Biol. Inl. Wat. Suppl. 3, 157-168, M. Yoshimura and Y. Takemon (eds.)

SEM and Digital Morphology of *Siphluriscus chinensis* Ulmer (Ephemeroptera: Siphluriscidae)

Changfa ZHOU[1]*

[1]The Key Laboratory of Jiangsu Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing 210023, China.
(*) Corresponding author: zhouchangfa@njnu.edu.cn

Abstract

The SEM and digital photos of selected structures on both nymphs and adults of *Siphluriscus chinensis* are provided for the first time. They present some details of those structures, clarify some confusion and confirm that this species possesses a large number of plesiomorphies, and as well some automorphies. Morphologically, the sister group relationship between Siphluriscidae and Nesameletidae is weakened by their different mandibles and symplesiomorphies or convergence. In the light of these facts, the evolutionary trends and character changing direction should be reassumed or revalued in the Ephemeroptera.

Key words: *Siphluriscus chinensis*, Siphluriscidae, Nesameletidae, Ephemeroptera, Evolution

Introduction

Zhou and Peters (2003) described the nymphs and provided more imaginal characters of *Siphluriscus chinensis* Ulmer (1920). Based on its characteristics, this species as well as the family Siphluriscidae is believed to represent the most plesiomorphic clade in Ephemeroptera. This point has been restated and confirmed by several others (Kluge, 2004; Zhou, 2007; Ogden et al., 2009).

In recent years, the reconstruction of higher phylogeny within the Ephemeroptera has based on more detailed morphology, like the dentiseta of the maxillae and sutures on the imaginal thorax (sensu Kluge, 1998, 2004). Even in the discussion on phylogenetic position of the order Ephemeroptera in the Insecta, the nymphal structures such as mandibles are also used in some way (Staniczek, 2000). However, in the paper of Zhou and Peters (2003), those structures are not shown clearly because they cannot be seen under the optical microscope. Additionally, based on a combined molecular and morphological database, Ogden et al. (2009) provided a new phylogenetic hypothesis for the Ephemeroptera. In their summary tree, the Siphluriscidae were separated from other mayflies as a sister group. In the present study, scanning electronic microscope (SEM) photos and digital pictures of *Siphluriscus chinensis* are presented. The accessory gill details of this species have been discussed by Staniczek (2010) and Zhou (2010) to certain degree. Hopefully, those detailed characters in this series of related papers can be used in discussions of phylogeny and clarify some problems in mayfly classification and character polarization.

Results

Siphluriscus chinensis Ulmer, 1920

(Figs. 1-28)

Adult: Thorax (dorsal, ventral and lateral view, Figs. 1-3)

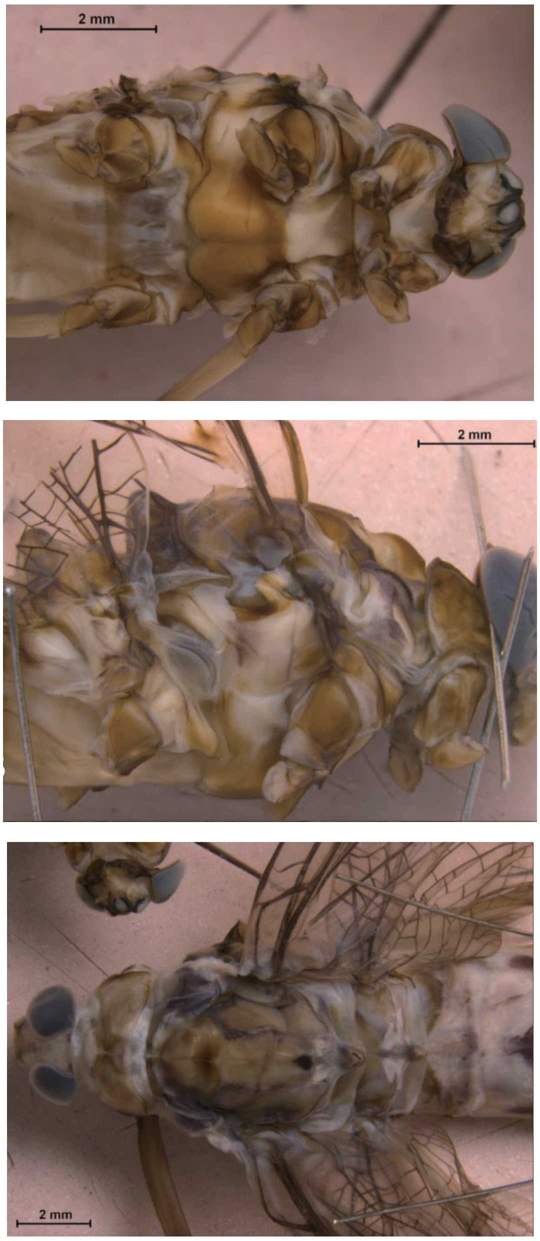


Fig. 1-3. Female imago of *Siphluriscus chinensis* (1. ventral view, 2. lateral view, 3. dorsal view).

