

Comparative morphology of gill cover microtrichia in the Caenidae (Insecta: Ephemeroptera)

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Based on SEM pictures the different evolutionary stages of shape and arrangement of microtrichia on the ventral side of the operculate gills (gill cover) in Caenidae are shown. There are a number of tendencies that can be observed in the evolution of these structures. The presence of different evolutionary stages of these structures in the Caeninae, Madecocercinae, and the newly postulated *Clypeocaenis* group is discussed and parallel developments in these taxa are assumed.

Keywords: mayfly; Caenidae; operculate gills; microtrichia arrangement

Introduction

Operculate gills on the abdominal segment II are present in several different higher taxa of Ephemeroptera (McCafferty and Wang 2000, see also Kluge 2004, Table 8). In the Caenoidea the operculate gills are medially touching, as in Neophemeridae, or even overlapping as in Caenidae. Only in Caenidae they are provided with a row or band of microtrichia on the ventral side. These microtrichia therefore can be regarded a unique synapomorphy for the Caenidae (Kluge 2004). Malzacher and Staniczek (2006) showed that different evolutionary stages of these structures occur within each subfamily and interpreted this as a parallel development. In the present paper this conclusion is reinforced by showing the evolutionary tendencies in the different lineages.

Materials and methods

Objects used for SEM were dehydrated through stepwise immersion in ethanol and then dried by critical point drying. The mounted material was coated with a 20 nm Au/Pd layer and examined with an ISI-SS40 scanning electron microscope at 10 kV. Digital photographs were directly acquired by using DISS 5 (point electronic) and subsequently enhanced by using Adobe Photoshop 7.

In those figures showing the entire gill opercula (Figures 6, 9, 11, 14, 17, 20, and 23), the apical end of the respective microtrichia band is always marked by a small arrow.

Material used for this study was taken from the author's collection (*Caenis pusilla*, *C. rivulorum*, *C. beskidensis*, *C. luctuosa*, *C. lactea*, *C. horaria*, *C. robusta*).

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Additional material was made available by following colleagues and institutions: Michel Sartori, Musée de Zoologie, Lausanne (*Afrocerus guinensis*, *Trichocaenis inexperta*, *Callistellina* spec., *Madecocercus tauroides*, *Barnardara demoori*, *Clypeocaenis afrosetosa*, *Caenis* spec. from Madagascar, undescribed genus and species from Guinea), Jan Peters, Florida A&M University, Tallahassee (undescribed genus and species from Thailand), Roland Grimm, Tübingen (*Caenis hoggarensis*), Thomáš Soldán, Czech Academy of Sciences, České Budějovice (*Clypeocaenis oligosetosa*), Arwin Provonsha, Purdue University, West Lafayette (*Amercaenis ridens*), and the Natural History Museum, London, coll. Gillies (*Caenopsella meridies*).

Results

Shape and arrangement of microtrichia

In the Caenidae, microtrichia are in general evenly distributed across the ventral surface of the operculate gills (Figure 1). Additionally, banded clusters of microtrichia in different shapes can be observed in proximity to the lateral and often to the hind margins.

In *Afrocerus*, a genus of the Madecocercinae, a very simple stage of microtrichia arrangement can be seen (Figure 2): there is a broad sublateral field of small spines present. The spines are evenly distributed in the lateral part, but form clusters in the median direction. At its medial end even early stages of scale-like microtrichia can be observed. A transition from the above mentioned very small spines can also be seen on the central surface. The whole structure can be interpreted as a result of two opposite evolutionary tendencies (see Table 1): (1) an enlargement of spines, becoming effective in centrifugal direction, and (2) the differentiation in centripetal direction.

In *Trichocaenis inexperta* (Malzacher 2009) there is a very similar field of microtrichia present, just a little narrower (see Table 1 (3)) and closer to the lateral margin. These are the same elements as in *Afrocerus*: single spines laterally, clusters and simple scales medially (Figure 3).

