

THE MORPHOLOGY OF *SIPHLAENIGMA JANAE* PENNIKET (EPHEMEROPTERA, SIPHLAENIGMATIDAE), AND ITS SIGNIFICANCE FOR THE GROUND PLAN OF THE BAETOIDEA

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The endemic New Zealand mayfly *Siphlaenigma janae* is generally regarded as the sister group of the Baetidae. Hitherto supposed synapomorphies between Siphlaenigmatidae and Baetidae are re-evaluated and the monophyly of the Baetoidea is confirmed. Based on this evidence a comparison between the abdominal morphology of *Siphlaenigma* and species of the Baetidae has been made to unravel the ground plan of the abdominal organisation of the Baetoidea.

It is shown that *Siphlaenigma janae* has retained baetoid ground plan conditions regarding the structure of eggs, penes, larval compound intersegmental muscles, Malpighian tubules, distribution of abdominal nerve ganglia, and tracheae. The results imply as a consequence for the higher phylogeny of Ephemeroptera, that the Pisciforma + Setisura are at present no longer substantiated by apomorphic characters.

INTRODUCTION

Since the discovery of *Siphlaenigma janae* by PENNIKET (1962) this species usually has been referred to as being closely related to the Baetidae (EDMUNDS *et al.*, 1963; DEMOULIN, 1969; TSHERNOVA, 1970; EDMUNDS, 1972, 1973; RIEK, 1973; EDMUNDS *et al.*, 1976; MCCAFFERTY & EDMUNDS, 1979; LANDA & SOLDÁN, 1985; GILLIES, 1991; KLUGE *et al.*, 1995). However, some authors did not base their assumptions on the uniquely shared apomorphic characters of these taxa. Besides that, there have been authors who assumed a closer relationship of *Siphlaenigma* to the Siphonuroidea rather than to the Baetidae (KOSS & EDMUNDS, 1974). These conflicting assumptions indicate the necessity of a re-evaluation of larval and imaginal characters by phylogenetic analysis.

Our knowledge of the internal anatomy of *Siphlaenigma* has been sparse. EDMUNDS (1972, 1973) noted «a *Metamonius*-like nerve cord and Malpighian tubules», but gave no detailed information about the distribution of abdominal ganglia. LANDA & SOLDÁN (1985) briefly stated a nearly identical arrangement of internal organs with that of the Baetidae, and referred to differences concerning the visceral tracheae and the arrangement of gonads. KOSS & EDMUNDS (1974) investigated the egg structure of *Siphlaenigma*. The morphology of the reproductive system in Baetidae is well known (SNODGRASS, 1936; GRANDI, 1941, 1960; KEFFERMÜLLER, 1972; SOLDÁN, 1981; GRIMM, 1985), whereas only the external genitals of *Siphlaenigma* are described (PENNIKET, 1962). The abdominal musculature of

Siphlaenigma has never been investigated so far. There is at least some information about the abdominal musculature in Baetidae (DÜRKEN, 1907; EASTHAM, 1958; GRANDI, 1962), but previous authors partly came to quite different results though studying identical species. Therefore the abdominal musculature of *Baetis rhodani* is reinvestigated and compared to the situation in *Siphlaenigma*.

Regarding the crucial phylogenetic position of *Siphlaenigma janae* at the base of the Baetoidea (*sensu* KLUGE *et al.*, 1995), detailed knowledge of its morphology could reveal important clues for the evolution of the Baetoidea. The study of additional abdominal characters in *Siphlaenigma* has been done to widen our knowledge of the baetoid ground plan.

METHODOLOGY, MATERIALS AND METHODS

The term «ground plan» is used herein *sensu* HENNIG (1981), comprising the whole character set (both plesiomorphic and apomorphic characters) of the last common ancestor of a monophyletic group (KÖNIGSMANN, 1975). Also the term «monophyletic group» is used herein *sensu* HENNIG (1950), meaning a group that contains all the known descendants of a single stem-species.

According to recent phylogenetic studies, the Siphonuroidea probably represent a paraphyletic taxon at the base of the Ephemeroptera (MCCAFFERTY, 1991; KLUGE *et al.*, 1995). Thus in the phylogenetic analysis of the Baetoidea, the Siphonuroidea *sensu* KLUGE *et al.* (1995) chiefly were chosen as an outgroup to determine the character polarity. Fully grown larvae of *Siphlaenigma janae* were collected from three locations throughout New Zealand. Male imagines were provided by Mr T. Hitchings, Canterbury Museum, Christchurch. Larvae and adults of *Baetis rhodani* (PICTET, 1843) were collected near Tübingen, Ger-

many. The material was fixed by a mixture of 95% ethanol, 35% formaldehyde, and acetic acid (66:33:10). After 24 hours or longer it was transferred to 80% ethanol.