Fig. 4-28. Nymph of *Siphluriscus chinensis*.

Nymph: Body (Fig. 4): minnow-like, streamlined, resemble general pattern of Siphlonuroidea, but much larger than other Chinese mayflies. The head definitely hypognathous, tail very strong.

Antennae (Fig. 5): compared to other mayflies, antennae of *Siphluriscus chinensis* are very short, 8 segmented. Pedicel shortest among all segments, while scape broadest and longest. Flagella 6 segmented, progressively longer from first segment to fifth one. The apical segment much slimmer than others, some hair-like.

Fig. 4. Habitus (body, lateral view)

Fig. 5. Antenna.

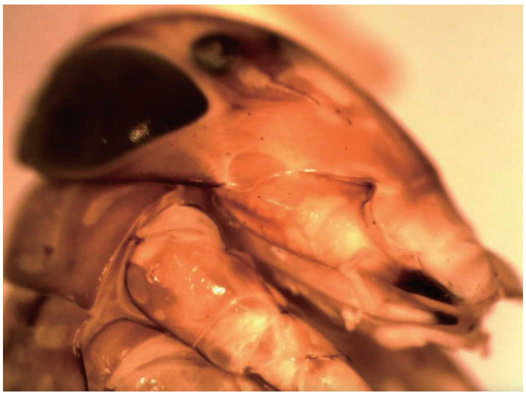
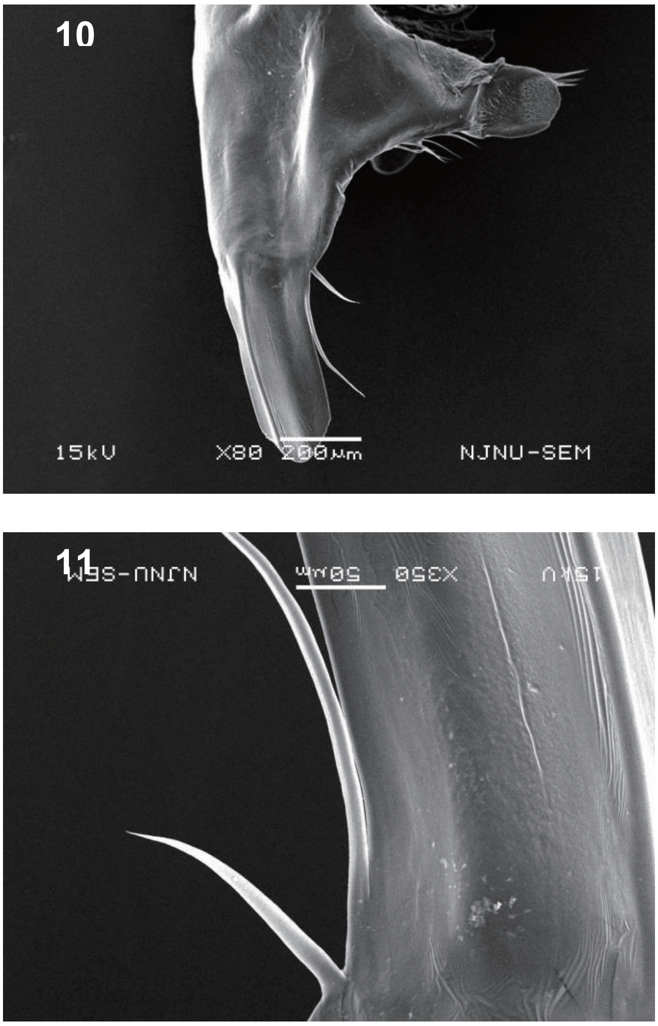


Fig. 6. Head (lateral view)

Figs. 7-9. Labrum (7. dorsal view, 8. ventral view, 9. center part enlarged in ventral view)



