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Morphology and ultrastructure of the molar area in the mandible of mayfly (Ephemeroptera) larvae

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The main objective of the study is to describe in detail morphological structures on the mandibular molae of different larval Ephemeroptera. Therefore, representative samples of species of 80 genera from 28 families have been investigated by scanning electron microscopy. Six basic types of molar surface are differentiated. The respective types of molae differ in the cuticular structures of individual ridges. Relationships between the molar type, feeding categories, and phylogenetic position of individual taxa are discussed. The functional significance of various microstructures on the molar surfaces is explained.

Keywords: Ephemeroptera; morphology; mandible; molar area

Introduction

In the winged stages of the order Ephemeroptera, mouthparts are generally reduced and non-functional. Nevertheless, mayfly larvae possess well-developed mouthparts. Although the morphology of larval mouthparts in most species of the Ephemeroptera is well described, the ultrastructure of the molar area of the mandible is generally very poorly known. Only a few studies dealing with the functional morphology and anatomy of the mouthparts of individual species include details on the molar surface structures (e.g. Strenger 1953, 1970, 1975, 1977; Elpers and Tomka 1994a).

Even in studies including the characteristics of molar surface, these were only studied in a very restricted number of species, and comparative aspects were largely omitted. Accordingly, a comparative study of taxa throughout the Ephemeroptera is lacking.

The structure of the mandibular molae is also mentioned in several studies dealing with the feeding habits of particular mayfly species, e.g. Brown (1961b) studied *Cloeon dipterum* (Linnaeus, 1761) and *Baetis rhodani* (Pictet, 1843–1845).

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Murphy (1922), in a first attempt, classified the molar surfaces of mandibles into three types. Nevertheless, her study was based exclusively on Nearctic species, and so naturally did not cover the variability of mayfly mouthparts on a worldwide scale.

Elpers (1997) studied the molar surfaces of mayfly mandibles by means of scanning electron microscopy and described their surface structures in detail. However, this study was limited to only seven species of the suborder Ephemeroidea, which exhibit a quite uniform arrangement of the molar surface.

The objective of the present study is to provide more information on the ultrastructure of molar surfaces in representatives of various phylogenetic lineages within the Ephemeroptera. It is aimed at distinguishing different types of molar surfaces, and discussing functional and phylogenetic factors that may have an influence on the different arrangements of the molar areas.

Materials and methods

The ultrastructure of mandibular molae was studied by means of scanning electron microscopy (SEM). The mandibles used for this study were dissected from larvae that were usually fixed in 75% EtOH. The mandibles were gradually transferred to acetone, critical point dried, sputter-coated with gold in a Polaron PS100 sputterer, and observed with a Jeol JSM-6300 scanning electron microscope at an accelerating voltage of 10–15 kV. Photos used in the present study were taken with a scanning electron microscope Jeol JSM-7300 at an accelerating voltage of 3 kV.

Gut contents were analysed according to the procedure used in McShaffrey and McCafferty (1991) by removing the foregut and macerating the contents in glycerin on a microscope slide. Slides were examined using a microscope magnification of $400 \times$, with the proportions of each type of food determined by measuring the area covered by each type. The area was measured by using a 10×10 ocular grid. Ten randomly selected fields were evaluated for each slide. Gut contents were classified in five categories: animal remains, filamentous algae, diatoms, mineral particles and detritus. A single mature larva of each species with sufficient amount of ingested food in the foregut was examined.

Samples of 80 species (representing 80 genera and 28 families) were studied (see below). The material used for this study is deposited in the collection of the Institute of Entomology, České Budějovice, Czech Republic.

Results

The mandible of mayfly larvae consists of an elongated basal part, broadened distally toward the molar surfaces and incisors.

The molar parts of the mandibles are situated at the inner edge of the distal mandibular portion. In most species the molar parts of the right and left mandible are strongly asymmetrical. The molar surface of the left mandible is most projected in its distal part and follows the longitudinal axis of the mandible. The surface of the

right mandible is most projected in its proximal part and oriented parallel to the transverse axis of the mandible. Exceptions to this are found only in selected taxa, the mandibles of which are a mirror image of the standard arrangement. From the taxa studied, this situation occurred in *Arthroplea congener* from the family Arthropleidae. In rare cases the asymmetry of mandibles is lost and the shape of molar area is highly derived (many carnivorous species), or molae are completely reduced (*Prosopistoma* spp.).

The movement of mandibles during the feeding process consists of lateral and medial adduction. During the medial adduction stage, molar surfaces are moved towards each other. In spite of their shape, they fit precisely against each other when pressed together.

The position of the mandibles in the head capsule, their three-dimensional orientation and the fitting of molar areas is also apparent from Figures 1–4.

The molar surface in mayfly larvae consists, in most species, of a system of alternating ridges and grooves. In addition, cuticular projections of various shapes may be seen on the surface and/or between individual ridges.