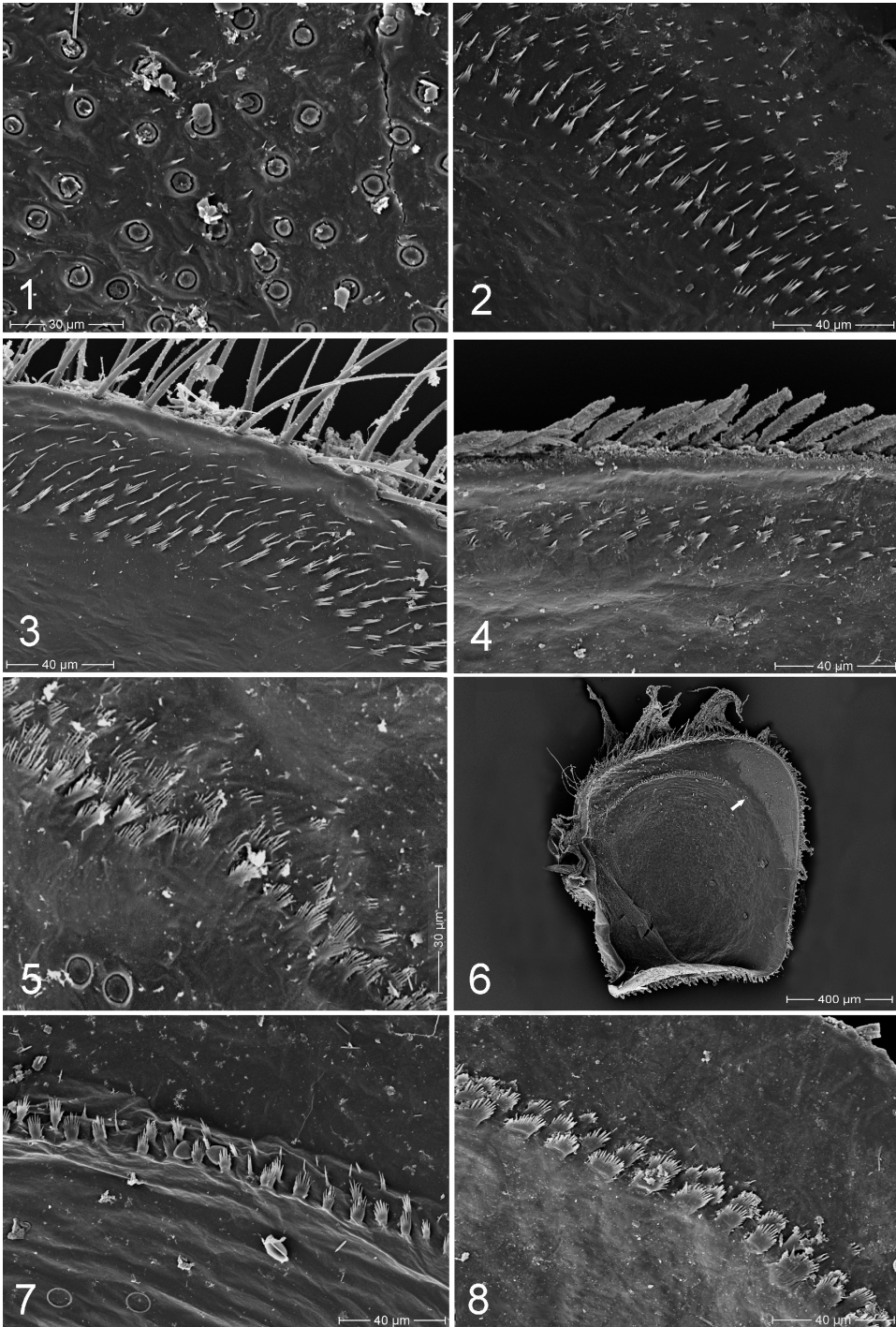
The same arrangement is seen in *Clypeocaenis oligosetosa*, the number of microtrichia however, particularly that of the single spines, is reduced. The band is relatively narrow and, like in *Trichocaenis*, close to the lateral margin (Figure 4).

In *Callistellina*, scale-like microtrichia are more frequent and more developed. The dense and highly irregular band shows different transitional stages, among them comb-like clusters of up to 10 spines, but these are not fused together basally (Figure 5).