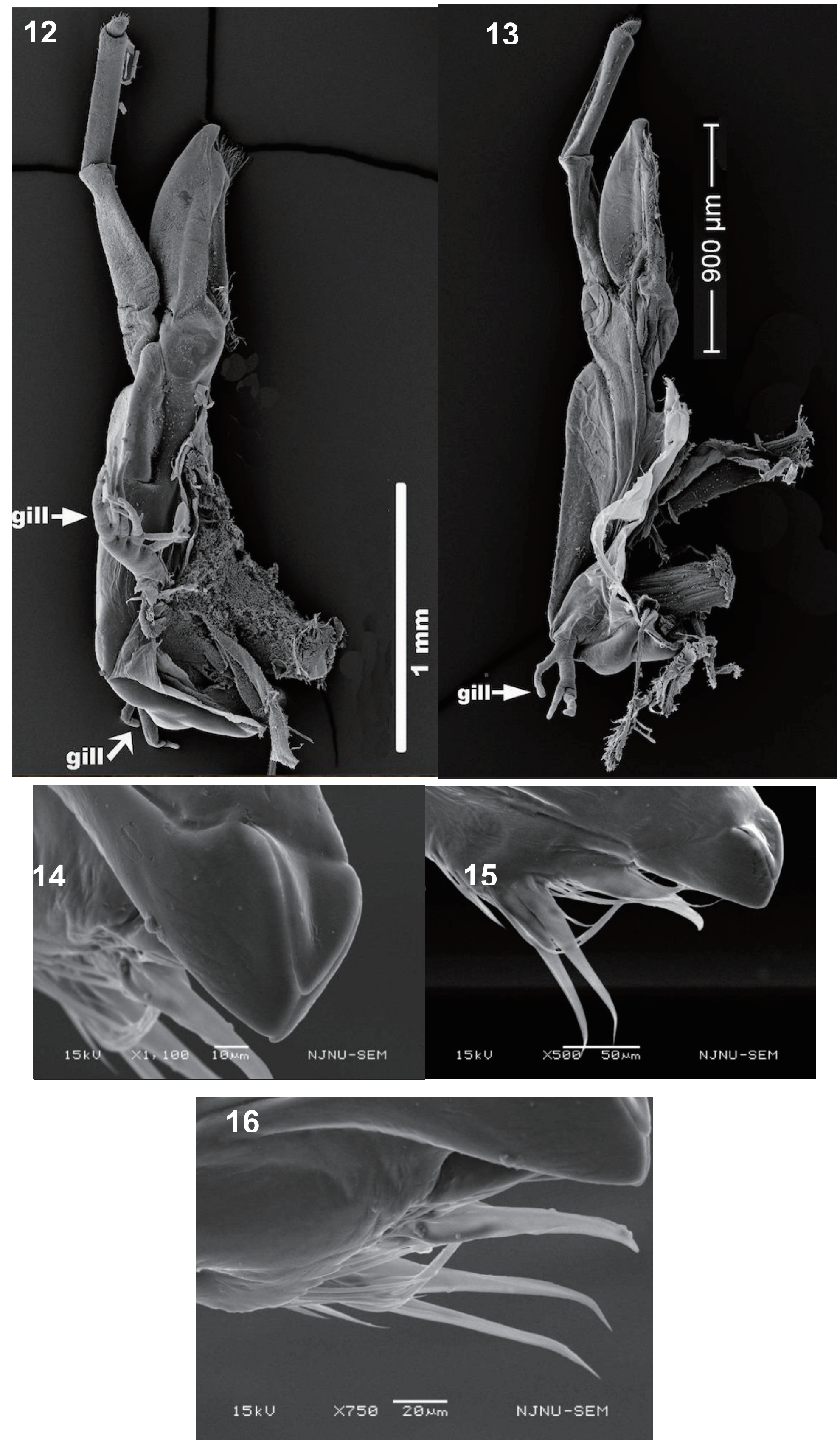
Figs. 10-11. Right mandible (10. whole view, 11. incisor)

Clypeus (Fig. 6): enlarged dramatically, looks like a distinct lobe above labrum.

Labrum (Figs. 6, 7-9): clearly divided into two lobes, they are separated and connected by a median Y-shaped connective tissue. All of them have setae on both dorsal and ventral surfaces as well as free margins, those on ventral surface slightly denser than dorsal surface. Marginal setae longer and more dense than others.

Mandible (Figs. 6, 10-11): outer incisor blade-like; inner incisor spine-like, with common base with outer one; prostheca, also one spine-like projection, located very close to inner incisor; a row of shorter and smaller hairs between mola and prostheca; a tuft of spines at apex of mola; surface of mola with dense bristles, grooves and ridges as well.

Maxilla (Figs. 12-16): with two tufts of accessory gills, one ventral, one dorsal, both of them located on the membranous area between stipes and cardo, the ventral one actually between maxilla and labium (Figs. 12, 18). A clear longitudinal suture between galea and lacinia. Setae on maxillary surface are very tiny except those on inner margin of galea-lacinia. Three larger maxillary canines (sensu Kluge, 1998) at medio-apical corner. Three additional long and big spines situated near canines, and some smaller and soft setae around and near the spines, they located irregularly. A row of smaller setae situated



Figs. 12-16. Left maxilla (12. dorsal view, 13. ventral view, 14. maxillary canines, 15. dorsal view of spines on the top of maxilla, 16. ventral view of spines on the top of maxilla)

Fig. 17. Hypopharynx (front view)

