 mm.

Colouration of cuticle: Beige. Head, pro- and mesonotum, operculate gills, and femora with a brownish shading, caused by the brown microtrichia.

Epidermal pigmentation: No pigments visible.

Morphology

Cuticle: Large parts of dorsal side densely covered with small, compact, shield-shaped microtrichia (Fig. 20). A conspicuous pattern of pale marks, blotches, and lines results from the lack of microtrichia on these places (Figs. 9, 10).

Head: Outline of head bulged in lateral view (Fig. 1m), clypeus slightly protruding anteriorly; genae strongly bulging out (Fig. 10). Mandibles with only one or two long bristles and a ventrobasal field densely covered with broad multi-branched bristles (as in Fig. 21a). Galealacinia apico-medially broadly rounded (Fig. 11). Segment 3 of maxillary palp ventrally with numerous very long, thin and medially shortly combed bristles; peeking out beneath them some broad comb-shaped bristles with very long and thin tips (Fig. 12). Segment 3 of labial palp about 0.8 times as long as segment 2 (Fig. 13). Postmentum with similar bristles as on maxilla (Fig. 21a).

Thorax: Sides of pronotum straight and parallel, sometimes fore corner laterally a little protruding and side slightly S-shaped; sides of mesonotum with broad rounded antero-lateral projections (Figs. 1n, 9). Femora broad and flattened (Fig. 14). Fore femur with a very dense transverse row of 15–18 bristles which are broadly spatulate, bifurcated and pinnate (Figs. 14a, 15). Coxal processes flatly semi-elliptical and slightly bulging out (Fig. 1i, j). Mid and hind legs marginally with similar bristles like that from the transverse row on fore femur (Fig. 14b). Tibiae with comb-shaped, unipinnate bristles (Fig. 16b), apically additionally with strongly bipinnate ones (Fig. 16a). There are a lot of different shapes from short and very broad bristles to those of moderate length and also transitional stages to tuft-shaped forms (fore tibia) (Fig. 21). Fore tarsus with an inner row of about 8 relatively long simple bristles. Mid tarsus with inner row of about 7 strong simple bristles. Hind tarsus with inner row of about 10 strong simple or slightly unipinnate bristles and an outer row of about 5 small and unipinnate bristles. Claws short and basally broad, with sides clearly converging anteriorly; apically bent, without denticles (Fig. 1k, l).

Abdomen: Posterolateral processes of moderate length (Fig. 1f–h). Posteromedian process of tergum II broadly triangular with rounded tip. Terga VII–IX with medio-longitudinal ridge (Fig. 9). Hind margin of tergum VII with short spatulate and pinnate bristles, VIII–X with long denticles apically more or less rounded. Tergum VII covered with spatulate bifid bristles that are slightly pinnate. Posterior part of sternum IX shortened and cut, sometimes medially slightly indented (Fig. 1f), dorsal shagreen not discernible. Gill I very short, about 0.2 the length of operculate gill. Lateral and hind margins of operculate gill with broadly spatulate and pinnate bristles (Fig. 18), inner margin with thin, pointed and finely frayed ones. Y-shaped ridge keeled, the inner branch a little stronger, with 3–4 short and strong bristles at the base. Row of microtrichia on ventral side of operculate gill running in regular distance from the lateral and hind margins, not reaching the latter (Fig. 17). The row consists of short and rounded simple microtrichia (Fig. 19). Gills III(–V) with only 1 filament with 3 branches; all the other filaments simple or two-branched.

Eggs

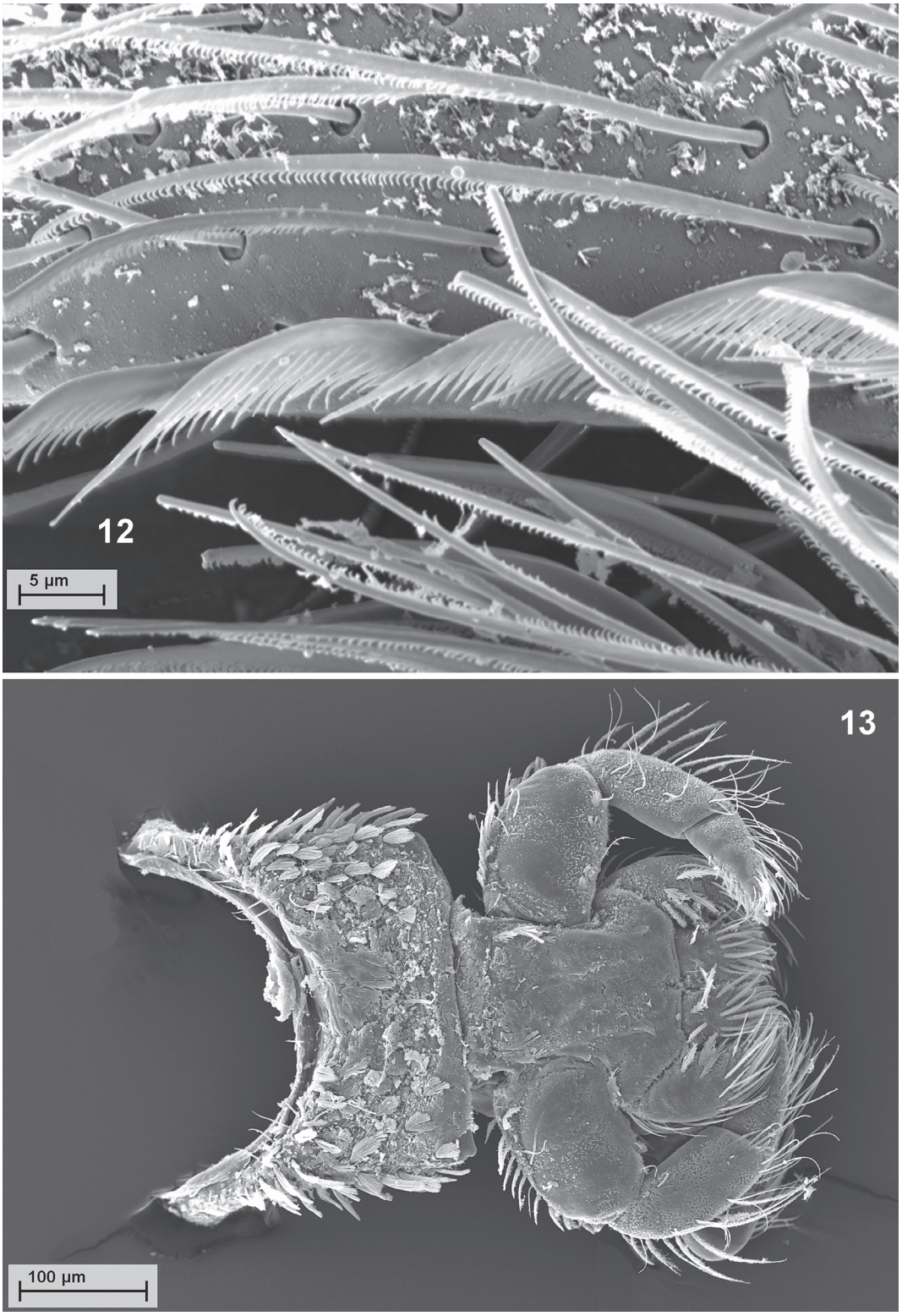
[The eggs were taken from a last instar female larva. In this developmental stage, parts of the egg surface, particularly the epithemata, are still covered by a thin protecting membrane.]