Detailed observation of the molar surfaces by SEM revealed that it is possible to distinguish six basic types of surface according to their ultrastructure:

> *Type I*: The space between individual ridges is filled with tiny hairs, which are distributed evenly or in groups (Figures 5, 11, 12).
> *Type II*: The ridges are provided with non-articulated teeth, overlapping neighbouring grooves (Figure 6).
> *Type III*: Each groove is equipped with a row of individual projections that are at least partially separated from the ridges (Figures 7 and 8).
> *Type IV*: The ridges and grooves are without teeth or any other projections between ridges (Figure 9).
> *Type V*: The surface is without a structure of ridges and grooves, being instead provided with numerous individual tubercules (Figures 10, 17, 18).
> *Type VI*: The asymmetry of left and right mandible is lost, molar surfaces are provided with long bristles.

Although the molar area of any individual species in most cases can be unambiguously associated with one of these different types of molar surface, transitional states between individual categories also exist. An intermediate state between categories II and V occurs, when the structure of ridges and grooves is mostly retained, grooves however disintegrate into individual projections in some areas, mainly marginally (from the sample studied, this situation occurred in the genera *Metamonius* (Nesameletidae) and *Tindea* (Leptophlebiidae) (Figure 15). Transition between types I and II can be observed in *Coloburiscoides* sp. and *Murphyella* sp. (Coloburiscidae), where molae exhibit types I and II in different parts of the molar area.

