## Distribution of accessory gills in mayfly larvae (Insecta: Ephemeroptera: Siphlonuroidea, Eusetisura)

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#### Abstract

Mayfly larvae are usually characterised by the presence of paired abdominal gills on the first seven abdominal segments. Numerous taxa assigned to different families however possess additional membranous cuticular outgrowths on different body parts that are generally referred to as accessory gills. These accessory gills can be located on maxillae, labium, thoracic sterna or coxa. The present study compares the different structures of those accessory gills that occur in some taxa of Siphlonuroidea and Eusetisura. The homology of these outgrowths is discussed, and their possible phylogenetic relevance is evaluated.

K e y w o r d s : Accessory gills, coxal gills, labial gills, maxillary gills, sternal gills, mouthparts, larva, mayflies, morphology, phylogeny.

#### Zusammenfassung

Eintagsfliegenlarven besitzen in der Regel paarige Tracheenkiemen an den ersten sieben Abdominalsegmenten. Daneben kommen bei verschiedenen Familien zusätzlich membranöse Ausstülpungen der Kutikula an verschiedenen Körperteilen vor, die im allgemeinen als akzessorische Kiemen bezeichnet und gedeutet werden. Diese akzessorischen Kiemen können an Maxillen, Labium, thorakalen Sterna oder Coxen vorhanden sein. Die vorliegende Arbeit vergleicht die unterschiedliche Ausprägung dieser akzessorischen Kiemen bei Vertretern der Siphlonuroidea und Eusetisura. Die Homologie dieser Strukturen und deren mögliche phylogenetische Relevanz werden diskutiert.

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#### **1** Introduction

Aside from abdominal gills, mayfly larvae of certain taxa can be equipped with membranous outgrowths on such different body parts as coxae, thoracic sterna, maxillae, or labium. These outgrowths are commonly referred to as accessory gills, although their respiratory function has not been experimentally confirmed for most of these taxa. As these different accessory gills are mostly simple membranous tubules or tufts and rarely distributed within mayfly larvae, little attention has been paid to these structures. Information on accessory gills in mayfly larvae is scarce and widely scattered in descriptive literature.

Maxillary gills are, however, present throughout several families of Eusetisura and are also known from some families of Siphlonuroidea. The present study aims to investigate the distribution of different accessory gills in these taxa, to compare their structures, and to discuss their possible homology and phylogenetic relevance. The question of the presence of accessory gills in the groundplan of mayflies is also addressed.

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#### 2 Methods and materials

Photograph series with different focal depths were made from larval heads, legs and dissected mouth parts using a Leica DFC320 digital camera on a Leica Z16 APO microscope and subsequently processed with Leica LAS software to obtain photographs with extended depth of field. The combined photographs were then digitally enhanced using Adobe Photoshop 7.

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/Pd layer, examined with a ISI-SS40 scanning electron microscope at 10 kV, and subsequently photographed. Digital photographs were directly acquired by using DISS 5 (point electronic).

Specimens used for microscopic sectioning were dehydrated in ethanol and then stored three times at 50 °C in propan-2-ol for 24 hours each time. Then the material was gradually transferred to paraffin at 50 °C and finally transferred to Paraplast Plus<sup>TM</sup> at 60 °C. There the specimens were kept under vacuum conditions for 24 hours to optimise their penetration. Finally the material was embedded in Paraplast Plus<sup>TM</sup>. Sections of 5 µm thickness were obtained by using a Leitz 1516 rotation microtome. Sections were stained with Delafield's hematoxylin and counterstained with eosin. Photographs of sections were taken with a Canon Powershot S45 digital camera on a Leica DMR microscope and digitally processed with Adobe Photoshop 7.

The following material (all deposited in SMNS) was used in this study (number of examined specimens in brackets):

#### Nesameletidae

*Nesameletus ornatus*: New Zealand, Coromandel Peninsula, Whangaeterenga Stream, 6.IV.1993, STANICZEK leg. (20).

Ameletoides lacusalbinae: Australia, New South Wales, Hedley Creek, 9.II.1966, RIEK leg. (3).

#### Rallidentidae

*Rallidens mcfarlanei*: New Zealand, Northland, Wekaweka, Ngatahuna Stream, 10.IV.1993, STANICZEK leg. (5).

*Rallidens* sp.: New Zealand, South Canterbury, Otaio River, 23424, 56223, 8.II.2000, T. R. HITCHINGS leg. (1).