Eggs short oval or barbell-shaped. Chorion with irregularly distributed, wide pores (Fig. 24). Two large epithemata; except for a narrow equatorial area covering the whole egg surface (Figs. 22, 23), not corresponding to types of epithemata hitherto described for Caenidae. A large number of single threads, each with a terminal tassel-shaped knob, running from the polar region along the meridians towards the equator (single-thread-type). After dissolution of the protecting membrane threads floating in the surrounding water (Fig. 23b). One (or two?) micropyle(s) with a short or very short visible channel and a small, irregular sperm-guide (Fig. 24).

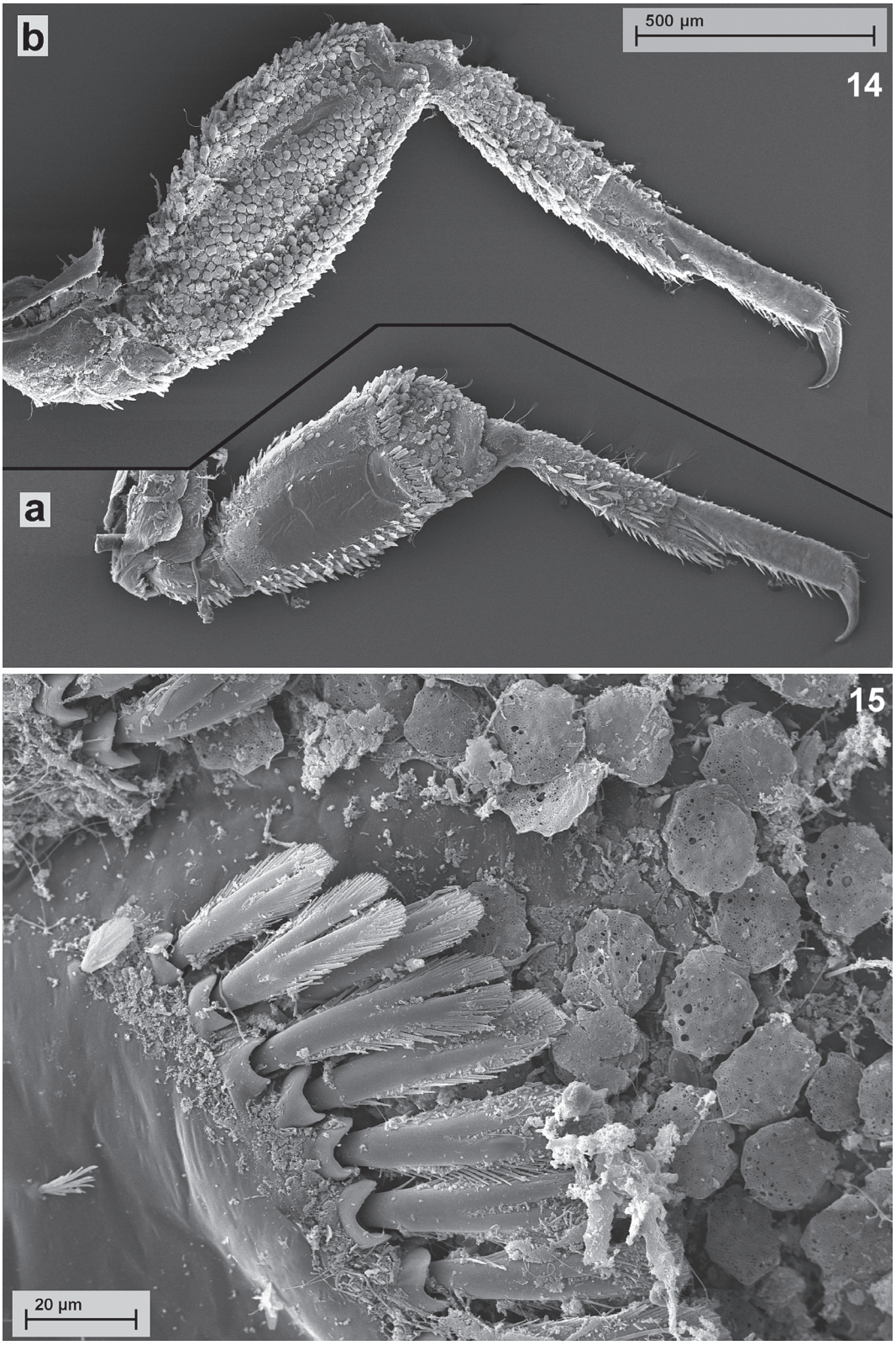
Imaginal stages unknown.