Another specific case of the molae occurs in type II, where ridges can fuse either marginally (genus *Elassoneuria* (Oligoneuriidae), Figure 16) or to distinct spots occurring on the whole surface, where individual overlapping teeth are in contact with the neighbouring ridge (genus *Lachlania* (Oligoneuriidae)).
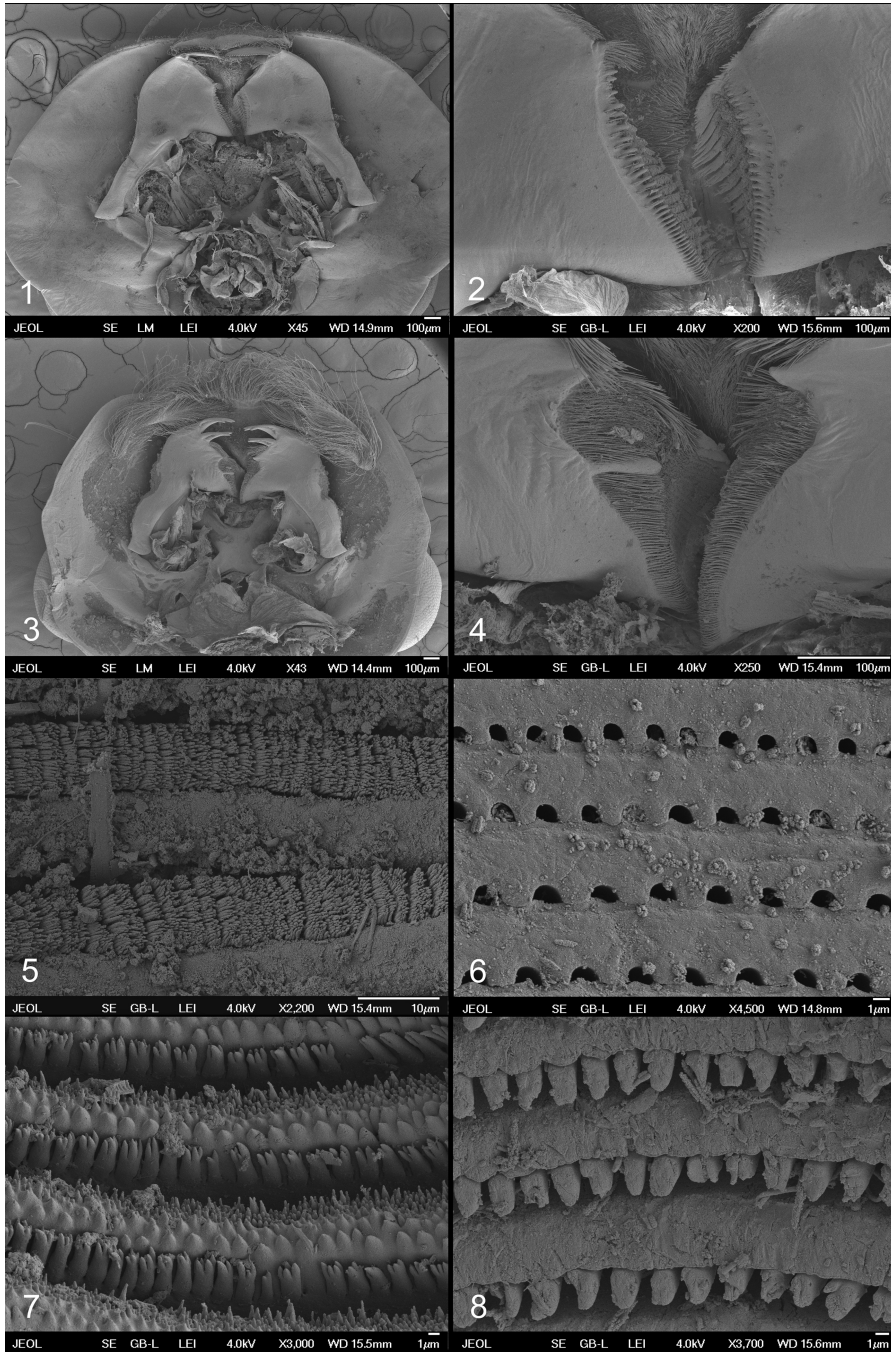
Figures 1–8. (1–2) *Rhithrogena semicolorata*, (1) head in ventral aspect, all mouthparts except mandibles removed, (2) detail of molar areas; (3–4) *Arthroplea congener*, (3) head in ventral aspect, all mouthparts except mandibles removed, (4) detail of molar areas. (5–10) Detailed arrangement of the (5) molar surface type I (*Penaphlebia chilensis*); (6) molar surface type II (*Elassoneuria trimeniana*); (7) molar surface type III (*Kimminsula* sp., freshly moulted); (8) molar surface type III (*Tindea* sp., before moulting).
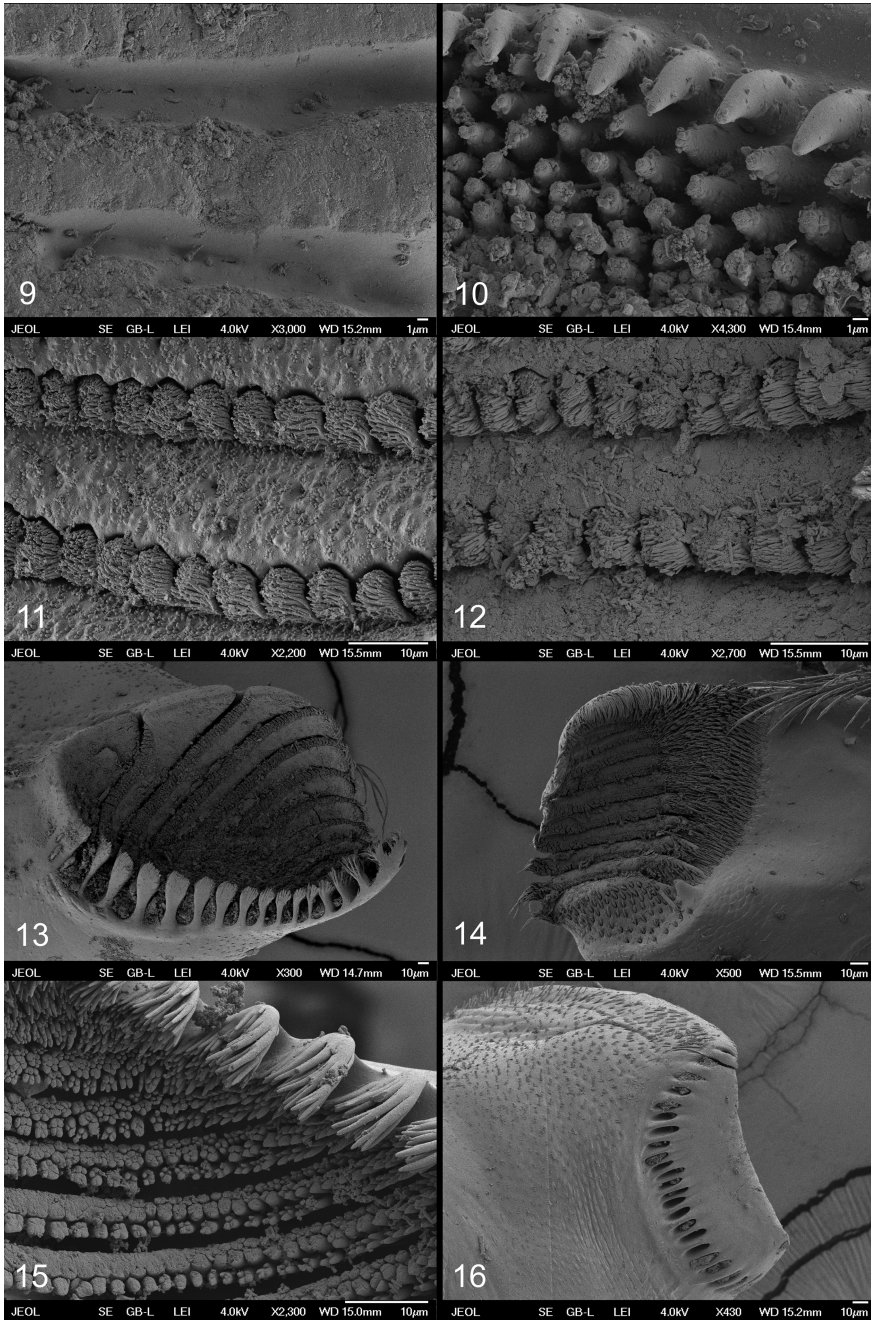
Figures 9–16. (9) Molar surface type IV (*Teloganodes* sp.); (10) molar surface type V (*Dactylobaetis* sp.); (11–12) molar surface type I of *Heptagenia sulphurea*, (11) freshly moulted larva, (12) larva before moulting; (13) right mola of *Palingenia fuliginosa* (type IV; apparent concavity of the molar surface); (14) left mola of *Dactylophlebia* sp. (type IV; apparent marginal setation); (15) marginal area of molar surface type III (*Tindea* sp.), with the ridges splitting to the individual tubercules; (16) molar surface type II (*Elassoneuria trimeniana*), lateral molar margin with apparent fusion of ridges.
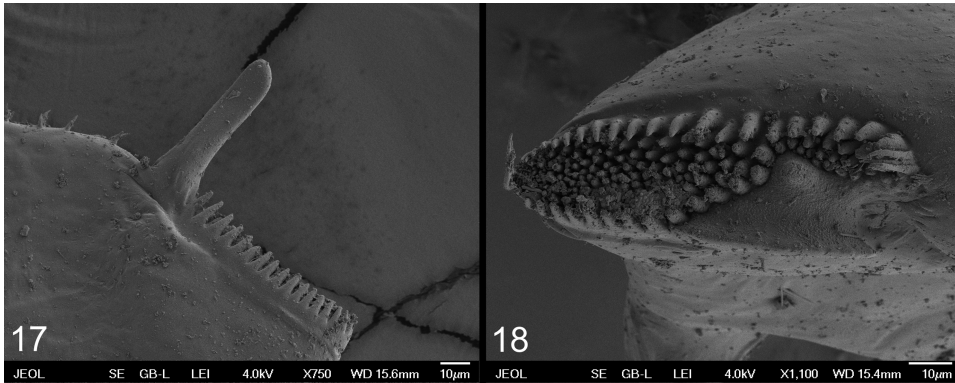
Figures 17–18.   Molar surface type V, (17) left molar of *Dactylobaetis* sp.; (18) right molar of *Dactylobaetis* sp.