The shortened microtrichial band in *Madecocercus tauroides* (Figure 6) shows a number of spines and few clusters laterally, and one to two irregular rows of scale-shaped microtrichia medially. The latter are relatively complex, basally narrowed and more or less elongated, and consist of up to 10 fused spines (Figure 7).





The arrangement in *Barnardara demoori* is similar to that in *Madecocercus*, but the microtrichia band is denser with only a few lateral spines and transitional stages. Scales are clearly broader than in *Madecocercus*, consisting of up to 15 spines (Figure 8).

In *Clypeocaenis afrosetosa* there is only one relatively short row of about 35 broad, irregularly shaped microtrichia, laterally with only few clusters or simple scale-like stages (Figures 9 and 10).



Figures 1–8. Ventral sides of operculate gills in Caenidae. (1) *Afrocercus guinensis*, with evenly distributed small spines; (2–5) microtrichia in sector of submarginal field in (2) *Afrocercus guinensis*; (3) *Trichocaenis inexperta*; (4) *Clypeocaenis oligosetosa*; (5) *Callistellina* spec.; (6) band of microtrichia in *Madecocercus tauroides*; (7–8) microtrichia in sector of submarginal band in (7) *Madecocercus tauroides*; (8) *Barnardara demoori*.

Table 1. Evolutionary tendencies in shape and arrangement of microtrichia and their realisation in different taxa.

Brachycercinae	Madecocercinae	Caeninae		
		<i>Clypeocaenis</i> group	Other genera	
				<p style="text-align: center;">Evolutionary tendencies of microtrichia on the ventral side of operculate gill II in the Caenidae</p> <ol style="list-style-type: none"> (1) Enlargement and sublateral concentration of micro-spines, evenly distributed at first. (2) Differentiation from simple spines up to scale-like microtrichia, consisting of a great number of spines which are basally fused. (3) Narrowing of a wide band of microtrichia to a simple row of scales. (4) Increase in regularity of band or row. (5) Increase in complexity of scales, particularly in length and number of fused spines. (6) Increase in number of scales leading to a distal prolongation of the row. (7) Differentiation of bands consisting of regular transverse rows of scale-shaped microtrichia.

A single row of about 45 scales is also present in *Amercaenis ridens*, but without lateral clusters or spines (Figures 11 and 12). As in the above described species the row ends well separated from the hind margin of the gill (see arrow in Figure 11). This is the most advanced stage within the hitherto described species and genera.