4 Larval key to the genera of Clypeocaenini

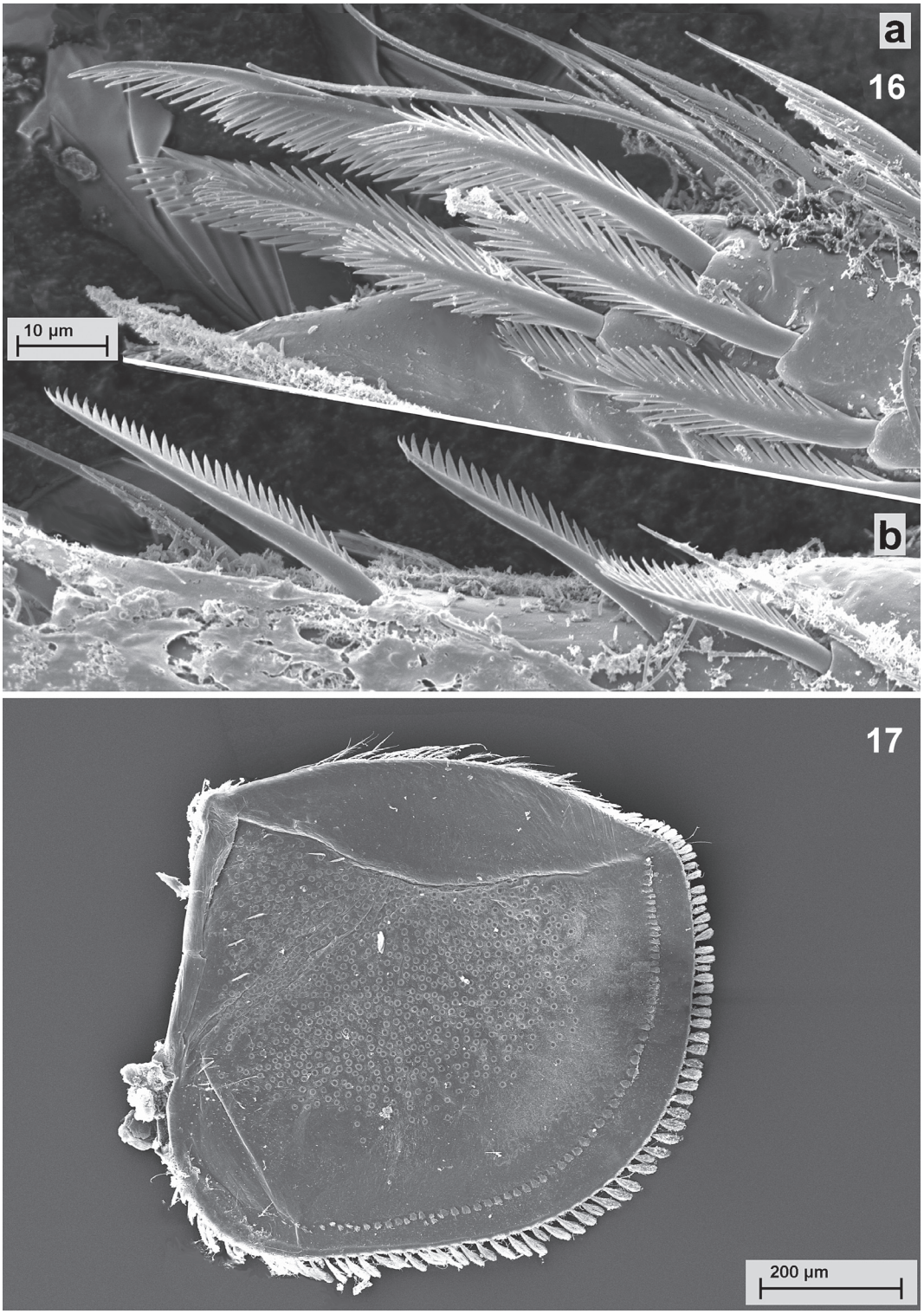
1 Fore tibia and fore tarsus with long filtering setae..... **2**
– Fore tibia and fore tarsus without filtering setae..... **5**
2 Filtering setae reaching to about half the length of tibia, densely arranged medially on fore tibia and fore tarsus. .... ***Amercaenis***
– Filtering setae nearly as long as tibia, not densely arranged and also present laterally on tibia and tarsus..... **3**
3 Maxillary palp three-segmented. Body cuticle with strong long spines. Mid and hind tibia ventrally with numerous long bristles (Malzacher 2014: fig. 1c). .....***Provonshara***
– Maxillary palp two-segmented. Body cuticle without strong long spines. Mid and hind tibia ventrally without long bristles. **4**



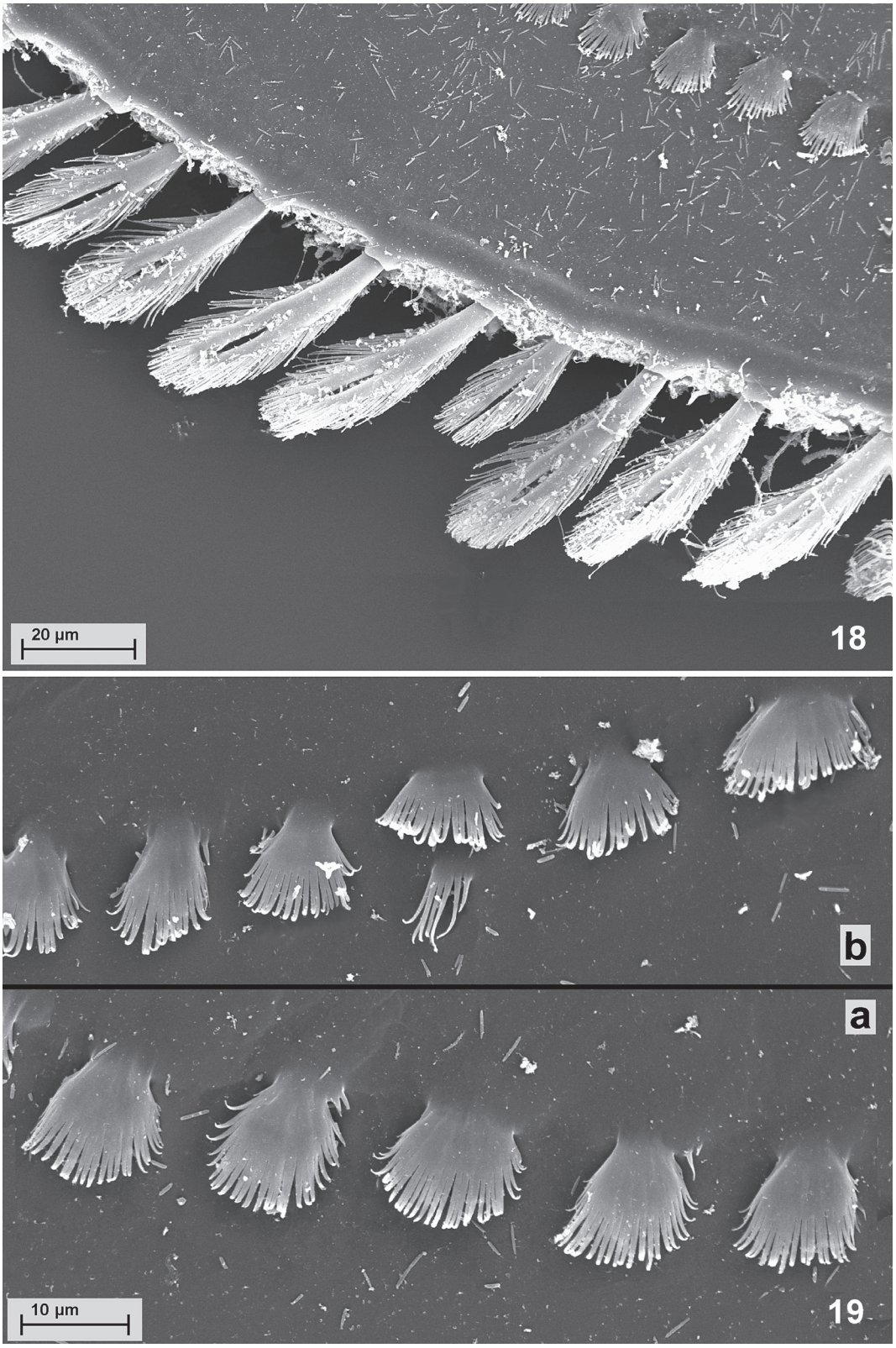
Figs. 12–13. *Papuaenis balkei* n. sp., larva. – **12**. Comb-shaped bristles from segment 3 of maxillary palp. **13**. Labium.