#### Siphluriscidae

Siphluriscus chinensis: China, Zhejiang Province, Long-Quan county, Guan-Pu-Yang, Nang-Ju, 15.VIII.1994, ZHOU leg. (1).

#### Coloburiscidae

Coloburiscus humeralis: New Zealand, Stewart Island, unnamed creek near Long Harry Hut, 4.III.1993, STANICZEK leg. (45).

Coloburiscoides sp.: Australia, Victoria, Powelltown, Ada River, 22.IV.1993, STANICZEK leg. (12).

*Murphyella needhami*: Chile, Nuble, Las Trancas, 2.III.1968, PEÑA & FLINT leg. (1).

#### Isonychiidae

*Isonychia tusculanensis*: USA, Tennessee, Great Smoky Mountains National Park, Little River at Metcalf Bottoms Trailhead, 7.XII.2001, JACOBUS leg. (3).

#### Oligoneuriidae

*Oligoneuriella rhenana*: Germany, Baden-Württemberg, Oberriexingen, River Enz, 22.VII.1996, STANICZEK leg. (20).

#### Abbreviations

- ca cardo cc chloride cell
- cx coxa
- cxg coxal gill

galeolacinia galc lbg labial gill lbr labrum md mandible mga anterior maxillary gill posterior maxillary gill mgp musculus tentorio-cardinalis mtc maxilla mx maxillary gill mxg paraglossa pgl plb labial palp postmentum pm pmx maxillary palp prm prementum stipes st trachea tra trochanter tr

#### **3 Results**

## Nesameletidae Nesameletus ornatus (Eaton, 1833) (Figs. 1–9)

The Nesameletidae is a small mayfly family of amphinotic distribution that includes the three described genera *Nesameletus* (New Zealand, see HITCHINGS & STANICZEK 2003), *Ameletoides* (Australia, see TILLYARD 1933), and *Metamonius* (South America, see MERCADO & ELLIOT 2004). Phenetically, these genera are very similar to each other, so the investigation of the New Zealand species *Nesameletus ornatus* may stand representatively for the entire Nesameletidae.

The head of *N. ornatus* is slightly opisthognathous, i. e. the mouth parts are directed in posteroventral direction (Fig. 1). As usual in mayflies, the larval maxilla is composed of cardo, a stipes bearing a 3-segmented maxillary palp, and distal maxillary lobes that are medially fused to form a so-called galeolacinia. Different from other siphlonuroid taxa, however, is the membranous connection between cardo and stipes that is everted to form a membranous tubule. The base of this tubule is particularly formed by the lateral and anterior membrane between cardo and stipes. The basal half of the tubule is directed dorsally. About at half length the tubule bends so that its apical half proceeds in anterolateral direction (Figs. 1-3). The tip of the maxillary tubule thus points outwards and often can be seen in lateral view (see also Fig. 9 for spatial location within the head). The tubule itself is entirely membranous and, except of very few tiny setae that are only recognised in high SEM magnification, not equipped with any other cuticular structure. The maxillary gill is also lacking chloride cells.

Slide preparations of the maxilla do not reveal any peculiarities in the structure of the maxillary gill, but in histological cross sections the branching of a small trachea from the main maxillary trachea and its subsequent invasion into the lumen of the maxillary gill can be observed (Figs. 4–8).

## Rallidentidae Rallidens mcfarlanei Penniket, 1966; R. sp. (Figs. 10–13)

The Rallidentidae is a family endemic to New Zealand with one described species, *Rallidens mcfarlanei*. However, the material for this investigation is partly also taken from a second species that will be described elsewhere (STANICZEK & HITCHINGS in prep.).

Larvae of *Rallidens* (Fig. 11) are superficially very similar to *Nesameletus*, but equipped with additional ventral fibrillar gill tufts at the abdominal gills. The mouthparts are orthognathous and of different shape: The entire maxilla of *Rallidens* appears rather broad when compared to other taxa of Siphlonuroidea. The stipes considerably widens in its apical half, the galeolacinia is almost of square shape (Figs. 12, 13). Stipes and galeolacinia only meet on a short distance in their medial halves. The insertation of the maxilla (Fig. 13), so the palp is not visible in posterior view (Fig. 12).