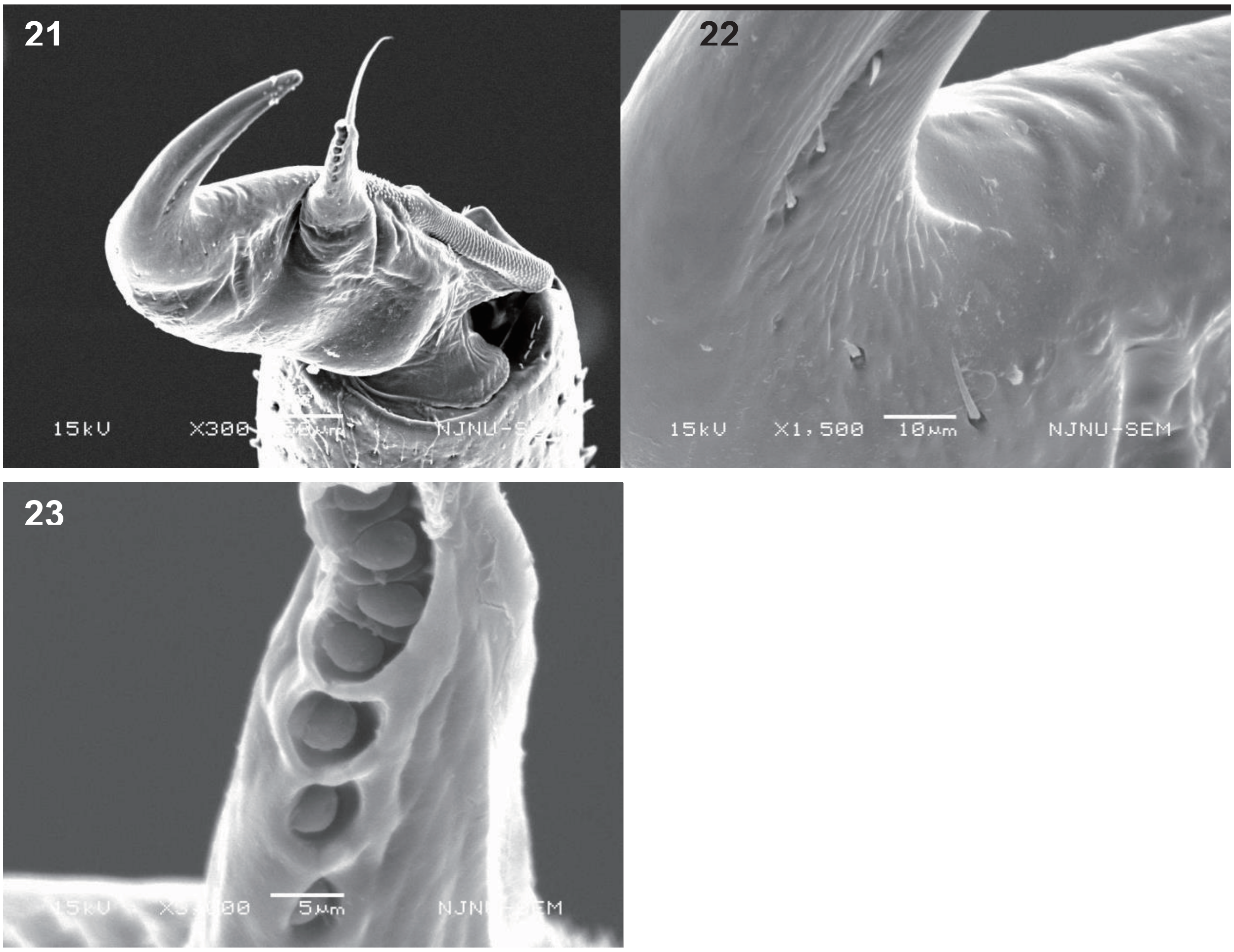
Figs. 18-19. Labium (18. ventral view, 19. apex of the labial palpus)

along inner margin of galea-lacinia. Maxillary palpi 3-segmented, with sparse and tiny setae on surface, those of apex slightly longer; apical segment much shorter than others, with more setae.