Manual dissection: Larvae were dissected under 80% ethanol on a layer of paraffin in a Petri dish. To observe internal organs, larvae were dorsally opened, pinned on minutiae, and stained with basic fuchsin. To observe cuticular structures, larvae were kept in 7% potassium hydroxide under room temperature for several days, until the soft tissues dissolved. Then the cuticula was stained with Chlorazol Black.

Light microscopy: 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™ at 60 °C. There the specimens were kept under vacuum conditions for 24 hours to optimize their penetration. Finally the material was embedded in Paraplast Plus™. Sections of 5–7 µm thickness were obtained with a rotation microtome. Sections were stained with Delafield's hematoxylin, counterstained with erythrosin, and observed with a Zeiss-Axioplan microscope. Photographs were taken with a Zeiss-MC 100 camera.

Scanning electron microscopy (SEM): Mouth parts, legs and eggs were dissected from final instar larvae, dehydrated through a stepwise immersion in ethanol and acetone, and then dried by critical point drying. The mounted material was coated with a 20 nm Au/Pd layer and examined with a Cambridge Stereoscan 250 MK 2 scanning electron microscope at 10 kV.

RESULTS

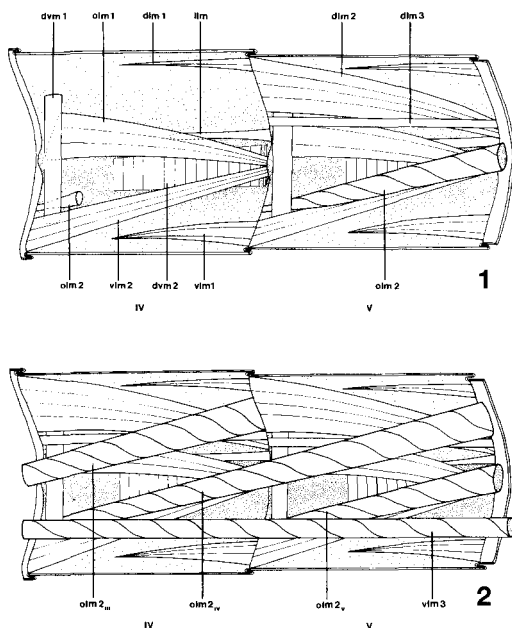
Pregenital abdominal musculature of the larva

In *Siphlaenigma* (Figs 1, 2) each half of the first seven abdominal segments is equipped with a set of dorsal longitudinal muscles (dlm), ventral longitudinal muscles (vlm), and dorsoventral muscles (dvm). In general these muscles have their origin and insertion on the medial ventral respective dorsal parts of each segment. In addition there are muscles that arise from the lateral parts of each segment. These lateral parts are heavily bulged. BÖRNER (1908) refers to this region as subcoxal, but as there is no general agreement about its leg-derived origin (DÜRKEN, 1909; KUKALOVÁ-PECK, 1983), I shall refer to this region as lateral lobe. The lateral lobe is the place of origin for the remaining muscles. These muscles are termed gill muscles (gm), lateral longitudinal muscles (llm), and oblique longitudinal muscles (olm) herein. At their points of insertion all muscles are attached to the epidermis by tonofibrillae. There are no apodemes or

other specific cuticular structures present, but the muscles merely insert at the anterior border of the subsequent segment.

The dlm1 is a delicate muscle that runs parallel to the middorsal line through the posterior half of the tergum. The insertion of the dlm2 the anterior border of the segment is broad and runs obliquely in a lateral direction to its small insertion at the anterior border of the subsequent segment. The dlm3 is a small muscle, that originates ventrolaterally in relation to the dlm2 at the anterior border of the segment. It runs medially of the dvm1 and olm1 in a straight longitudinal direction and inserts together with the dlm2 at the anterior border of the subsequent segment. The cross cut of the three muscles mentioned above reveals their rather flat profiles (Figs 1, 2).