Apart from these differences, there is a maxillary gill developed that has a striking similarity to the corresponding structure in Nesameletidae. As in *Nesameletus*, the anterior and lateral membrane between cardo and stipes is everted to form a membranous tubule that is directed anterolaterally. In side view onto a head of *Rallidens*, the tip of the maxillary gill can be seen from externally (Fig. 10). The maxillary tubule is neither equipped with chloride cells nor with other cuticular structures, but a tracheal supply can be assumed.

## Siphluriscidae

## Siphluriscus chinensis Ulmer, 1920 (Figs. 14–18)

The enigmatic larva of the sole species of Siphuriscidae was only recently discovered by ZHOU and described in detail by ZHOU & PETERS (2003).

The larva of *Siphluriscus chinensis* resembles very much the larvae of Nesameletidae in general appearance and opisthognathous mouthparts, i. e. the mouthparts are slightly directed backwards (Fig. 14), the mandibular incisors are fused to form a scraping tool, and the maxilla is of similar proportions as in Nesameletidae.

As in Nesameletidae and Rallidentidae, a membranous outgrowth can be observed at the anterolateral membranous area between cardo and stipes (Figs. 15, 16). This anterior maxillary gill has a broad base that branches off after a short distance, and apically each branch splits off again. The entire anterior maxillary gill is directed dorsolaterally. In contrast to the previously decribed taxa, in *S. chinensis* another, posterior maxillary gill tuft can also be found (Fig. 17). In the single specimen that was available to me this posterior maxillary gill is pectinate, i. e. comblike. On both anterior and posterior maxillary gill, coniform chloride cells are scattered on the membranous surface (Fig. 15).

The general shape of the labium, especially the elongated postmentum, is also very similar to *Nesameletus*, but the labium of *Siphluriscus* laterally bears two distinct pairs of membranous fringes with successively branched tubules (Fig. 18). As the medial insertion of the long musculus tentorio-praementalis clearly denotes the border between post- and prementum, it becomes obvious that both pairs of labial gills originate at the lateral borders of the postmentum. On the labial gill surface also numerous coniform chloride cells can be observed.

Finally, there are also coxal gill fringes developed in the pro- and mesothorax at the medial membrane between each coxa and sternal sclerite (Fig. 14). The coxal gills are also supplied with a trachea. Tracheation of maxillary and labial gills can also be observed.

## Coloburiscidae

## Coloburiscus humeralis Walker, 1853; Coloburiscoides sp.; Murphyella needhami Lestage, 1930 (Figs. 19–31)

Coloburiscidae is another amphinotic family with three described genera: *Coloburiscus* (New Zealand, see WISELY 1961), *Coloburiscoides* (Australia, see SUTER et al. 2009), and *Murphyella* (South America, see Dominguez et al. 2006). In the different genera of Coloburiscidae there is a variety of different accessory gills present, although the general appearance of these genera is very similar. This is for instance reflected in the mouthparts by a peculiar arrangement of the maxillary and labial palps (Figs. 20, 26, 28). The palps insert on the posterior (aboral) side of stipes and prementum. The basal palp segments are directed downwards. The apical palp segments are elongated and directed anteriorly, thus covering the mouthparts ventrally (Figs. 20, 25, 31).

In *Coloburiscus humeralis* (Figs. 19–23) the posterior (aboral) side of the maxilla is equipped with two tubular, membranous outgrowths that have a short common stem (Fig. 20). This common stem originates at the membranous area between cardo and stipes, medially extending to the likewise membranous area between maxilla and labium (Fig. 20). Each branch of the maxillary gill forms a simple, membranous, finger-like protuberance. The maxillary gills are directed ventromedially. The membranes of both branches and stem are pigmented in some specimens; internally the gill is supplied with a trachea that branches off the main trachea supplying the maxilla (Fig. 21). There are no coxal or labial gill tubules present.



**Figs. 1–3.** *Nesameletus ornatus.* – **1**. Exarticulated head in posterior view. **2**. Left maxilla in posterior view. **3**. Left maxilla in anterior view. – Abbreviations see chapter 2. – Scale bars: 0.2 mm.



**Figs. 4–9.** *Nesameletus ornatus.* – **4–8**. Histological cross sections through left maxilla from caudal to cranial. **9**. Histological cross section through lower head. – Abbreviations see chapter 2. – Scale bars:  $50 \,\mu m (4-8)$ ,  $70 \,\mu m (9)$ .