Table 1.   Overview of the studied species.

Family	Species	Molar type	Figure No.	Gut content
Acanthametropodidae	*Analetris eximia* Edmunds, 1972	VI		91-0-0-9-0
Ameletopsidae	*Chiloporter* sp.	VI		
	Chaquihua sp.	VI		
	Mirawara sp.	VI		
Arthropleidae	*Arthroplea congener* Bengtsson, 1908	II	3, 4	0-0-0-0-100
Baetidae	*Baetis rhodani* (Pictet, 1943–1845)	V		0-39-0-11-50
	Centroptiloides sp.	V		100-0-0-0-0
	Cloeon (Similicloeon) simile Eaton, 1870	II		0-18-0-26-56
	Dactylobaetis sp.	V	10, 17, 18	0-6-0-10-84
	Heterocloeon petersi (Müller-Liebenau, 1974)	V		0-68-0-13-19
	Nesoptiloides sp.	V		100-0-0-0-0
Baetiscidae	*Baetisca rogersi* Berner, 1940	II		
Behningiidae	*Dolania americana* Edmunds & Traver, 1959	VI		
Caenidae	*Afrocaenis* sp.	III		
	Brachycercus harrisellus Curtis, 1834	I		0-17-0-35-48
Coloburiscidae	*Coloburiscoides* sp.	I + II		0-10-0-9-81
	Coloburiscus humeralis (Walker, 1853)	I		0-21-0-26-53
	Murphyella sp.	I + II		
Ephemerellidae	*Caudatella hystrix* (Traver, 1934)	II		
	Cincticostella sp.	II		0-13-6-40-41
	Ephacerella longicaudata (Uéno, 1928)	II		0-1-0-71-28
	Ephemerellina sp.	I		
	Hyrtanella sp.	I		0-3-2-90-5
	Timpanoga hecuba (Eaton, 1884)	III		0-4-1-43-52
	Torleya sp.	IV		

(*continued*)

Table 1. (*Continued*).