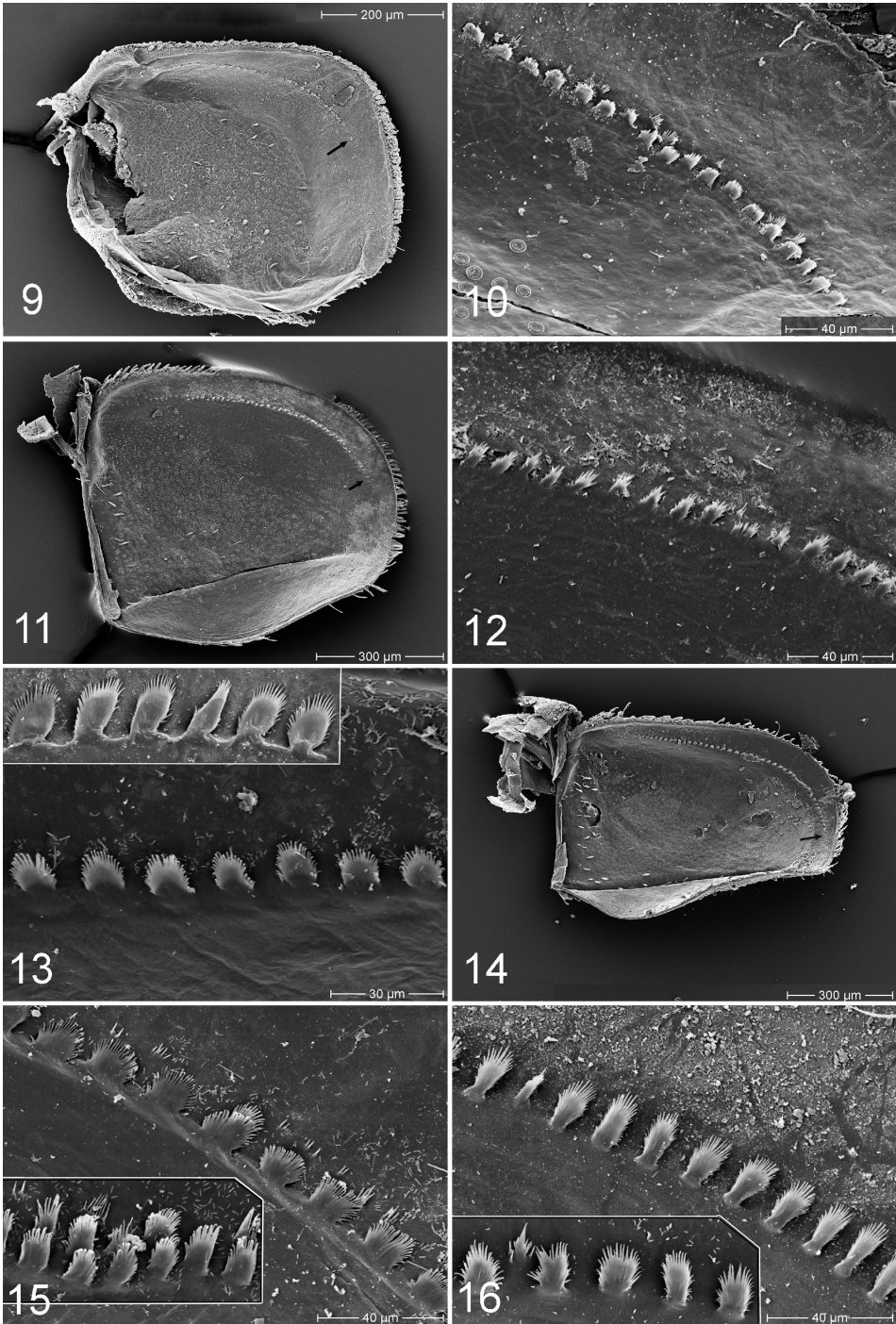
In the following species, all belonging to the genus *Caenis*, both shape and arrangement of microtrichia are clearly more differentiated. There are scales of very similar shape, consisting of a great number of basally fused spines ending in free filaments. They form very regular rows reaching the hind margin of the operculate gills in nearly all species except *Caenis pusilla* (see arrow in Figure 14).

The microtrichia in *Caenis pusilla* are semicircular with about 20 apical filaments (Figure 13). The row consists of about 45 scales (Figure 14).