**Figs. 10–13.** *Rallidens* spp. – **10**. *R. mcfarlanei*, head in lateral view. **11**. *R. mcfarlanei*, larva in dorsal view. **12**. *R.* sp., left maxilla in posterior view. **13**. *R.* sp., left maxilla in anterior view. – Abbreviations see chapter 2. – Scale bars: 0.2 mm (10), 2 mm (11), 0.1 mm (12, 13).



**Figs. 14–18.** *Siphluriscus chinensis.* – **14**. Head and thorax in ventral view. **15**. Anterior maxillary gills. **16**. Right maxilla in anterior view. **17**. Right maxilla in posterior view. **18**. Labium in posterior view. – Abbreviations see chapter 2. – Scale bars:  $250 \mu m$  (14),  $100 \mu m$  (15),  $50 \mu m$  (16, 17),  $200 \mu m$  (18).



**Figs. 19–23.** *Coloburiscus humeralis.* – **19**. Larva in lateral view. **20**. Exarticulated head in posterior view. **21**. Right maxilla in medial view. **22**. Labium in lateral view. **23**. Left maxilla in medial view. – Abbreviations see chapter 2. – Scale bars: 2 mm (19), 0.5 mm (20), 0.2 mm (21), 0.5 mm (22), 40 µm (23).



**Figs. 24–29.** *Coloburiscoides* sp. – **24**. Larva in lateral view. **25**. Head and thorax in ventral view. **26**. Exarticulated head in posterior view. **27**. Right maxilla in medial view. **28**. Labium in posterior view. **29**. Labium in lateral view. – Abbreviations see chapter 2. – Scale bars: 1 mm (24), 0.5 mm (25–29).



**Figs. 30–34.** *Murphyella needhami, Isonychia tusculanensis.* – **30**. *M. needhami*, head and thorax in lateral view. **31**. *M. needhami*, head and thorax in ventral view. **32**. *I. tusculanensis*, head and thorax in lateral view. **33**. *I. tusculanensis*, left foreleg in anterior view. **34**. *I. tusculanensis*, right foreleg in anterior view. – Abbreviations see chapter 2. – Scale bars: 1 mm (30, 31, 34), 0.5 mm (32), 0.1 mm (33).



**Figs. 35–38.** *Isonychia tusculanensis.* – **35**. Coxal gill. **36**. Base of maxilla with maxillary gill. **37**, **38**. Right maxilla in posterior view. – Abbreviations see chapter 2. – Scale bars: 0.2 mm (35, 36, 38), 0.3 mm (37).



**Figs. 39–43.** *Oligoneuriella rhenana.* – **39**. Larva in lateral view. **40**. Histological horizontal section through head. **41**, **42**. Horizontal sections through maxilla at different levels. **43**. Section through maxillary gill tubules. – Abbreviations see chapter 2. – Scale bars: 2 mm(39), 70 µm(40), 150 µm(41, 42), 50 µm(43).



**Figs. 44–47.** *Oligoneuriella rhenana.* – **44**. Head and thorax in ventral view. **45**. Chloride cells on surface of maxillary gills. **46**. Left maxilla in dorsal view. **47**. Right maxilla in ventral view. – Abbreviations see chapter 2. – Scale bars:  $400 \mu m$  (44),  $50 \mu m$  (45),  $500 \mu m$  (46, 47).

In *Coloburiscoides* sp. (Fig. 24) two pairs of simple tubular outgrowths can be observed, each at the posterior (aboral) side of maxilla and labium (Fig. 26). Both tubules have separate origins: The dorsal tubule originates from the same position as the maxillary gill in *Coloburiscus*, namely from the membranous area close to cardo and basal part of stipes (Fig. 27). The ventral tubule is approximated to the labium. It originates laterally of the labial palp at the lateral side of the basal prementum and is also darkly pigmented (Figs. 28, 29). A trachea can be observed that deeply reaches into the lumen of the tubules (Fig. 27). Both gill tubules are not equipped with chloride cells, and on the surface only very few short setae can be observed.

A yet different arrangement of these outgrowths can be found in the mouthparts of the sole known species of the third genus, Murphyella needhami. While this is the only described mayfly species entirely lacking abdominal gills, there are multiple accessory gills present. The distribution of these accessory gills can be best described as a mix of characters that occur in the previous two genera: The maxillary gill has the same structure as in Coloburiscus, a pair of two tubules with common stem, but additionally a labial gill as in Coloburiscoides can be observed. Both labial and maxillary gills can be seen in lateral view (Fig. 30). Additionally, there is also a pair of procoxal gills present. Each gill originates as simple short tubule from the inner membrane between procoxa and prosternum (Fig. 31). Finally, there are even three unpaired sternal gills present that protrude as medial tubule from the middle of each thoracic sternum (Fig. 31). However, ŠTYS & SOLDÁN (1980) and ZHOU (2010) report certain variability in the formation of maxillary and labial gills in this species.