Family	Species	Molar type	Figure No.	Gut content
Ephemeridae	*Ephemera glaucops* Pictet, 1843–1845	I		0-0-0-54-46
	Hexagenia munda Eaton, 1883	I		0-12-0-70-18
Euthyplociidae	*Campylocia anceps* (Eaton, 1883)	I		
	Euthyplocia sp.	III		0-4-1-60-35
Heptageniidae	*Afronurus* sp.	II		0-8-0-14-78
	Cinygma sp.	I		0-3-0-62-35
	Ecdyonurus subalpinus (Klapálek, 1907)	II		0-5-1-9-85
	Ironopsis permagnus Traver, 1935	III		
	Heptagenia sulphurea (Müller, 1776)	I	11, 12	0-0-0-11-89
	Iron sp.	III		0-10-0-12-78
	Ironodes sp.	II		
	Rhithrogena semicolorata (Curtis, 1834)	I	1, 2	0-31-34-18-17
	Stenonema sp.	I		0-3-0-2-95
Ichthybotidae	*Ichthybotus* sp.	I		0-0-0-21-79
Isonychiidae	*Isonychia* sp.	II		0-2-0-0-98
Leptophlebiidae	*Adenophlebia* sp.	I		
	Choroterpes picteti (Eaton, 1871)	I		
	Choroterpides sp.	I		0-0-0-24-76
	Celiphlebia caledoniae Peters & Peters, 1980	III		
	Dactylophlebia sp.	I	14	0-20-0-7-73
	Hagenulus sp.	I		
	Hermanella sp.	I		0-31-0-13-56
	Kimminsula sp.	III	7	0-0-4-46-50
	Lepegenia lineata Peters, Peters & Edmunds, 1978	III		
	Massartella sp.	I		0-20-0-23-57
	Meridialaris sp.	I		0-3-0-47-50
	Notachalcus corbassoni Peters & Peters, 1981	I		
	Paraleptophlebia submarginata (Stephens, 1835)	I		0-9-0-46-45
	Penaphlebia chilensis (Eaton, 1884)	I	5	0-0-0-17-83
	Poya sp.	I		
	Thraulus sp.	I		
	Tindea sp.	III	8, 15	
Metretopodidae	*Siphloplecton speciosum* Traver, 1932	I		
Neoephemeridae	*Neoephemera youngi* Berner, 1953	I		
	Potamanthellus chinensis Hsu, 1936	I		0-10-0-62-28
Nesameletidae	*Metamonius* sp.	II		
Oligoneuriidae	*Oligoneuriopsis skhounate* Dakki & Giudicelli, 1980	II		
	Elassoneuria trimeniana McLachlan, 1868	II	6, 16	
	Homoeoneuria dolani Edmunds, Berner & Traver, 1958	II		
	Lachlania sp.	II		

Table 1.   (*Continued*).

Family	Species	Molar type	Figure No.	Gut content
Oniscigastridae	*Oniscigaster* sp.	II		0-0-0-54-46
	Siphlonella sp.	I		0-1-0-42-57
	Tasmanophlebia sp.	I		0-2-0-40-58
Palingeniidae	*Palingenia fuliginosa* (Boeber [in Georgi], 1802)	I	13	0-2-0-60-38
Polymitarcyidae	*Ephoron virgo* (Olivier, 1791)	I		
	Asthenopus sp.	III		
Potamanthidae	*Potamanthodes* sp.	I		
	Potamanthus luteus (Linnaeus, 1767)	I		0-11-0-46-43
	Rhoenanthus sp.	I		0-14-0-63-23
Pseudironidae	*Pseudiron centralis* McDunnough, 1931	VI		
Rallidentidae	*Rallidens mcfarlanei* Penniket, 1966	III		0-40-1-16-43
Siphlonuridae	*Parameletus minor* (Bengtsson, 1909)	I		
	Siphlonurus lacustris Eaton, 1870	II		
Teloganodidae	*Teloganodes* sp.	IV	9	0-90-0-0-10
Tricorythidae	*Tricorythus* sp.	III		0-41-0-9-50

Classification of families follows Brittain and Sartori (2003) except for several changes according to the current updated nomenclature, which includes recognition of the families Ichthybotidae and Nesameletidae. Respective types of the molar surfaces associated with individual species and the results of the gut content analysis are provided. Concerning the gut analysis results, numbers in the respective column indicate a relative percentual proportion of ingested food particles in the order as follows: animal remains – filamentous algae – diatoms – mineral particles – detritus. Families are listed in alphabetical order.

Discussion

The functional aspect of the molar area

The molar area of the mandible has been traditionally considered to be determined for crushing food particles, or in other words, "The primary function of the May-fly mandible is to grind food. For this purpose a molar surface is developed" (Murphy 1922).

However, taking into account the ultrastructure of molar surfaces as revealed by SEM, we can assume a function far beyond simple grinding. The molae of mayfly larvae living on detritus and various types of algae are adapted for straining water from food and its compression rather than for grinding. Brown (1961b) investigated the composition of different mayfly diets through gut content analyses and remarked that the ingestion of large quantities of water should be expected, but gut analyses reveal that the food must be swallowed in a relatively dry condition as food particles are found to be very tightly packed in the foregut.