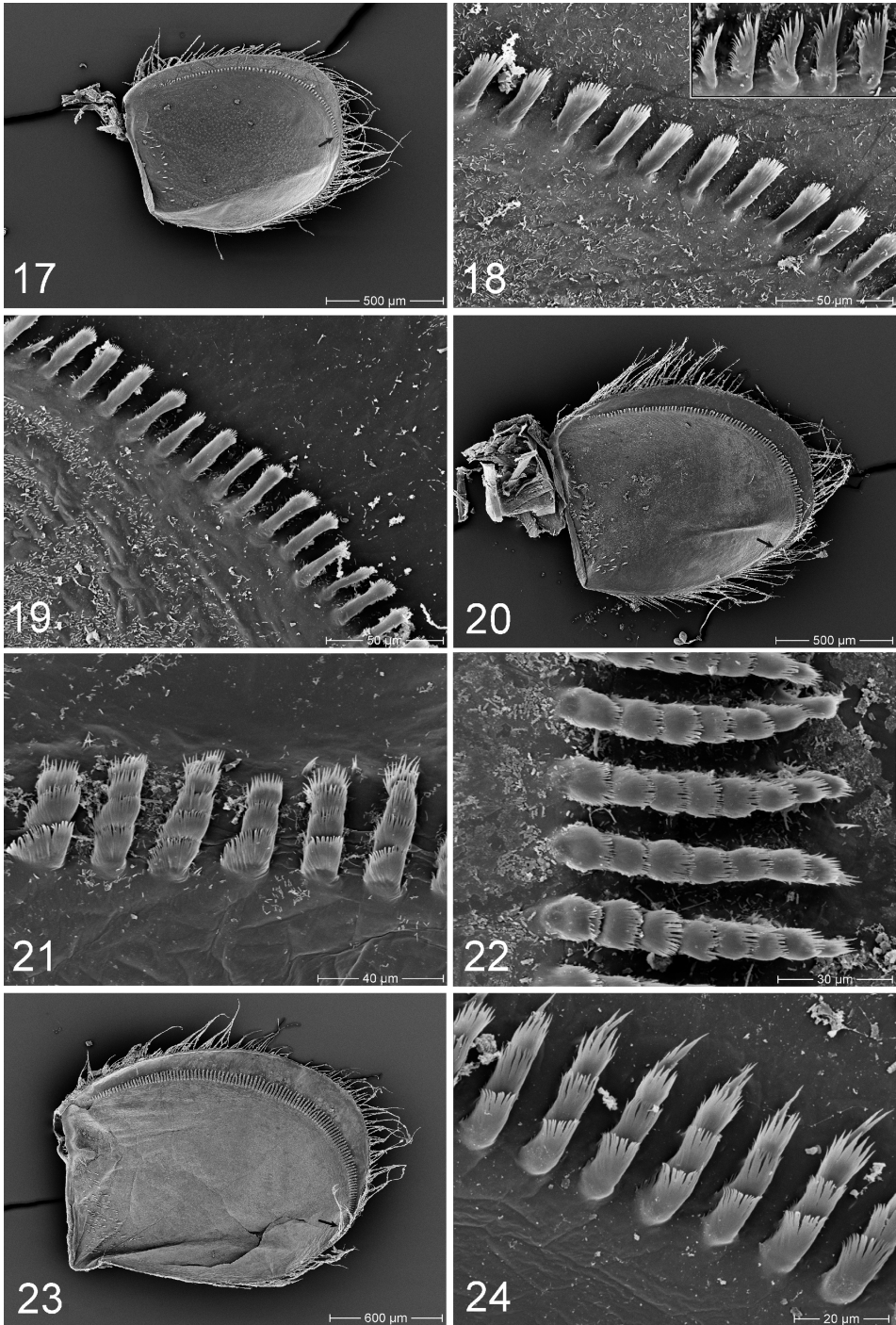
Microtrichia in *Caenis rivulorum* are similar to those in *Caenis pusilla*, sometimes a little elongated, with up to 30 filaments. In a few specimens the rows are partly duplicated (Figure 15, small box). In some specimens clusters of spines can be observed at the lateral margin of the row (Figure 15). The row reaches the hind margin medially from the median axis of the gill and consists of about 55 scales.

Microtrichia of *Caenis beskidensis* are circular (Figure 16, small box) or more or less elongated showing up to 25 filaments (Figure 16). The row is formed by about 70 scales ending near the middle of the hind margin (Figure 17). Elongated and elliptical scales can be found in *Caenis* spec. from Madagascar (Figure 13, small box).

Caenis luctuosa shows clearly elongated microtrichia (Figure 18), and the scales of the closely related *Caenis hoggarensis* are highly asymmetrical (Figure 18, small box).