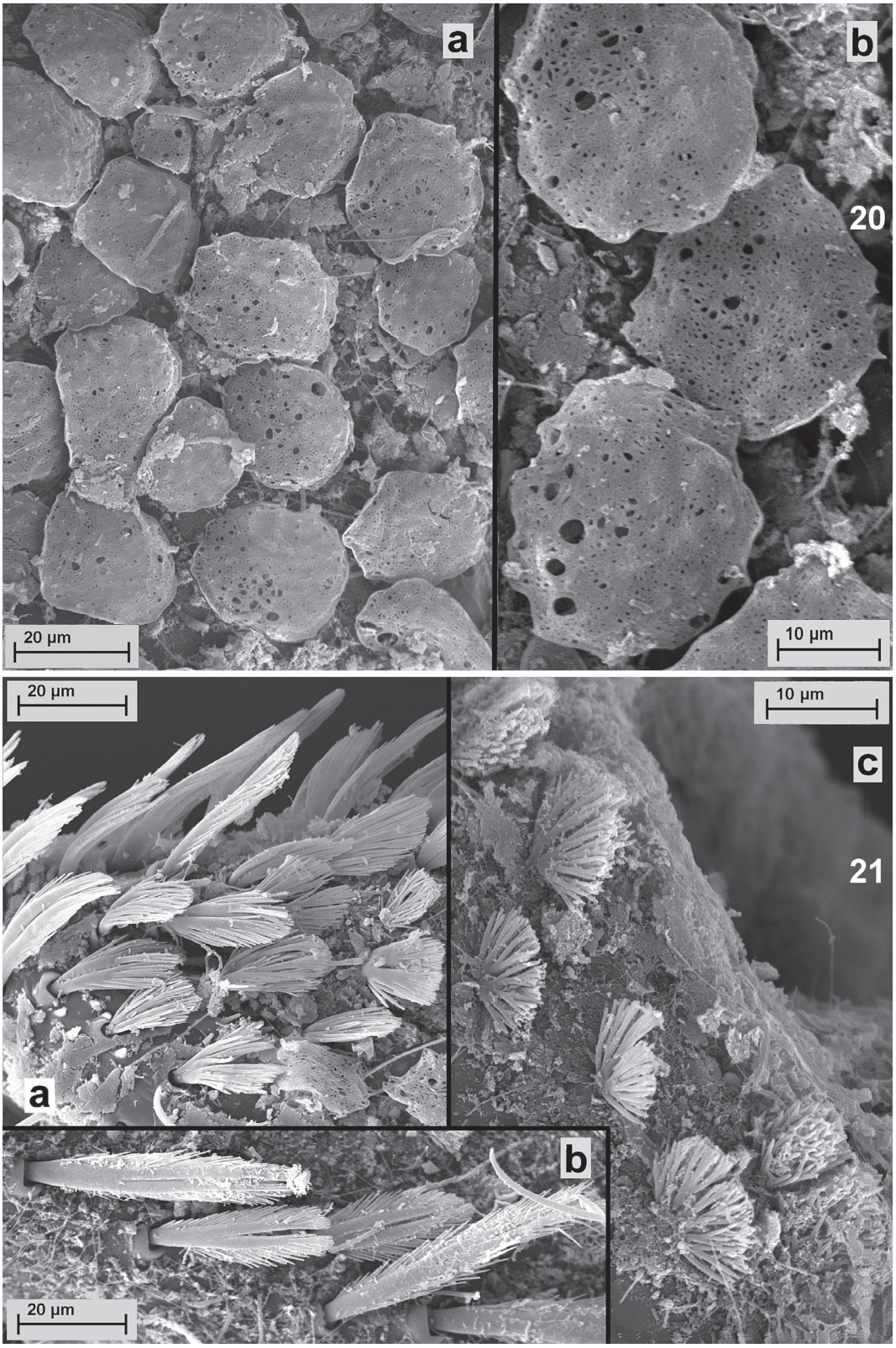
Figs. 14–15. *Papuaenis balkei* n. sp., larva. – **14**. Fore leg (a) and hind leg (b). **15**. Sector from transverse row of bristles on fore femur.



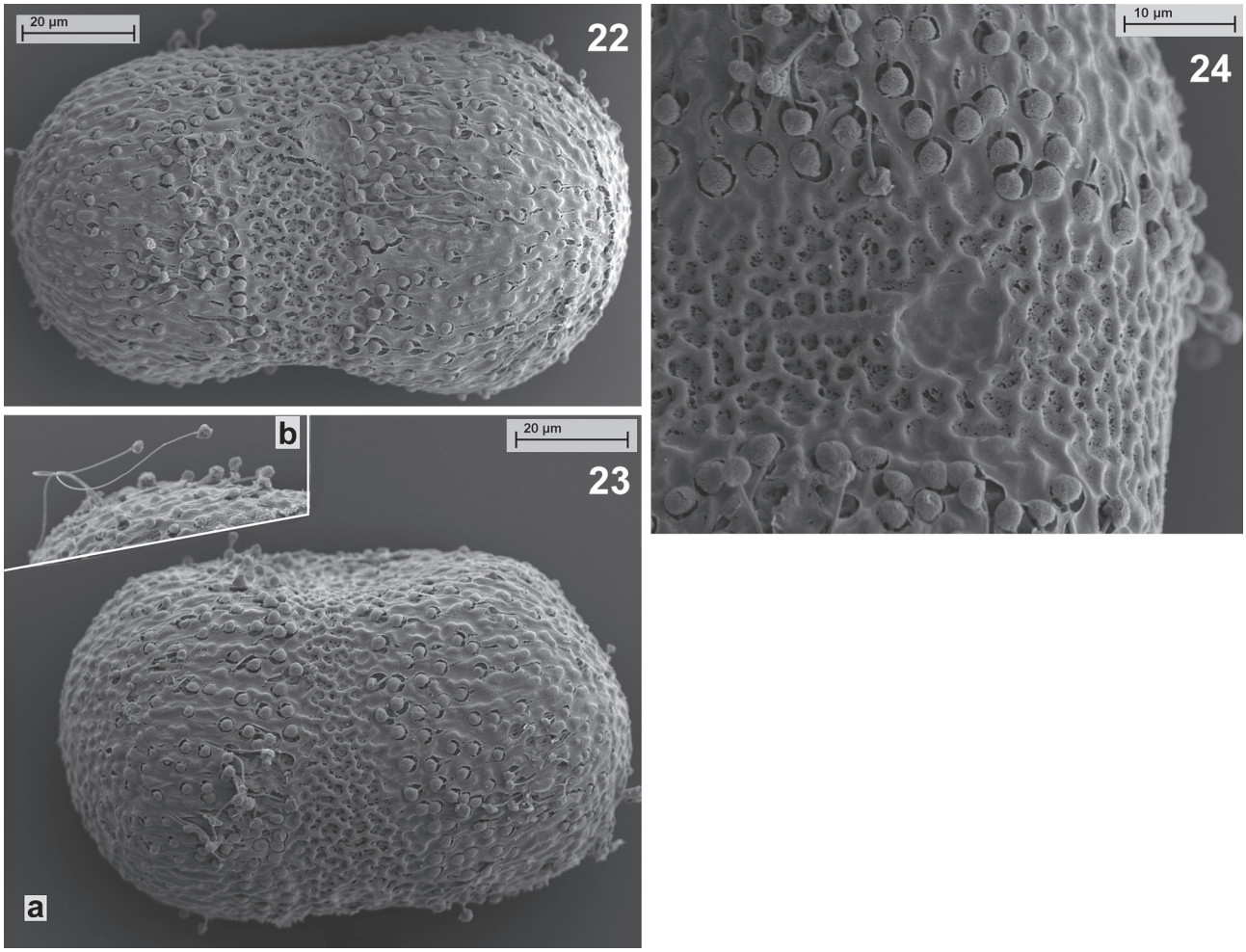
Figs. 16–17. *Papuaenis balkei* n. sp., larva. – **16**. Different bristles from fore tibia, apical protrusion (a), inner margin (b). **17**. Operculate gill, ventral view.