The mechanism for straining the water out of food particles can be generally described as an interaction of several factors.

In many species both molar surfaces are concave, so when the molae are tightly connected in their marginal zone, there is still some space left in between them in the central part of the molae (Figures 13 and 14).

During the feeding process, the free space between the molar surfaces is filled with food particles. When the mandibles close, the food particles are locked in the central part in between the molae and compressed. Excessive water is thereby strained through the molar grooves. During the straining process, hairs or teeth in

the grooves are of great importance. Water is free to pass through little gaps between these tiny structures deep into the grooves and subsequently out through the peripheral vents of individual grooves, which actually function as draining channels. Thus various teeth and hairs are present in the grooves to prevent the outflow of food particles. Interspaces between these tiny structures are big enough to allow water to escape, but food particles are retained. In the marginal parts of molae there are ridges that are often serrated, or areas with numerous bristles can occur (Figure 14). These structures prevent the outflow of food through any eventual narrow discontinuity between the left and right mola.

The draining function of mandibles was already assumed by Elpers (1997) for the molae of Ephemeroidea. Landolt et al. (1995) even found food particles of *Palingenia longicauda* packed in the foregut in the form of discoid nutritional pellets. Each individual pellet originated from a single enclosure of food between molae.

A stout conical projection is often present at the edge of the left molar surface (Figure 17). When the mandibles close, this peg projects over the edge of the right molar surface. This structure occurs in many species and was already noticed by Brown (1961b) in *Cloeon dipterum* and *Baetis rhodani*. Brown (1961b) considered the peg to be a protective device to prevent damage from overclosure of the molar surfaces. However, such a projection often fits into a respective hollow on the opposite mandible and therefore more likely acts as a kind of guiding structure to enable mandibles to be closed in the exact position in which molar surfaces precisely fit into each other. A pronounced form of such an arrangement can be observed in the mandibles of the genus *Dactylobaetis* (Figures 17 and 18). In *Arthroplea* (a genus where the mandibles are a mirror image of the typical arrangement), this projection occurs on the right mandible, in a similar position as on the left mandible in the majority of other species (Figure 4).

Abrasion of the molar surface

Specimens approaching ecdysis sometimes show apparent signs of abrasion on the molar surfaces. The deterioration of the feeding apparatus in larvae of aquatic insects was investigated by Arens (1990), who found many examples of extensive wear mainly in specimens obtaining their food by scraping rough substratum. The efficiency of the feeding apparatus is probably reduced at the end of each instar, although there is no evidence that the rate of abrasion has an effect on moulting intervals (Arens 1990). Molar surfaces usually do not become as much abrased as the parts of mouth apparatus which are actually functioning as detaching structures during the feeding process (cuticular brushes, rakes, gouges etc.). However, signs of wear on the molae can also be observed as the deterioration of tiny and minute cuticular projections on the surface of ridges and/or the ridges themselves (mainly in marginal areas). The difference between the molar surfaces of a freshly moulted larva and a larva just before moulting is obvious from Figures 11 and 12 (molar type I) and from Figures 7 and 8 (molar type III).

Food processing in different types of molae

The crucial question is which factors influence the specific type of molar surface in each species and how the type of molar surface is connected with the preferred nutrition and/or phylogenetic position of the species.

The relevancy of food preferences in mayfly larvae was always questionable. Coffmann et al. (1971) suggested that the "availability of food is the dominant factor influencing the composition of the diet". In contrast, food analyses by Brown (1960) showed that the larvae of *Cloeon dipterum* preferred some species of algae to others available to them in the habitat. Laboratory experiments arranged by Cianciara (1980) also revealed a clear preference for some types of food.

Further, some mayflies show at least a very narrow specialisation in the way they obtain food and this subsequently determines to a certain degree which particles they ingest (filter-feeders, grazers of epilithic vegetation etc.). Such species have developed morphological adaptations in their mouthpart arrangement (including molae) for processing their particular diet.

Obviously the most specialised mouthparts are present in species which are obligatory carnivores. However, even in many other less specialised taxa some level of morphological adaptations for the processing of the preferred diet can be found on the molar surface, when results of the gut analysis are compared with the ultrastructural characteristics of molae.

The results of the gut analyses in the present study provide only basic outlines and are aimed at distinguishing the dominant components of the diet of individual taxa. It is not the intention to provide a complex analysis of the food preferences, since only a restricted sample of individuals was used and the effects of seasonality, local food source supply and the size of larvae have not been taken into account, although these factors surely also have some effect on the composition of the diet (see Brown 1961a; Cianciara 1980; Baekken 1981; Gibs and Mingo 1985).