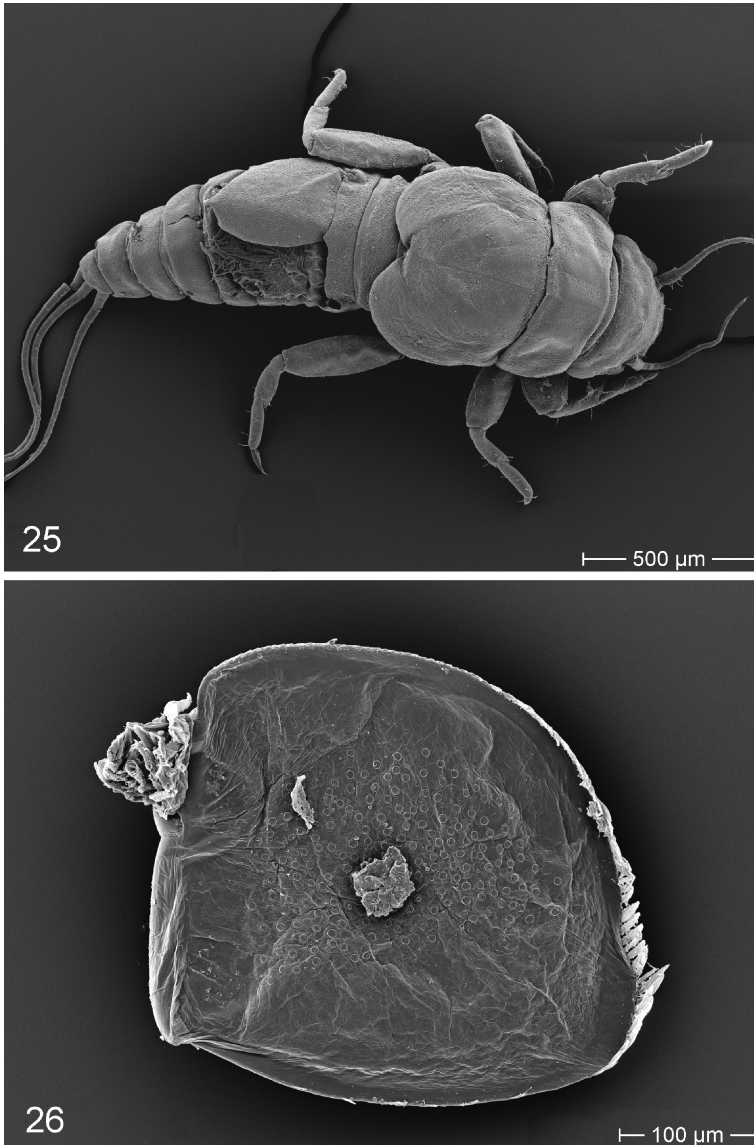
Figures 9–16. Ventral sides of operculate gills in Caenidae. (9) *Clypeocaenis afrosetosa*; (10) *Clypeocaenis afrosetosa*; sector of submarginal row; (11) *Amercaenis ridens*; (12) *Amercaenis ridens*, sector of submarginal row; (13) *Caenis pusilla* (large picture) and *Caenis* spec. from Madagascar (small box), sector of submarginal row; (14) *Caenis pusilla*; (15) *Caenis rivulorum*, simple (large picture) and duplicated (small box) row of microtrichia, sector of submarginal row; (16) *Caenis beskidensis*, specimens with different forms of microtrichia in sector of submarginal row.



Figures 17–24. Ventral sides of operculate gills in Caenidae. (17) *Caenis beskidensis*; (18) *Caenis luctuosa* (large picture) and *Caenis hoggarensis* (small box), sector of submarginal row; (19) *Caenis lactea*, sector of submarginal row; (20) *Caenis lactea*; (21) *Caenis horaria*, sector of submarginal band of transverse rows; (22) *Caenis robusta*, sector of submarginal band of transverse rows; (23) *Caenis robusta*; (24) undescribed genus and species from Thailand, sector of submarginal band of transverse rows.

In both species only a few filaments are single. Most of them form band-shaped structures consisting of a more or less great number of them. In *Caenis luctuosa* the row of microtrichia consists of about 85 scales and reaches the inner hind corner of the operculate gill.

Very long and slender scales finally can be found in *Caenis lactea*. The filaments are almost entirely fused (Figure 19). The row of microtrichia is apically prolonged, slightly bent backwards at the inner margin of the gill and numbering up to 100 scales (Figure 20).



Figures 25–26. Undescribed genus and species (Guinea). (25) Larva, dorsal view; (26) operculate gill, ventral view.