Figs. 18–19. *Papuaenis balkei* n. sp., larva, operculate gill. – **18**. Bristles from lateral margin. **19**. Microtrichia from ventral side, sector from median part (a) and from basal part (b) of the row.



Figs. 20–21. *Papuaenis balkei* n. sp., larva. – **20**. Microtrichia from dorsal cuticle (a), in higher magnification (b). **21**. Different bristles, from postmentum (a), fore tibia (b) and fore femur (c).



Figs. 22–24. *Papuaenis balkei* n. sp., eggs. – **22**. Barbell-shaped egg. **23**. More rounded egg (a), floating threads (b). **24**. Micropyle.

4 Filtering setae forming regular rows (Provonsha & McCafferty 1995: fig. 16). Clypeus anteriorly strongly protruding, with long setae..... ***Clypeocaenis***
– Filtering setae irregularly arranged (Provonsha & McCafferty 1995: fig. 5). Clypeus anteriorly without long setae. ..... ***Barnardara***
5 Legs long and slender, femora narrowed (Malzacher 2013: fig. 8b–d). Maxillary palp elongated and coiled (Malzacher 2013: fig. 12a). Mesonotum not broadened. ..... ***Kalimaenis***
– Legs not elongated, femora broad. Maxillary palp shorter and not coiled. Mesonotum broad, outline of body more or less evenly curved. **6**
6 Great parts of cuticle densely covered with shield-shaped microtrichia (Figs. 6, 20). **7**
– Cuticle without densely arranged shield-shaped microtrichia. **8**
7 Sternum IX posteriorly shortened, hind margin straight (Fig. 1f). Claws short, without denticles (Fig. 1k–l)..... .....***Papuaenis***
– Sternum IX not shortened, hind margin bowed, with median incision (Fig. 1a). Claws longer, with about 12 denticles (Fig. 1d). .....***Mandelara***
8 Body surface provided with many very long hair-like bristles (Malzacher 2009a: fig. 4). Thoracic notae smooth..... .....***Trichocaenis***
– Body surface without long bristles. Thoracic notae with ridges or bulges (Sun & McCafferty 2001: fig. 1). ....***Callistellina***

5 Cladistic analysis

For data matrix see Tab. 1. Characters used for cladistic analysis are as follows:

1 (I) Mesonotal ommation: **(0)** absent; **(1)** present.
2 (I, L) Hind wing (buds): **(0)** present; **(1)** absent.
3 (I) Segmentation of forceps: **(0)** 4-segmented; **(1)** 1-segmented.
4 (I, L) Styliger (anlagen): **(0)** fully exposed; **(1)** partly retracted into abdominal segment IX.
5 (I) Procoxae: **(0)** broadly separated; **(1)** approximated.

Tab. 1. Data matrix for 14 species of Caenidae and *Neoephemera youngi* (Neoephemeridae).

	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Neoephemera youngi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Madecocercus tauroides	1	1	1	1	0	0	1	1	1	2	3	3	2	1	0	0	0	2	0	0	1	1	0	0
Brachycercus harrisella	1	1	1	1	0	0	1	1	1	2	1	2	1	1	0	0	0	1	1	2	1	1	0	0
Caenoculis bishopi	1	1	1	1	0	0	1	1	1	4	2	1	0	1	0	0	0	0	0	1	1	1	0	0
Trichocaenis inexperta	?	1	?	?	?	?	?	1	1	1	3	0	0	1	0	1	1	0	0	0	1	1	1	0
Mandelara immutata	?	1	?	?	?	?	?	1	1	2	3	0	0	1	0	1	1	0	0	0	1	2	1	1
Papuaenis balkei	?	1	?	?	?	?	?	1	1	3	3	0	0	1	0	1	1	0	0	0	1	2	1	1
Callistellina panda	?	1	1	1	?	?	?	1	1	2	3	0	0	0	0	1	1	0	0	0	1	2	1	0
Provonshara spinifera	?	1	?	?	?	?	?	1	1	2	3	0	0	1	2	1	1	0	0	0	1	2	1	0
Barnardara demoori	?	1	?	?	?	?	?	1	1	2	3	0	0	0	2	1	1	1	0	0	1	2	1	0
Clypeocaenis oligosetosa	1	1	1	1	1	1	0	1	1	2	3	0	0	0	3	1	2	1	0	0	1	3	1	0
Amercaenis ridens	1	1	1	1	1	1	0	1	1	3	2	0	0	0	1	1	1	0	0	0	1	2	1	0
Kalimaenis sibylliana	1	1	1	1	1	1	0	1	1	3	2	0	1	0	0	1	1	0	0	0	1	2	0	0
Caenis macrura	1	1	1	1	1	1	0	1	1	3	1	0	0	0	0	0	0	0	0	0	2	1	0	0
Tasmanocoenis tillyardi	1	1	1	1	1	1	1	1	1	3	1	0	0	0	0	0	0	0	0	0	2	1	0	0

6 (I) Metathorax: **(0)** without transversal ridge; **(1)** with transversal ridge.
7 (I) Shape of forceps: **(0)** without ridges or keels; **(1)** with longitudinal ridge; **(2)** with single ventral longitudinal groove; **(3)** with dorsal and ventral longitudinal groove.
8 (L) Gill cover medially: **(0)** not overlapping; **(1)** overlapping.
9 (L) Gill cover: **(0)** bilobed; **(1)** without ventral lobe.
10 (L) Ventral side of gill cover: **(0)** without microtrichia; **(1)** with field of irregularly arranged microtrichia; **(2)** with band of irregularly arranged microtrichia; **(3)** with single row of regularly arranged microtrichia; **(4)** with transverse rows of regularly arranged microtrichia.
11 (L) Gill III with multiple branchings: **(0)** in nearly all filaments; **(1)** in 15–25 filaments; **(2)** in about 8 filaments; **(3)** in 1–3 filaments.
12 (L) Abdominal posterolateral projections in abdominal segments: **(0)** (2)3–9; **(1)** 4–7; **(2)** 3–7; **(3)** 2, 5–9.
13 (L) Femora: **(0)** broad and stout; **(1)** thin and elongated; **(2)** extremely broadened and stout.
14 (L) Profemora dorsally: **(0)** with transverse row of setae; **(1)** without transverse row of setae.
15 (L) Protibiae: **(0)** without filter setae; **(1)** with irregularly arranged short filter setae; **(2)** with irregularly arranged long filter setae; **(3)** with regularly arranged rows of filter setae.
16 (L) Outline of head: **(0)** evenly bowed; **(1)** bulged.
17 (L) Clypeus anteriorly: **(0)** not protruding; **(1)** slightly protruding; **(2)** heavily protruding.
18 (L) Maxillary palps: **(0)** 3-segmented; **(1)** 2-segmented; **(2)** 1-segmented.
19 (L) Labial palps: **(0)** 3-segmented; **(1)** 2-segmented.
20 (L) Ocelli: **(0)** without tubercles; **(1)** with small tubercles; **(2)** with large tubercles.
21 (L) Operculate gill, ventral row of microtrichia: **(0)** lacking; **(1)** not reaching hind margin; **(2)** reaching hind margin.
22 (L) Operculate gill, medial ridge on dorsal side: **(0)** absent; **(1)** not keeled; **(2)** keeled; **(3)** extremely keeled.
23 (L) Mesonotum: **(0)** not broadened, laterally with distinct angle to abdomen; **(1)** broadened, laterally confluent with abdomen.
24 (L) Shield-shaped microtrichia on cuticle: **(0)** absent; **(1)** present.