When confronted with the most recent phylogeny of the order Ephemeroptera, based on morphological and molecular data (Ogden et al. 2009), individual types of mayfly molae, as described in the present study, often appear repeatedly in various phylogenetic lineages within the order (for details see discussion concerning individual types of molar surfaces below; majority-rule consensus phylogenetic tree of Ogden et al. (2009) is taken as a reference of mayfly phylogeny in further text). Moreover, Arens (1994) described almost identical structures as the molar surfaces of some mayflies, but found them in completely unrelated taxa of aquatic insects, probably feeding on a similar diet as the respective mayfly larvae.

In the molar surface of type I, the space between individual ridges is filled with tiny hairs, distributed evenly or in groups (Figures 5, 11, 12). Cuticular hairs in ridges, functioning as an obstacle for food particles, are elastic. It is the most widespread arrangement throughout Ephemeroptera. It can be found in representatives of 15 families from the sample used in this study (see Table 1). Several families uniformly show this type of molar surface in all species studied (e.g. Ephemeridae, Neoephemeridae, Potamanthidae, most of Leptophlebiidae). It is the most frequent arrangement in many basal groups of Furcatergalia (Leptophlebiidae, Potamanthidae and Fossoriae). However, this arrangement can be found in many other non-related phylogenetic lineages of mayflies (Heptageniidae, Siphlonuridae etc.). According to the gut content analysis of the species examined in the present study, molar surface type I can be associated with species showing a diet composition consisting mainly of detritus with a large portion of mineral particles. This assumption is consistent with the findings of other authors, who examined the gut contents of mayfly larvae with this type of molar surface (e.g. Kłonovska 1986). Highly elastic cuticular hairs between ridges may be an adaptation to withstand the

effect of tough, unbreakable mineral particles which might otherwise cause damage to rigid molar surfaces.

The molar surface of type II has ridges provided with teeth, overlapping the grooves (Figure 6). It corresponds to the ''grating-like molar surfaces'' according to Arens (1994). The teeth may be in close contact or even fuse with the neighbouring ridge, leaving only small openings between them (e.g. in *Lachlania* sp.). Moreover, the ridges themselves may be fused in some species (Oligoneuriidae), in the marginal areas, but vents for the straining of water are present which function as estuaries of the draining channels formed by individual ridges (Figure 16).

This type of molar surface is typical for Oligoneuriidae, Isonychiidae and Arthropleidae. It can also be found also in several representatives of Heptageniidae, Ephemerellidae, and Siphlonuridae.

Most species with molar surface type II exhibit different nutrition than those with type I. These taxa are either filter-feeders (Oligoneuriidae, Isonychiidae and Arthropleidae) with nutrition composed of small particles of detritus and algae, or scrapers of epilithic vegetation (Heptageniidae) living on algae and detritus attached to submerged objects. In both cases, their diet contains a relatively small percentage of mineral particles. There is no risk of unbreakable mineral particles causing damage to the molar surfaces. Therefore there is no demand for particular elasticity of the molae and retaining structures (hairs, tiny articulated projections) between grooves.

Molar arrangement type II has certainly evolved several times independently and can also be observed in other aquatic invertebrates presumably adapted to living on a similar diet (e.g. dipteran larvae of the genus *Odontomyia* and isopods of the genus *Ligia*; see Arens 1994). Among lineages of the order Ephemeroptera, taxa with this type of molae are scattered along the phylogenetic tree and almost all lineages contains some representatives with this molar arrangement. However, it is in general a rare arrangement in Furcatergalia (missing in Fossoriae and present only in some Ephemerellidae from the taxa studied).

The molar surface of type III is characterised by a row of articulated projections in each groove, not rigidly attached to the ridges as in the case of type II, but some elasticity is retained (Figures 7, 8). This type is probably a compromise between type II (overlapping teeth on ridges) and type I (tiny hairs between ridges). It is not as rigid as type II, but is however less elastic than type I. Thus, species living on a diet of intermediate stiffness can be supposed to possess this type of molar structure arrangement.

Gut content analysis of the species exhibiting this type of molar area proved approximately equal amounts of mineral particles and detritus (*Timpanoga* sp., *Kimminsula* sp.), or distinct amount of algae in some species (*Rallidens mcfarlanei*, *Tricorythus* sp.). The ridges are sometimes partly disintegrated into individual tubercules, mainly in marginal areas (Figure 15). This can be interpreted as transition to type V, where individual tubercules cover the entire molar surface. The disintegration of ridges can also be observed in type IV. From the species studied, type III has been found in representatives of the families Caenidae, Ephemerellidae, Euthyplociidae, Leptophebiidae, Polymitarcyidae, Rallidentidae, and Tricorythidae. Most of these families represent the lineage of Furcatergalia, with the exception of Heptageniidae and Rallidentidae.