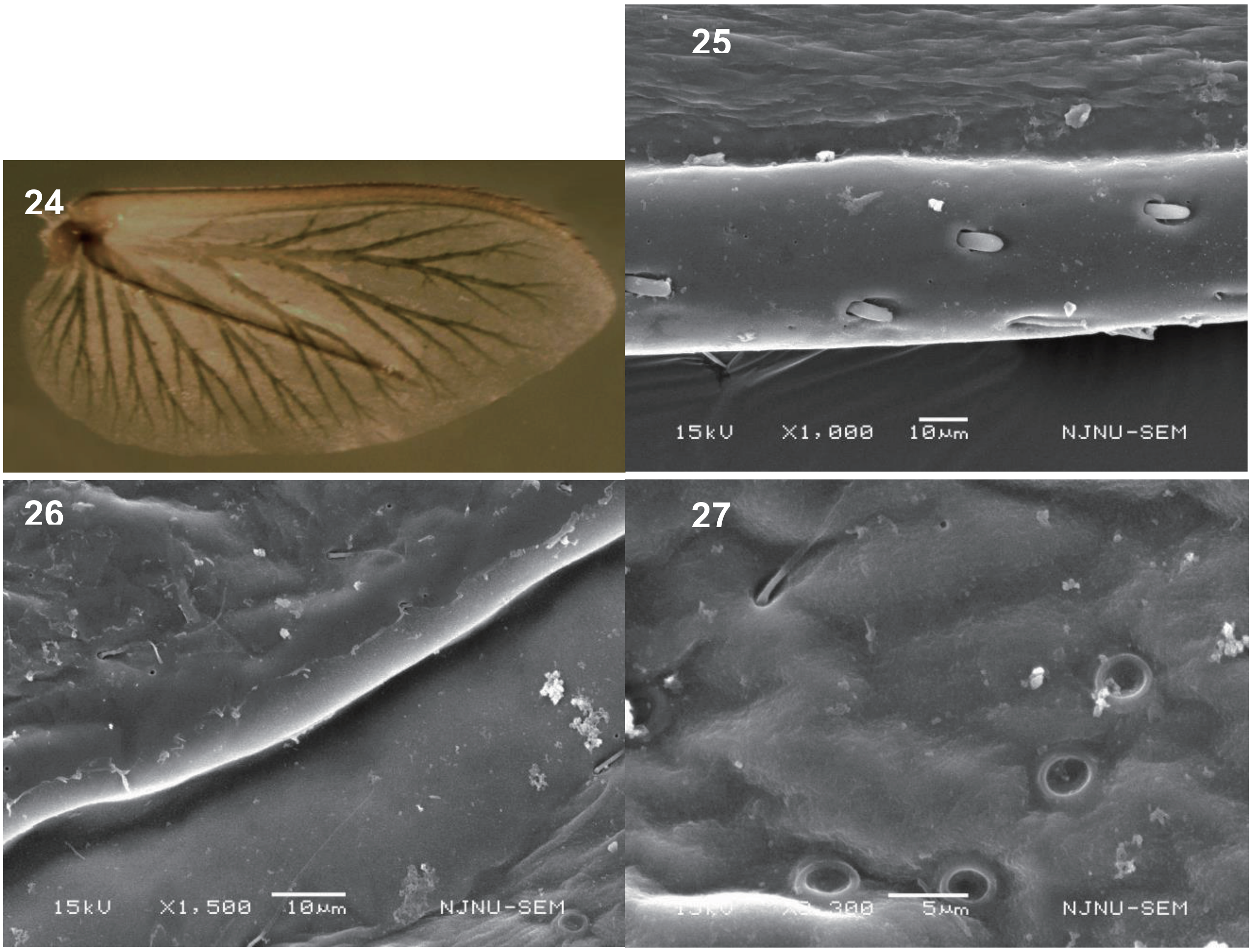
Hypopharynx (Fig. 17): three lobes with dense but tiny setae on free margins only.

Labium （Figs. 18-19）: a pair of heavily branched accessory gills on the lateral margin of postmentum; dense setae on surface of labium and labial palpi; glossae and paraglossae long and narrow; labial palpus 3 segmented, apical one shortest, with more setae on surface and a small apical projection, no suture between apical segment and projection.

Fig. 20. Legs



Figs. 21-23. Claw (21. front view of claw, 22. surface of claw, 23. inner surface of claw appendage)



Figs. 24-27. Gills (24. gill shape, 25. leading margin, 26. surface sclerotized line, 27. surface pits)

Legs (Fig. 20): fore- and midcoxae with gill tufts respectively; femora of all legs with obvious 3 distal lobes; tarsi slight shorter than tibiae; patellar-tibial fusion suture of mid- and hind tibiae clear.

Claw (Figs. 21-23): with very tiny setae on surface; the moveable dactyl-like appendages of all claws with a row of small holes or pits on inner surface, some sphere-shaped materials in every pit.

Gills (Figs. 24-27): leading margin sclerotized obviously, with tiny blunt spines; an additionally sclerotized line on surface; some tiny setae and circle shallow pits situated on gills surface irregularly.

Paraproct (Fig. 28): with obvious and sharp spine-like projections on mesal corner, no suture between them.