The most complex differentiation in microtrichia arrangement is represented by a band of transverse rows of microtrichia. For a long time it has been known only from two Palaearctic species, *Caenis horaria* and *Caenis robusta*. Recently I obtained two larvae representing two species of an undescribed genus, one from Thailand and the other from Nigeria. In both species the operculate gills are also provided with bands of transverse rows.

Caenis horaria shows transverse rows of about four scale-shaped microtrichia (Figure 21). The band numbers about 70 transverse rows and reaches the inner hind corner of the operculate gill.

In *Caenis robusta* there are about 110 transverse rows with up to eight rounded scales (Figures 22 and 23). Transverse rows of the undescribed species from Thailand consist of about four slightly elongated scales (Figure 24).

Finally I obtained a number of specimens of an enigmatic, undescribed larva from Guinea, West Africa (Figure 25): this specimen is clearly a pannota larva, most probably belonging to the Caenidae. Its gill covers are overlapping medially, but they are without any trace of microtrichia on the ventral side (Figure 26).

Discussion

Evolutionary tendencies

In many species within all subfamilies a great number of evenly distributed very small spines can be observed on the ventral surface of the operculate gills (Figure 1). A concentration and enlargement of these structures towards the lateral and posterior margin, forming the mentioned row or band of microtrichia, probably serves as a protection against mud and detritus. Table 1 shows tendencies that can be observed in the development and arrangement of those microtrichia (compare with descriptions of the different species):

The first tendency, the enlargement of spines and the formation of dense sublateral bands of strong spines (1), is very common in the Brachycercinae. Only in a few species of Brachycercinae the development from spines to simple scale-like microtrichia consisting of few spines (2) has taken place (e.g. in *Brachycercus gilliesi*, Malzacher unpublished). This kind of differentiation along with the basal fusion of many spines is common among the Madecocercinae. In the investigated genera of the Madecocercinae, namely *Madecocercus* and *Afrocerus*, and in five genera of the Caeninae s. str. (Malzacher and Staniczek 2006), namely *Clypeocaenis*, *Amercaenis*, *Barnardara*, *Callistellina* and *Trichocaenis*, the evolutionary tendencies (1)–(4) can be observed. The latter five genera are those with striking larval apomorphic characters such as filtering setae on the fore legs, ridges on the head and thoracic nota, or an unusual body shape. Consequently, these species should be united in a *Clypeocaenis* group.

An even more complex character state as described above for *Caenis* can be additionally observed in the genera *Afrocaenis* (Malzacher unpublished), *Caenopsella* (together with *Caenis* belonging to the Caeninae s. str.), and in the Australian genera *Tasmanocoenis* (Suter 1984; Alba-Tercedor and Suter 1990), *Wundacaenis* (Suter 1993) and *Irpacaenis* (judging from a rather schematical drawing in Suter 1999). From *Caenopsella* there are only two larval exuviae available (Coll. Gillies, BMNH) prepared on micro slides. The very delicate microtrichia are hardly visible under light microscope but they seem to be equal in shape and forming a regular row. So in all the above mentioned genera, tendencies (5)–(7) on microtrichia development are

realised, originating from an initial stage as seen in *Amercaenis* (Figure 10). The highest differentiation in this group, a band of transverse rows of microtrichia, is realised in two genera, namely *Caenis* and the yet undescribed genus from Thailand and Nigeria, which are not closely related to each other. Therefore a parallel development of these structures seems to be very likely.

A parallelism can also be assumed for development in the Madecocercinae and the *Clypeocaenis* group. Even within the *Clypeocaenis* group parallelism has to be assumed judging from the very different developmental stages in *Clypeocaenis oligosetosa* and *C. afrosetosa*.

The assumption of Malzacher and Staniczek (2006) that regular and prolonged rows of scale-shaped microtrichia occur in all Caeninae can no longer be maintained. As shown here this highly developed character is present only in the *Caenis*-like genera and the Australian Caeninae, but not in the *Clypeocaenis* group. This would point to a sistergroup relationship between these genera and the *Clypeocaenis* group. On the other hand the experience that parallel developments are common in lower taxa legitimates the presumption that genotypes of those taxa are often very similar to each other. Phenotypical differences could then result e.g. from different molecular genetic processes such as specific transcription mechanisms (Malzacher 1997). This aspect makes it difficult to assess the probability of a synapomorphic evolution of a certain character at a lower taxonomical level. Even the highly developed rows of microtrichia (tendencies (5)–(7)) in *Caenis* and the Australian genera could be the result of such a parallel development, regardless of a probable sistergroup relationship of these taxa as assumed by Ogden and Whiting (2005) and Ogden et al. (2009).