6 Phylogenetic discussion

The cladistic analysis confirmed the monophyly of Caenidae, which was unchallenged before. Within Caenidae, the Brachycercinae + Madecocercinae were recovered as sistergroup to the monophyletic Caeninae. Within Caeninae, the Caenini + Tasmanocoenini and Clypeocaenini were recovered as well-supported sistergroups.

Within Clypeocaenini, *Kalimaenis sibylliana* branches off first, followed by *Amercaenis ridens*. The remaining genera within Clypeocaenini form a weakly supported monophyletic group. Within this group, however there are two well supported monophyla, namely *Papuaenis balkei* + *Mandelara immutata* and *Provonshara spinifera* + (*Clypeocaenis oligosetosa* + *Barnardara demoori*). The positions of *Callistellina panda* and *Trichocaenis inexperta* in relation to these other two groups remain unresolved (see Fig. 25). For apomorphies determined in the cladistic analysis see Fig. 26.

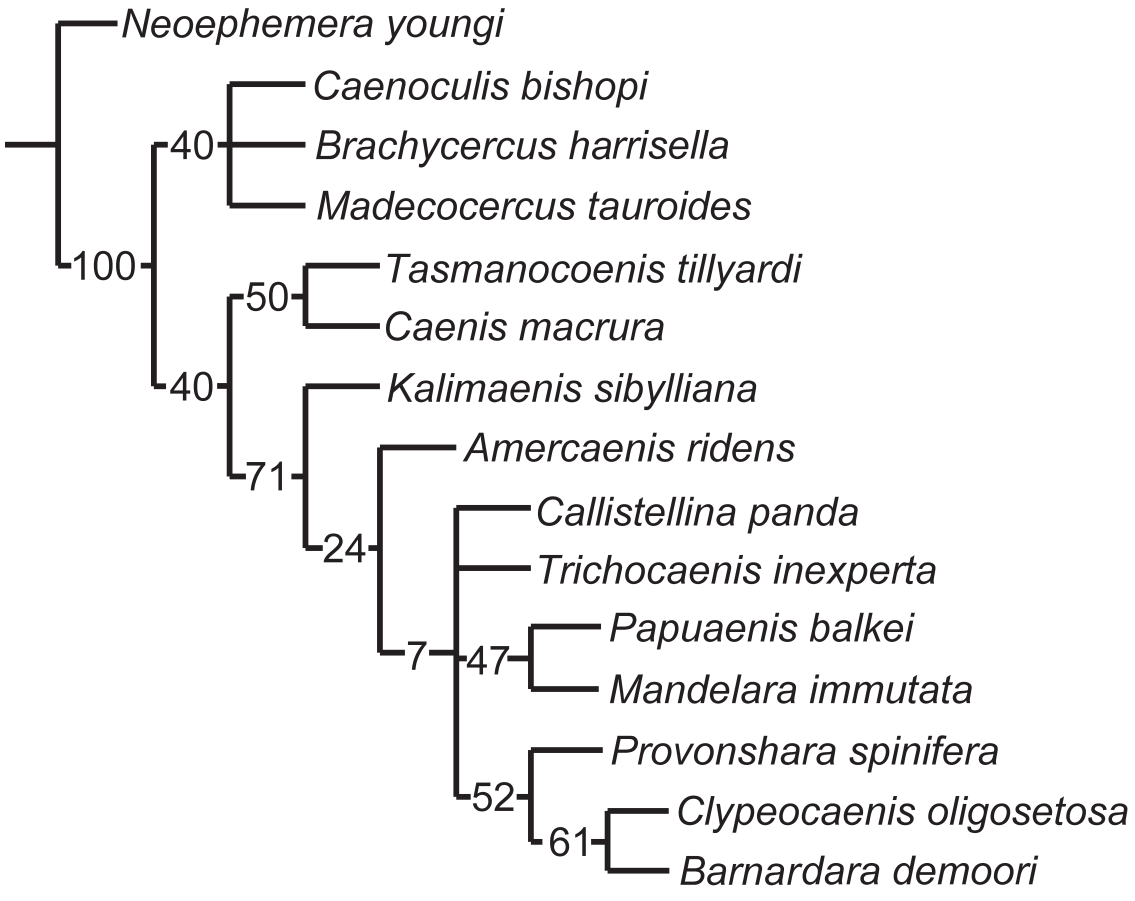


Fig. 25. Cladistic analysis of Clypeocaenini, Strict Consensus, Jacknifing (P = 36). – GC Values, 10000 replicates, cut = 1 (tree 0).

The cladistic analysis confirms the mental analysis due to Phylogenetic Systematics sensu Hennig (1966) that results in the very same phylogeny (based on larval characters).

Due to mental analysis (Fig. 27), the genera of the tribe Clypeocaenini share two synapomorphies:

(1) Gill III (first of the four respiratory gills) possesses at most eight filaments (in most cases only one or two), each made up of three or more branches, in contrary to 15–25 filaments in the Caenini and Tasmanocoenini (character 11). In the outgroup Neoephemeridae there are numerous filaments present, each with 5 and more branches, so that a reduction of filament branches can be regarded as apomomorphic for Clypeocaenini.

(2) Outline of head in lateral view with bulges, clypeus more or less protruding (character 14, see also Malzacher 2013: fig. 13c–h). In the above mentioned outgroups and in Ephemerellidae the outline of the head in lateral view is evenly bowed (Malzacher 2013: fig. 13a, b).