#### Isonychiidae

## Isonychia tusculanensis Berner, 1948 (Figs. 32–38)

The Holarctic Isonychiidae is a widespread taxon with currently 34 described species in a single genus (see KONDRATIEFF & VOSHELL 1984, TIUNOVA et al. 2004).

The maxilla of the North American species *Isonychia tusculanensis* (Fig. 32) bears on its posterior side a membranous gill tuft (Figs. 37, 38). The maxillary gill tuft has a common stem that originates within the posterior maxillary membrane medially of the stipes and ventrally of the cardo. The distal part of the accessory gill is split up into a bundle of membranous tubules. The maxillary gill is supplied with a thick and dark pigmented tracheal branch that invades deeply into the accessory gill (Fig. 38). *I. tusculanensis* is also equipped with a pair of prothoracic coxal gills that are located medially of each procoxa (Figs. 33, 34, 35). Each coxal gill is orientated ventrally and distally split up into a bunch of tubules. The gill tufts are supplied by a darkly pigmented tracheal branch that splits off into numerous, also darkly pigmented tracheoles (Fig. 38). Although there are numerous coniform chloride cells present on trochanter, coxa, and stipes, the chloride cells do not extend to the maxillary and coxal gill membranes (Figs. 35, 36).

## Oligoneuriidae Oligoneuriella rhenana (Imhoff, 1852) (Figs. 39–47)

The Oligoneuriidae is – apart from the Australian Realm – worldwide distributed with a distributional centre in the Oriental Realm. It includes about 50 described species in 12 genera (see KLUGE 2004, PESCADOR & PETERS 2007).

The head of the European species Oligoneuriella rhenana (Fig. 39) is prognathous, i. e. the mouthparts are directed anteriorly. They are ventrally covered by a highly modified labial shield (Fig. 44), so the maxilla is hardly seen in ventral view. Only the voluminous maxillary gill tufts are visible covering the prosternum between the forecoxae. The maxillary gills are directed posteroventrally and surround the labial shield from posterior. The maxillary gill tuft has a common stem that occupies the entire membranous area medially of the stipes (Figs. 46, 47). In aboral (ventral) view the cardo is not even visible and superposed by the massive development of the maxillary gill (Fig. 47). Only in oral (dorsal) view a cardinal sclerite can be observed (Fig. 46). High SEM magnification reveals that the maxillary gills are entirely laced with numerous coniform chloride cells (Fig. 45). Between the coniform chloride cells, there are fewer bulbiform chloride cells scattered. Histological cross sections show the massive development of the trachea supplying the maxillary gills (Figs. 40-43). Almost the entire lumen of the maxillary gills is filled by the voluminous maxillary trachea that branches into multiple tracheoles, deeply invading each maxillary tubule (Fig. 43).

#### **4** Discussion

Membranous tubules or tufts associated with other tagmata than the abdomen are known to occur in six mayfly families and are commonly referred to as accessory gills. In Baetidae, these structures are only present in a few genera. ZHOU (2010) lists species of *Afrobaetodes*, *Baetiella*, *Baetodes*, *Dicentroptilum*, and *Heterocloeon* that possess accessory gills. They are mostly located between coxae and sterna, or sometimes between coxae and trochanteres. Apart from these genera, accessory gills in Baetidae are also known from *Camelobaetidius* (Dominguez et al. 2006). ZHOU (2010) also noted additional maxillary gills in *Afrobaetodes berneri*. There is even the presence of a tubular outgrowth on the first segment of the labial palp reported in a species of *Afrobaetodes* (GATTOLLIAT & SARTORI 1999). So there are at least three different types of accessory gills reported in Baetidae. However, the vast majority of Baetidae do not possess accessory gills at all.