Discussion

The adults of *Siphluriscus chinensis* have a long series of plesiomorphies, such as larger body, longer hindwings (Zhou and Peters, 2003), independent ScA brace, relic traces of basal Rs, MA and Cu stem (Zhou, 2007). According to Kluge et al. (1995), the followings are also plesiomorphic: the mesonotum with clear mesonotal suture, furcasternal protuberances of mesothorax contiguous over their entire length, paracoxal suture dividing episternum completely, tarsi 5-segmented.

Among the characters used by McCafferty and Wang (1994), some primitive ones are also found in *Siphluriscus*. For instance, the shorter tibiae, penes furcated, forewing with long cubital field, well developed terminal filament, larger clypeus, hindwing with MA forked at mid-length, forewing with MP stem not connected to CuA by crossveins. However, most of these characters should be used further very carefully because they can vary dramatically within one family or evolved or lost frequently (Zhou and Peters, 2003).

Compared to imaginal characters, the status of most nymphal characters of *Siphluriscus* are more controversial. Only two characters are considered here as plesiomorphies with certain confidence: the larger body and the labrum. The divided two lobes of labrum must represent the primitive condition of their two separated ancestral appendages. In addition, from fossil records, the older mayflies were usually larger (Kukalová-Peck, 1985). Paraproct having obvious spines in *Siphluriscus* may be a possible plesiomorphy (Zhou and Peters, 2003). The suture near the lateral margin of head capsule may be plesiomorphic too if the head capsule is fused from several sclerites.

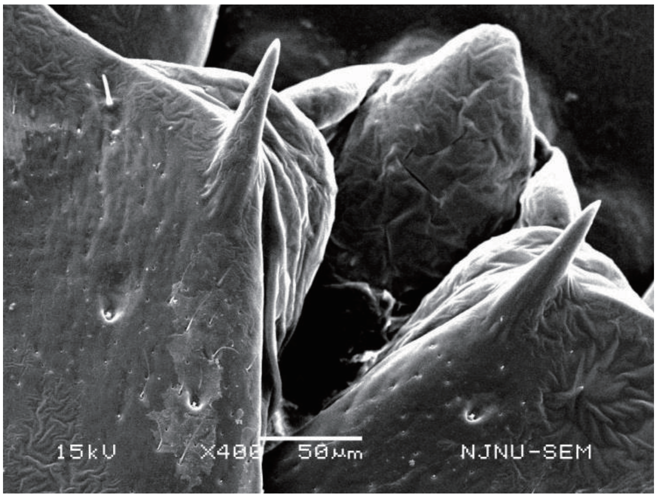


Fig. 28. Paraproct

Kluge et al. (1995) suggested that the nymphal streamlined body and paddle-like tail with three filaments plus long setae may be plesiomorphic. Riek (1973) and Kluge et al. (1995) thought the plate-like gill with two sclerotized lines was another nymphal plesiomorphy. However, if we accept that the mayflies as winged insects that originated from a terrestrial ancestor, at least in the beginning, they cannot have strong and perfect swimming abilities or bodies. Kukalová-Peck (1991, 2008) hypothesized that the mayfly nymphal gills have a similar origin to the imaginal wings. If this is true, like the counterparts of imagos, the swimming gills must have evolved over a very long time to their present form.

The antennae of *Siphluriscus* are short and have less segments than in the Order otherwise. If one assumes that a more segmented flagella is a derived character, that of *Siphluriscus* is a plesiomorphy. However, the antennae are among the most variable structures. Siphluroidea are considered a papraphyletic group, all families have short antennae, even the Prosopistomatidae and Baetiscidae have very short antennae.

Like *Nesameletus*, *Siphluriscus* have a short apical segment of the maxillary palpi plus long and narrow glossae and paraglossae of the labium. This does mean they are similar. From figures 46-47 of Hitchings and Staniczek (2003) and figure 47 of Kluge et al. (1995), there are 3 dentisetae, sensu Kluge et al. (1995) and Kluge (1998, 2004) on the apex of maxillae, the first one being much larger and thicker than other two. However, in *Siphluriscus* that seta is only slightly thicker than the latter two. Unlike *Nesameletus*, the dentisetae of *Siphluriscus* located on a disc could not be found. Both of *Nesameletus* and *Siphluriscus* have a median suture on galealacinia of maxillae, but it is a plesiomorphy (Ogden et al., 2009). The labial palpi of *Siphluriscus* have additional apical projections.