The molar surface of type IV is not frequently found in Ephemeroptera. The basic structure of alternating ridges and grooves is retained, but minute cuticular

structures in individual grooves are absent (Figure 9). Among all investigated species type IV was only found in *Teloganodes* sp. and *Torleya* sp. The loss of tiny cuticular structures in the grooves, occurring frequently in other types of mayfly molae, is most probably secondary; the species concerned do not represent basal phylogenetic lineages of Ephemeroptera, both species belong to the advanced group of Ephemerelloidea. In *Teloganodes* sp., this structure of molae may be an adaptation to a diet consisting of almost exclusively filamentous algae. Their cells need to be broken up for successful digestion, and the outflow of food particles through grooves is unlikely because of coherence of the mass of algal filaments.

The molar surface of type V, the ''thorn-carpet molar surface'' according to Arens (1994), lacks the typical structure of ridges and grooves; instead the mola is covered with individual tubercules (Figures 10, 17, 18). The functional adaptation of such a molar arrangement and its advantages in comparison with other types remains unknown at present. Food is retained by these blunt structures and water is released through gaps between them. In this case the outflow of the food particles is more probable than in previous groups. However, the large bristles of the hypopharyngeal lingua and the respective bristles of the epipharynx may also help to retain food particles, as suggested by Brown (1961b). Moreover, marginal tubercules are often enlarged to prevent the outflow of food particles. Molar structure type V is typical for the family Baetidae and most probably represents a unique apomorphy in this basal lineage of mayflies. However, the tendency to develop this type of molar surface independently may be illustrated by several transitional states probably leading to this type (e.g. *Tindea* sp., Figure 15). Gut content analysis of the species, which possess this structure of molae (*Baetis rhodani*, *Dactylobaetis* sp., *Heterocloeon petersi*), revealed a heterogeneous diet with a small percentage of mineral particles and various amounts of algae and detritus.

The molar surface of type VI is adapted for carnivory. Its shape is highly derived, and the asymmetry of molae is completely lost. The molar surface is equipped with stout, sharp setae or thorns for capturing and processing prey. These adaptations go along with many other modifications of the mouthparts. The molar surface has lost its function as a draining device. The mouthparts of carnivorous larvae were studied in detail e.g. by Tsui and Hubbard (1979), Elpers and Tomka (1994b) or Staniczek (2000). Such a type of molae can be observed in several independent carnivorous lineages within Ephemeroptera (Acanthometropodidae, Ameletopsidae, Pseudironidae). From the phylogenetic point of view, adaptations for carnivory are generally not common in Ephemeroptera and show no apparent pattern in their position along the phylogenetic tree. They have evolved independently in various lineages of mayflies (Acanthametropodidae and Ameletopsidae represent independent lineages in one of the basal clades of the order, Behningiidae is placed at the base of Fossoriae (Furcatergalia)).

Several families are consistent in their type of mola. From the sample of species studied, type V occurs only in Baetidae. The families Ephemeridae, Neoephemeridae, Potamanthidae, and Leptophlebiidae have uniformly type I (with only a few exceptions in Leptophlebiidae). All members studied from the family Oligoneuriidae have molae of type II, investigated species of Polymitarcyidae and Euthyplociidae have type I or III. The molar types of Ephemerellidae and Heptageniidae are diversified as they are variable in their preferred diet. Assuming from the data gathered in the present study, the molar surface arrangement often corresponds with the type of ingested diet.

However, where dietary overlap is present although molar surface arrangement is different, or where molar surface structure is the same, although dietary preferences are different, some sort of phylogenetic constraint is to be expected.

The family Baetidae is unique in that no specific dietary components were recognised to explain the arrangement of their molar surface.

Several exceptions to the food composition and expected molar arrangement pattern were also found. Some species of Heptageniidae and Leptophlebiidae (e.g. *Heptagenia sulphurea*, *Rhithrogena semicolorata*, *Hermanella* sp.) exhibit molar surface of type I, however their diet consists of a very small percentage of mineral particles, with higher amounts of algae and/or detritus. The molar surface of type I here probably represents the ancestral state and the diversification of representatives of these families into various ecological niches is yet to be followed by specialisation of the molar surfaces.

However, variance in ingested diet composition can to a certain extent be caused by other factors involving ingested particles, such as the effect of the availability of food sources in the habitat.

From a comparison of obtained data with the present view of the phylogeny of Ephemeroptera and the composition of the ingested diet of the respective taxa, it is highly probable that all types of molar surface arrangement evolved several times independently as a result of selective pressure in forming diet processing solutions. As optimal diet processing is essential for the effective exploitation of the food sources, appropriate adaptations of the ultrastructure of the molar area takes place frequently according to the niche inhabited. Therefore a great heterogeneity in the type of molar surfaces can be observed throughout the order Ephemeroptera, as demonstrated above.

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