In contrast, all species of six other families of Ephemeroptera wear some kind of accessory gills: In the Siphlonuroidea it is the Nesameletidae, Rallidentidae, and Siphluriscidae, in the Eusetisura it is Coloburiscidae, Isonychiidae, and Oligoneuriidae. Apart from their accessory gills, these six families each have several morphological characters that can be interpreted as autapomorphic (KLUGE 2004), so we can consider each of these taxa to be monophyletic. The structure of the respective gills within the different families is quite uniform, so we can also assume that these structures are each homologous within the respective families. As these specific gills are reported from each species in the respective families, we can certainly also hypothesise the presence of specific accessory gills in the respective stem species of these taxa and thus attribute the specific accessory gills to the respective groundplans of these six groups. This leads to the questions if the different types of accessory gills are homologous to each other, if they could be interpreted as synapomorphic characters that account for a closer phylogenetic relationship of these taxa, if accessory gills are even plesiomorphic and already present in the groundplan of Ephemeroptera, or if these structures have evolved several times independently within the different groups. All these hypotheses were postulated by different authors, but to evaluate this problem, we have first to take a closer look at the different types of gills in the different families.

Though the maxillary tubules in Nesameletidae and Rallidentidae are rather simple structures, they resemble each other in position (both gills originate at the anterolateral edge of the maxilla), shape, and spatial orientation. Furthermore both maxillary gills lack chloride cells, are supplied by a branch of the maxillary trachea, and are equipped with few short setae. So these similarities would indeed point to an interpretation of the maxillary tubules of Nesameletidae and Rallidentidae as homologous structures, and this was also a reason for PENNIKET (1966) to assume a closer relationship of these taxa. At that time the larva of Siphluriscus chinensis was not yet known, which shares striking similarities of mandible, maxilla and labium with Nesameletidae (ZHOU & PETERS 2003). Based on these characters, HITCHINGS & STANICZEK (2003) rather assumed a phylogenetic sequence of Rallidentidae + (Nesameletidae + Siphluriscidae). The same phylogeny was confirmed by processing a morphological dataset in a comprehensive cladistic analysis of Ephemeroptera by OGDEN et al. (2009). However, adding molecular data to this morphological dataset suggested a sistergroup relationship of Nesameletidae + Rallidentidae, placing Siphluriscidae as sister to all other Ephemeroptera in the combined analysis (Fig. 48).

The presence of ventral abdominal gill tufts in Rallidentidae and Eusetisura, similarities in the wing venations of Rallidentidae and Isonychiidae, and the presence of maxillary gills in Nesameletidae, Rallidentidae, and Eusetisura led DEMOULIN (1969) to conclude a closer relationship of these taxa. However, KLUGE (2004) realised that the maxillary gills in Coloburiscidae, Isonychiidae, and Oligoneuriidae have a different origin from the maxillary gills present in Nesameletidae and Rallidentidae. In the latter, the maxillary gills evert from the anterolateral membrane between cardo and stipes, while in the former three families the maxillary gills originate from the posterior (aboral) side of the maxilla. Consequently, KLUGE (2004) assumed an independent origin of the maxillary gills in these groups and assessed the maxillary gills of Coloburiscidae, Isonychiidae, and Oligoneuriidae as autapomorphic character of Eusetisura.

In his phylogeny of Heptagenioidea, McCAFFERTY (1991) assumed maxillary gills as a derived groundplan character of this group and suggested their subsequent loss in Heptageniidae. Earlier also EDMUNDS (1973) assumed a common phylogenetic origin of Coloburiscidae, Isonychiidae, and Oligoneuriidae. While these authors differ in the specific branching sequences of these taxa, their underlying assumption is certainly a homology and derived character state of the maxillary gills.

However, if the phylogeny of OGDEN et al. (2009) is correct in assuming a paraphyletic Eusetisura, then the accessory gills in these taxa are either convergent developments, or are rather plesiomorphic characters that must be traced back to the groundplan of Ephemeroptera.

The evaluation of coxal and thoracic gills is even more difficult, as tufted coxal gills are only scattered within Baetidae and universally only present in Isonychiidae and Siphluriscidae. In Coloburiscidae, only *Murphyella needhami* has simple tubular coxal gills developed. So regardless if we consider Eusetisura as monophyletic or paraphyletic, the occurrence of coxal gills in the different taxa can likewise only be interpreted either as independently evolved or as a remnant of a plesiomorphic groundplan condition.

The latter view has been put forward by ZHOU (2010) who not only assumes a serial homology of coxal, labial and maxillary gills in all these taxa, but also assumes their presence already in the stem species of mayflies, thus interpreting these structures as plesiomorphic ground-plan characters of Ephemeroptera. Following KUKALOVA-PECK's (1983) ideas on insect leg segmentation, he derives coxal, labial and maxillary gills from "coxal rami (exites or endites)" of early Pterygota.