The sister group of Clypeocaenini, represented by the tribes Caenini + Tasmanocoenini shares two synapomorphies:

(3) Row of microtrichia on ventral side of gill II (operculate gill) extends to hind margin of gill (character 21; for Caenini see Malzacher 2009b: figs. 17, 20 and 23; for Tasmanocoenini see Suter 1984: fig. 6, Alba-Tercedor & Suter 1990: fig. 19, Suter 1993: figs. 16, 41 and 68, and Suter 1999: fig. 95). In Clypeocaenini, the row of microtrichia does not extend to hind margin of operculate gill. The distance from the end of the row to hind margin varies from $^1/_8$ to $^1/_{15}$ the length of gill II (Figs. 4, 17).

(4) Gill II with regular row of complex, very similar, scale-shaped microtrichia, which are semicircular or more or less elongated, consisting of 20–30, rather basally fused filaments or spines (character 10). In *Kalimaenis*, a similar arrangement is present, but this is regarded as a convergent development. The plesiomorphic character states in Clypeocaenini show very different developmental stages from a band of simple spines and clusters of spines up to scales with 5–20 filaments arranged in an irregular row

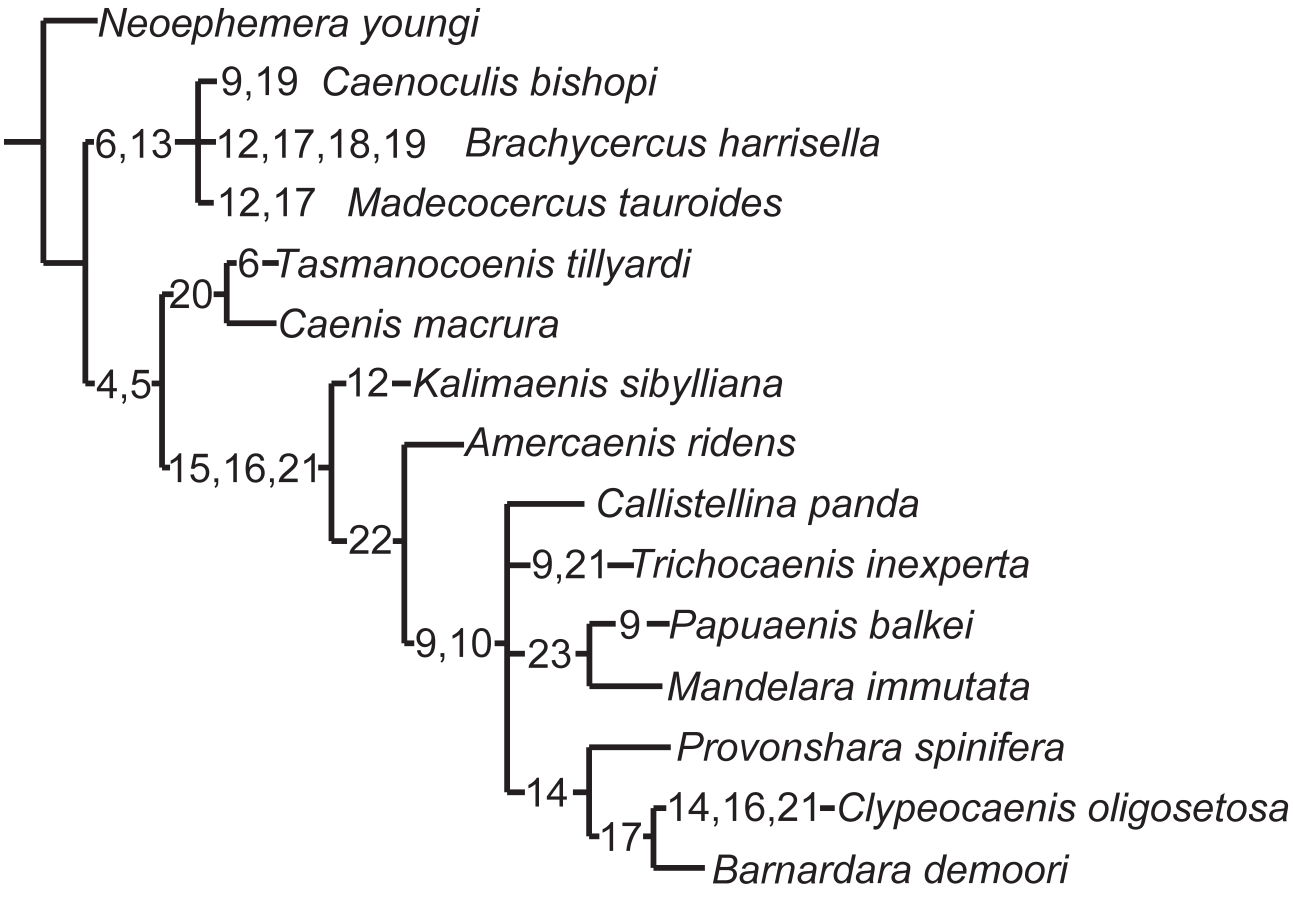


Fig. 26. Apomorphies of cladistic analysis. – Synapomorphies common to three trees.

(Malzacher 2009b: figs. 3–5, 8, 10, 12). The most plesiomorphic state, simple spines and clusters of spines, can also be found in Brachycercinae and Madecocercinae. In the outgroup Neoephemeridae there are no ventral microtrichia present at all.

Within Clypeocaenini the genus *Kalimaenis* branches off from the remaining genera that share a single apomorphic character (character 23):

(6) Mesonotum broad. Pronotum broadly attached to the mesonotum (Figs. 2, 9). Outline of body more or less evenly curved. (e. g. Malzacher 2013: fig. 47, Malzacher 2009a: fig. 4; Sun & McCafferty 2001: fig. 1). In *Kalimaenis*, the pro- and mesonotum is much narrower. In Malzacher 2013 and 2014 this was established erroneously also for *Amercaenis*, but it turns out that the mesonotum of the latter is clearly broader.

Kalimaenis shows three autapomorphies:

(8) Legs long and slender (character 13), particularly in the females, with narrow femora more or less parallel-sided (Malzacher 2013: fig. 8b–d). This shape shows a certain similarity to legs of Brachycercinae. The fore legs of *Kalimaenis* are reduced in length just like in the latter subfamily.

Two further characters were not included in the cladistic analysis, but are clearly autapomorphies of *Kalimaenis*:

(9) Maxillary palps strongly elongated and narrowed with a conical segment 1 and S-shaped coiled segments 2 and 3 (Malzacher 2013: fig. 12a). As far as we know, this shape is unique in the Caenidae or even in the Pannota, where a tendency of reduction can be observed in all families. Only in *Tricorythus* a long and slender maxillary palp can be observed, but it shows only two segments (McCafferty & Wang 2000: fig. 85).

(10) Hind claws with about seven groups of micro-denticles. The micro-denticles in each group are fused together to larger denticles, respectively (Malzacher 2013: fig. 8e, f). In the Caeninae there can be homodont or heterodont hind claws present. The former are equipped with a number of denticles equal in size, whereas the latter have an additional row of more or less fused microdenticles (e. g.

Malzacher 2009a: figs. 1p, 2h, 3g), a state that is apomorphic in some Caenini but cannot be found in the Clypeocaenini. On the other hand there is at least one *Caenis* species that shows a long row of fused microdenticles only (Malzacher, in preparation). Nevertheless groups of fused micro-denticles are unique in *Kalimaenis*.