The left and right mandibles of *Siphluriscus* are symmetric. Near to the blade-like outer incisor, there are two spine-like structures. Because the longer one has the common base of the outer incisor, it was interpreted as the inner incisor by Zhou and Peters

(2003) and in the present study. Nymphs of the three genera of Nesameletidae have particular kinds of mandibles. According to Hitchings and Staniczek (2003) and the specimens examined in this study, all of them are similar in structure: the prostheca of the left mandible consists of three separate bristles while that of right mandible has only two bristles, one of them with pinnate hairs. On the base of these characters and interpretations, we can see that the mandible structure patterns of Siphluriscidae and Nesameletidae are not the same, although the outer incisors are similar. The pictures and descriptions of Richter et al. (2002) clearly show that the prostheca and incisor originated independently from different ancestral structures. In the ground-plan of the maxilla *sensu* Kukalová-Peck (2008), the incisor (galea) and prostheca (lacinia) arise from different segments of a primitive appendage. I assume that the origin and structure of mandible are similar to the maxilla. Thus, if our interpretation and understanding of this structure is correct, the sister group relationship between those two families should be re-evaluated. Even though they are similar in structure, as Kluge (2004) believed that two spine-like structures on *Siphluriscus* mandible are prostheca, it can also be considered as the result of convergent evolution because several lineages of Baetidae have blade-like mandibular incisor, like in the Malagasy *Xyrodromeus*, *Scutoptilum* (Gattolliat, 2002a,b), and the Panama *Moribaetis* (Flowers, 1979) .

The nymphal heads of Siphluriscidae and Nesameletidae are all elongated. But we do not know if it is a plesiomorphy or just an adaption to their similar feeding habits.

Both siphluriscid and nesameletid nymphs have tibiopatellar sutures on mid and hind tibiae. According to Kluge (1997), this condition is present in most mayflies except the majority of baetids and *Rhithrogena* (Heptageniidae) species, all of whose legs have this kind of suture. Kluge (1997) considered this an apomorphy in at least some Baetidae. Nevertheless, on Kukalová-Peck's theory (1991, 2008), the original appendages of insects had independent patella and tibia. The fusion between them may be an apomorphy, but not the converse.

The accessory gills also have been discussed previously (Štys and Soldán, 1980; Staniczek, 2010; Zhou, 2010). Although Zhou (2010) believed possessing them is a plesiomorphy while others suggested it is an apomorphy.

The nymphal claw of *Siphluriscus* is definitely an automorphy. The holes and tiny ball-like structures found here suggest that the dactyl-like appendage of the claw may have sensory function.

Compared to the figures of eggs provided by Kluge et al. (1995) and Hitchings & Staniczek (2003), the eggs of *Siphluriscus* are unique because both poles are covered by attachment structures. The egg surfaces of Nesameletidae have no additional attachments. Koss (1968, 1973) provided some egg SEM pictures of the main lineages of Ephemeroptera and a phylogeny based on that. However, we do not yet know the general morphological pattern of each family or lineages, the evolution of mayfly egg morphology and phylogeny reconstruction requires more data.

From above discussion, we can see that *Siphluriscus chinensis* has more plesiomorphies than any other mayfly species so far known. However, compared to imaginal structures, the nymphal character status and the phylogeny of family Siphluriscidae need more investigation. A frequently asked question should be: in phylogeny reconstruction and character polarization, what kind of characters we should trust and use more, either imaginal or nymphal ones? Needham et al. (1935, p. 107) wrote "adult mayflies have a brief aerial existence, and the air is the same for all. There is, therefore, little chance for changes due to the impress of environment." Demoulin (1958) stated that since the nymphs of mayflies often show many specializations, in doubtful cases of classification the adult characters were used to make the final decision. Schaefer (1975) suggested that because of short imaginal life, the ancient double molts remain in most mayflies. From these points of view, mayfly imaginal characters may be more valuable than those of nymphs because they have been under less selection pressure in the evolution process. Unfortunately, most of the imaginal characters of Siphluriscidae are plesiomorphic, if not automorphic.

Acknowledgements

I am grateful to Janice Peters (Entomology, Florida A&M University, USA) and Arnold Staniczek (Staatliches Museum für Naturkunde, Stuttgart, Germany) for kindly providing specimens and literature for this research, for valuable discussions on this topic and for taking some of the pictures.

This work was supported by the National Natural Science Foundation of China (Grants 30300037, 31172124 and 31472023), Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and project supported by key project of Science-technology basic condition platform from The Ministry of Science and Technology of the People's Republic of China (Grant No. 2005DKA21402). Some data in this research comes from the database of National Digital-Museum of Animal Specimens.

References

Demoulin, G. 1958. Nouveau schéma de classification des Archodonates et des Éphéméroptères. Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, 34:1-19.

Flowers, R.W. 1979. A new species of *Baetis* from Panama. (Ephemeroptera: Baetidae). Pan-Pacific Entomologist, 55:187-191.

Gattolliat, J.L. 2002a. Three new Malagasy species of *Xyrodromeus* (Ephemeroptera: Baetidae) with the first generic description of the adults. Revue suisse de zoologie, 109:325-341.

Gattolliat, J.L. 2002b. Two new genera of Baetidae (Ephemeroptera) from Madagascar. Aquatic Insects, 24:143-159.