**Fig. 48.** Distribution of accessory gills in mayfly larvae. – Phylogeny after OGDEN et al. (2009). Explanation of squares and circles see chapter 4.

This theory at first hand seems to be supported by the phylogenetic analysis of Ogden et al. (2009), in which the first three branches are represented by Siphluriscidae, Baetidae, and Isonychiidae. However, if we assume the presence of maxillary and coxal gills in the stem species of Ephemeroptera, we must also assume a convergent loss of these structures at least seven times (indicated by circles in Fig. 48). On the other hand, if we postulate the independent acquisition of these characters within Ephemeroptera, we would have to assume this at least six times (indicated by squares in Fig. 48). Apart from parsimony, there are also other difficulties with the derivation of coxal and maxillary gills from leg exites: coxal gills of all investigated mayfly larvae always originate from the ventral (inner) side of the coxosternal membrane, but the leg exites as shown by KUKALOVÁ-PECK (1983) in Diaphanopterodea and later in generalised schemes of insect legs (KUKALOVÁ-PECK 1987) are always located at the dorsal (outer) sides of the leg segments. Thoracic styli in extant Archaeognatha, often also interpreted as remnants of leg exites, are also located at the dorsal side of the coxa. It is also difficult to imagine the evolutionary development of accessory gills from arthropod endites: In Crustacea, endites are generally associated with food processing and not with oxygen intake, so there are rather heavily sclerotised structures developed than membranous devices. Another point is the obvious lack of coxal gills in otherwise well preserved early Carboniferous (STANICZEK 2007) or Permian (KUKALOVÁ 1968) stem group fossils, in which most delicate abdominal gills are preserved and clearly visible. Finally, there are neither coxal endites reported from primarily wingless insects nor from Odonata or basal Neoptera. All these considerations rather do not point to the presence of coxal or maxillary gills in the groundplan of Ephemeroptera. After all, the sternal thoracic outgrowths in Murphyella needhami definitely cannot be interpreted as remnants of leg exites, but must be rather explained as new evolutionary acquisition. These tubular outgrowths are rather simple structures, so convergent developments may easily have taken place. A multiple convergent development of tracheal gills of different body parts is also assumed within other aquatic insect orders like Plecoptera (Zwick 2009). I do not categorically rule out the possibility that underlying discontinuous activity of ancient leg genes (see STURM 1994) may trigger the development of coxal or maxillary gills when needed, but I do not necessarily assume their presence per se in the groundplan of Ephemeroptera. However, with an improved phylogeny of Baetidae and Ephemeroptera in general it would be easier to assess if accessory gills were already present in earliest Baetidae or successively developed in the different genera.

Though these membranous accessory structures are generally referred to as accessory gills, their actual role in respiration has, to my knowledge, never been tested experimentally. The fact that all coxal and maxillary gills are membranous and supplied by tracheal branches makes it probable that it is indeed their prevailing function to aid in respiration. ZHOU (2010) also discusses a possible function in nutrition by filtering food particles, but I do not consider this to be likely, as the gill tubules are much too thick to serve as filtering device for detritus. I also do not know of any observation that would support this hypothesis. On the contrary, ELPERS & TOMKA (1992) in their detailed observation of the feeding behaviour of *O. rhenana* clearly proved that maxillary gills are not involved in food acquisition.

Osmoregulatory function can be ruled out for the accessory gills of most taxa, as only in Siphluriscidae and Oligoneuriidae the presence of chloride cells on the gill surface could be confirmed. In Oligoneuriidae, however, this may be a significant function of the voluminous gill tufts, as they are densely covered with coniform and bulbiform chloride cells. The main function of accessory gills in general is probably indeed to support respiration.

## 5 Conclusions

Recent phylogenetic studies (OGDEN et al. 2009) support a sistergroup relationship of Nesameletidae + Rallidentidae, but do not support a monophyletic Eusetisura. Although Siphluriscidae, Baetidae, and Isonychiidae in this phylogeny represent three basal branches of Ephemeroptera, the most parsimonious assumption would still be an independent acquisition of accessory gills in these taxa. This is also supported by the different position and fine structure of maxillary gills in Siphlonuroidea and Setisura. The main function of accessory gills may indeed be aiding in respiration, and a role in osmoregulation can be assumed for Oligoneuriidae.

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