The enigmatic larva from Guinea, West Africa (Figure 25), due to the medially overlapping gill covers a putative species of Caenidae, is entirely lacking microtrichia on the ventral side of its gill cover. In spite of a still forthcoming detailed investigation and description, there are three possibilities of interpretation:

- (1) This species does not belong to the Caenidae, and the medially overlapping gill covers are a result of a convergent development. This is however not likely, as the general morphology of this species is overall resembling a Caenid larva.
- (2) This species nests within the Caeninae, but a change of ecological niche led to a total reduction of microtrichia. This is debatable, because the specimens were found in a sample containing a lot of *Caenis* larvae. Thus it can be assumed that all these species inhabit the same habitat.
- (3) The lack of microtrichia on the ventral side of the gill cover reflects an early phylogenetical stage and corresponds to the plesiomorphic character state in the Neoephemeridae. In this case the new species could be considered as the sistergroup of the remaining Caenidae.

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References

- Alba-Tercedor, J., and Suter, P.J. (1990), 'A New Species of Caenidae from Australia: *Tasmanocoenis arcuata* sp. n (Insecta, Ephemeroptera)', *Aquatic Insects*, 12, 85–94.
- Kluge, N.J. (2004), *The Phylogenetic System of Ephemeroptera*, Dordrecht/Boston/London: Kluwer Academic Publishers.
- Malzacher, P. (1993), 'Caenidae der äthiopischen Region (Insecta: Ephemeroptera). Teil 2. Systematische Zusammenstellung aller bisher bekannten Arten', *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, 66, 379–416.
- Malzacher, P. (1997), 'Relationship in the Caenidae (Insecta: Ephemeroptera)', in *Ephemeroptera and Plecoptera: Biology – Ecology – Systematics*, eds. P. Landolt and M. Sartori, Fribourg: Mauron + Tinguely and Lachat SA, pp. 550–553.
- Malzacher, P. (2009), 'New larvae of Caeninae from Madagascar (Ephemeroptera: Caenidae)', *Stuttgarter Beiträge zur Naturkunde A, Neue Serie*, 2, 177–194.
- Malzacher, P., and Staniczek, A.H. (2006), 'Revision of the Madecocercinae (Ephemeroptera: Caenidae)', *Aquatic Insects*, 28, 165–193.
- McCafferty, W.P., and Wang, T.-Q. (2000), 'Phylogenetic Systematics of the major lineages of pannota mayflies (Ephemeroptera: Pannota)', *Transactions of the American Entomological Society*, 126, 9–21.
- Ogden, T.H., Gattolliat, J.-L., Sartori, M., Staniczek, A.H., Soldán, T., and Whiting, M.F. (2009), 'Toward a new paradigm in mayfly phylogeny (Ephemeroptera): combined analysis of morphological and molecular data', *Systematic Entomology*, 34, 616–634.
- Ogden, T.H., and Whiting, M.F. (2005), 'Phylogeny of Ephemeroptera (mayflies) based on molecular evidence', *Molecular Phylogenetics and Evolution*, 37, 625–643.
- Suter, P.J. (1984), 'A redescription of the genus *Tasmanocoenis* Lestage (Ephemeroptera: Caenidae) from Australia', *Transactions of the Royal Society of South Australia*, 108(2), 105–111.
- Suter, P.J. (1993), '*Wundacaenis*, a New Genus of Caenidae (Insecta: Ephemeroptera)', *Invertebrate Taxonomy*, 7, 787–803.
- Suter, P.J. (1999), '*Irpacaenis*, a new genus of Caenidae (Ephemeroptera) from Australia', *Australian Journal of Entomology*, 38, 159–167.