In the next step *Amercaenis* branches off from the *Clypeocaenis* group s. str. that shares the following apomorphy:

(5) In gill III nearly all filaments (up to 45) have one or two branches (character 11). Only one or two apical filaments show three or more branches. These are situated at the apex of the gill (Malzacher 2014: fig. 1k). In *Kalimaenis* and *Amercaenis* there are 7–8 filaments with up to 5 branches and only about 20 reduced ones.

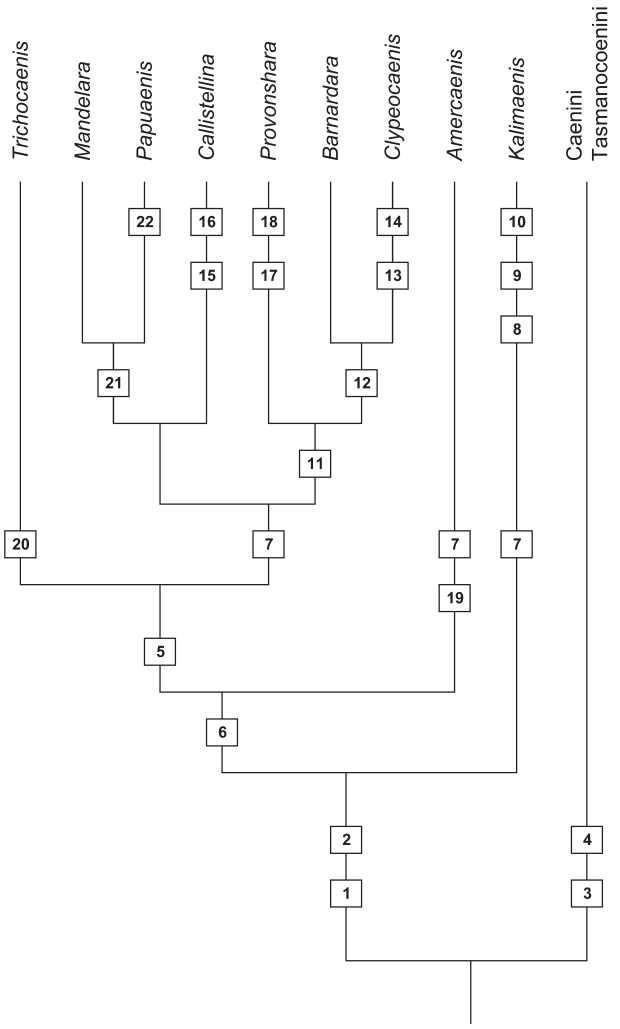


Fig. 27. Phylogenetic tree concluded by mental analysis with indicated apomorphies (see section 6 for detailed discussion on characters).

Amercaenis shows the apomorphic character:

(19) Densely arranged short filtering setae medially on tibia and tarsus of fore leg. This arrangement can only be found in Nearctic species and represents a parallel development to the African and Oriental genera with filtering setae.

The remaining genera of Clypeocaenini split into four groups: *Trichocaenis*, *Callistellina*, *Mandelara* + *Papuaenis*, and *Provonshara* + (*Barnardara* + *Clypeocaenis*). The phylogenetic relationship of these four groups to each other remains unresolved.

All Clypeocaenini except for *Trichocaenis* show the character:

(7) Gill II with a keeled inner ridge (character 22; see also Malzacher 2013: figs. 9d, 43, and Provonsha & McCafferty 1995: fig. 7). A keeled ridge is also present in some taxa of Caenini. It may be possible that this character has evolved independently in these groups, or it is even possible that it is present in their common groundplan, but reduced in *Trichocaenis* and most Caenini.

Trichocaenis shows the following apomorphic character:

(20) Body surface provided with a lot of very long hair-like bristles. This character is unique within the Clypeocaenini and otherwise can only be found in a few Caenini. Thus we conclude that this character has evolved independently in *Trichocaenis* and a few Caenini.

Callistellina is well-defined by the following two apomorphies:

(15) Head with microscopic pits each with a hand-shaped microtrichium (Sun & McCafferty 2001: figs. 9 and 10; Malzacher 2009a: fig. 10).

(16) Thoracic notae with ridges or bulges (Sun & McCafferty 2001: fig. 1). A single medio-longitudinal ridge is also visible in *Kalimaenis* (Malzacher 2013: fig. 32).

Mandelara and *Papuaenis* share the following synapomorphic character:

(21) Great parts of cuticle densely covered with shield-shaped microtrichia often overlapping themselves with their margins (Figs. 6, 20). Shield or funnel-shaped microtrichia seem to have developed independently in several *Caenoculis* (Malzacher, in preparation) and *Caenis* species.

Papuaenis shows one apomorphic character:

(22) Sternite IX posteriorly shortened, hind margin straight, not projecting beyond the posterolateral processes anteriorly. Such a shortening cannot be observed in other Caenidae.

At present, *Mandelara* cannot be defined by derived characters.

The *Clypeocaenis* group with the genera *Clypeocaenis*, *Barnardara* and *Provonshara* shares the following synapomorphy:

(11) Filtering setae on tibia and tarsus of fore leg. This is a parallel development to similar structures in *Amercaenis* (see character 9), but filtering setae are not arranged as densely as in *Amercaenis* and can also be found on the lateral part of tibia and tarsus, reaching more or less the length of tibia (Soldán 1978: fig. 8, Provonsha & McCafferty 1995: figs. 5, 16). In *Amercaenis*, on the other hand, filtering setae reach only a little more than half the length of tibia (Provonsha & McCafferty 1985: fig. 6, and 2006: fig. 5).

Provonshara is the sister to *Clypeocaenis* + *Barnardara*. The latter are united by a single apomorphy:

(12) Maxillary palp two-segmented. This character can also be observed as parallel development in the Brachycercinae and some families of the Pannota (see McCafferty & Wang 2000 and Kluge 2004).

Barnardara is not defined by unique apomorphic characters, while *Clypeocaenis* shows two apomorphies:

(13) Filtering setae arranged in rows. Few setae also often on inner margin of femur and in a basket-like arrangement on mandible (Soldán 1978: fig. 8, Provonsha & McCafferty 1995: figs. 13, 16).

(14) Clypeus strongly protruding and provided with long setae.

Provonshara has two unique apomorphies:

(17) Body cuticle with strong long spines (Malzacher 2014: fig. 2c). In all other Caeninae the cuticle is smooth, granulated, or shows short microdenticles (in *Kalimaenis* strongly granulated or scaly).

(18) Mid and hind tibia with numerous long bristles on ventral side (Malzacher 2014: fig. 1c). In other genera those bristles are not present. If at all, long bristles occur only on dorsal side or on the margins of tibia.

It is noteworthy that both cladistic and mental analyses end up with identical phylogenetic hypotheses. In summary, it can be concluded that Clypeocaenini form a well-defined monophyletic group within Caeninae.

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