Hitchings, T.R. and A.H. Staniczek. 2003. Nesameletidae (Insecta: Ephemeroptera). Fauna of New Zealand, 46:1-72. Manaaki Whenua Press, Lincoln, New Zealand.

Kluge, N.J. 1997. Classification and phylogeny of the Baetidae (Ephemeroptera) with description of the new species from the Upper Cretaceous resins of Taimyr. In eds. P. Landolt & M. Sartori "Ephemeroptera & Plecoptera: Biology-Ecology-Systematics", pp. 527-535. MTL. Fribourg.

Kluge, N.J. 1998. Phylogeny and higher classification of Ephemeroptera. Zoosystematica Rossica,7:255-269.

Kluge, N.J. 2004. The Phylogenetic System of Ephemeroptera. xiii + 442pp. Kluwer Academic Publishers, Dortrecht, Boston, London.

Kluge, N.J., D. Studemann, P. Landolt and T. Gonser. 1995. A reclassification of Siphlonuroidea. Bulletin de la Societe entomologique Suissee, 68:103-132

Koss, R.W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. Annals of the Entomological Society of America, 61:696-721.

Koss, R.W. 1973. The significance of the egg stage to taxonomic and phylogenetic studies of the Ephemeroptera. In eds W. L. Peters & J. G. Peters "Proceedings of the First International Conference on Ephemeroptera", pp. 73-78. Brill, Leiden.

Kukalová-Peck, J. 1985. Ephemeroid wing venation based upon new gigantic Carboniferous mayflies and basic morphology, phylogeny, and metamorphosis of pterygote insects (Insecta, Ephemerida). Canadian Journal of Zoology, 63:933-955.

Kukalová-Peck, J. 1991. Fossil history and the evolution of hexapod structures. In eds. C. Naumann et al. "The Insects of Australia", pp. 141-179. Melbourne University Press, Melbourne.

Kukalová-Peck, J. 2008. Phylogeny of Higher Taxa in Insecta: Finding Synapomorphies in the Extant Fauna and Separating Them from Homoplasies. Evolutionary biology, 35:4-51.

McCafferty, W.P. and T.Q. Wang. 1994. Relationships of the genera *Acanthametropus*, *Analetris*, and *Siphluriscus*, and a re-evaluation of their higher classification (Ephemeroptera: Pisciforma). Great Lakes Entomologist, 27:209-215.

Needham, J.G., J.R. Traver and Y.C. Hsu. 1935. The biology of mayflies, with a systematic account of North American species. 759pp. Comstock Publ., New York.

Ogden, T.H., J.L. Gattolliat, M. Sartori, A.H. Staniczek, T. Soldán and M.F. Whiting. 2009. Towards a new paradigm in mayfly phylogeny (Ephemeroptera): combined analysis of morphological and molecular data. Systematic Entomology, 34:616-634.

Richter, S., G.D. Edgecombe and G.D.F. Wilson. 2002. The *lacinia mobilis* and similar structures - a valuable character in arthropod phylogenetics? Zoologischer Anzeiger, 241:339-361.

Riek, E.F. 1973. The classification of the Ephemeroptera. In eds. W. L. Peters & J. G. Peters "Proceedings of the First International Conference on Ephemeroptera", pp. 160-178. Brill, Leiden.

Schaefer, C.W. 1975. The mayfly subimago: a possible explanation. Annals of the Entomological Society of America, 68:183.

Staniczek, A.H. 2000. The mandible of silverfish (Insecta: Zygentoma) and mayflies (Ephemeroptera): its morphology and phylogenetic significance. Zoologischer Anzeiger, 239:147-178.

Staniczek, A.H. 2010. Distribution of accessory gills in mayfly larvae (Insecta: Ephemeroptera: Siphlonuroidea, Eusetisura). Stuttgarter Beiträge zur Naturkunde A (Neue Serie), 3:85-102.

Štys, P. and T. Soldán. 1980. Retention of tracheal gills in adult Ephemeroptera and other insects. Acta Universitatis Carolinae-Biologica, 1978:409-435.

Ulmer, G. 1920. Neue Ephemeropteren. Archiv für Naturgeschichte (A), 85:1-80.

Zhou, C.F. 2007. The bracing and fusing pattern of longitudinal veins at base Ephemeroptera, Acta Entomologica Sinica, 50:51-56.

Zhou, C.F. 2010. Accessory gills in mayflies (Ephemeroptera). Stuttgarter Beiträge zur Naturkunde A (Neue Serie), 3:79-84.

Zhou, C.F. and J.G. Peters. 2003. The nymph of *Siphluriscus chinensis* and additional imaginal description: a living mayfly with Jurassic origins (Siphluriscidae new family: Ephemeroptera). Florida Entomologist, 86:345-352.