The slender vlm1 resembles its dorsal counterpart in shape, length, and direction. The vlm2



Figs 1-2. Medial view into the right half of abdominal segments IV and V of *Siphlaenigma janae*. The ventral and dorsal borders have been drawn asunder to enable a view onto the medial muscles. Muscles have been added successively to enable a view onto the lateral muscles. Gill muscles have not been drawn. Abbreviations: dlm: dorsal longitudinal muscle, dvm: dorsoventral muscle, llm: lateral longitudinal muscle, olm: oblique longitudinal muscle, vlm: ventral longitudinal muscle.

runs from its medial attachment at the anterior border of each segment obliquely to its ventrolateral attachment at the anterior border of the succeeding segment.

The dvm1 is a slim muscle that is situated in the anterior third of each segment. Its dorsal attachment lies lateral in relation to the dlm2, its ventral attachment is located lateral in relation to the vlm2 respectively. In this manner it spans the lateral lobe. The dvm2 runs laterally of the dvm1 and extends over the posterior two thirds of the lateral lobe. At its posterior end a minor part of this muscle is separated. The trachea branchialis passes through this cleft on its way to the tracheal gill.

Between dvm1 and dvm2 there are situated three muscles. The small llm runs dorsally of the dvm in the posterior half of the segment and inserts ventrolaterally of the dlm2. The olm1 is a big muscle that arises lateral in relation to the dvm1. It runs medially to the dvm2 and inserts dorsally of the vlm2. The olm1 like all the previous mentioned muscles has a rather flat profile, whereas the olm2 has a circular cross cut. The olm2 is one of the biggest abdominal muscles and arises laterodorsally of the dvm1 at the anterior border of the segment. From there it runs mediodorsally to the anterior border of the next but one segment. It is a compound intersegmental muscle that covers two segments in *Siphlaenigma*. The fibres of this muscle do not have a linear arrangement, but they appear to be twisted like a rope. The innermost fibres also appear to be attached at the anterior border of the second segment. Longitudinal sections reveal that at the segment border the whole muscle is interrupted by short tonofibrillae. This clearly points to the intrasegmental origin of this muscle.

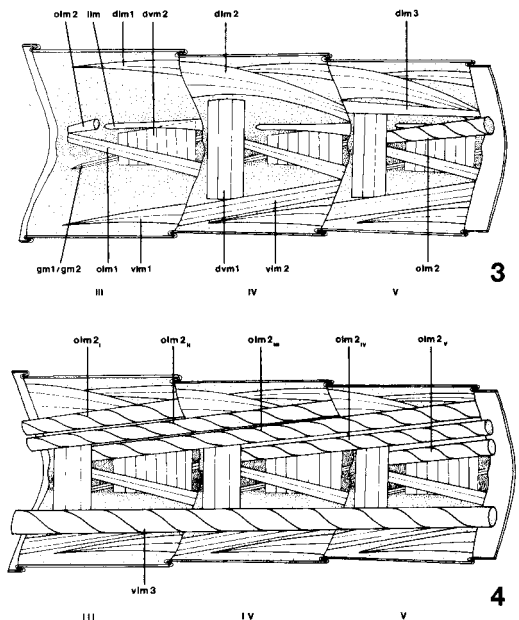
The vlm3 is a compound intersegmental muscle, too. It arises at the sternapophysis of the prothorax and runs through the pterothorax and the entire abdomen. Like the olm2, its fibres are twisted, and at every anterior border of a segment a few fibres are attached to the body wall. The fibres generally stretch over two entire segments until they are interrupted by short tonofibrillae.

In *Baetis rhodani* basically the same sets of muscles can be observed (Figs 3, 4). There are some differences regarding the proportions of certain muscles. In some of the specimens

investigated the vlm1 is split into two more or less separate bundles, but usually this muscle remains as a single unit. The dvm1 is considerably stronger compared to the condition in *Siphlaenigma*. The dvm2 instead is relatively short and covers approximately just the posterior half of the segment. Furthermore it does not extend beyond the branching of the trachea branchialis. The olm1 has a circular cross cut, but appears to be weaker than in *Siphlaenigma*. The olm2 covers even three segments in *Baetis*.

The gill muscles and their mode of attachment to the gill base

In *Siphlaenigma janae* there are two gill muscles located in the region of the lateral lobe (Fig. 5A). Both muscles have a common origin and run laterally from the dvm2 towards the gill base. The gm1 inserts at the medial side of the gill itself, the gm2 at the lateral side respectively. The points of attachment indicate



Figs 3-4. Medial view into the right half of abdominal segments III-V of *Baetis rhodani*. The ventral and dorsal borders have been drawn asunder to enable a view onto the medial muscles. Muscles have been added successively to enable a view onto the lateral muscles. Abbreviations: dlm: dorsal longitudinal muscle, dvm: dorsoventral muscle, gm: gill muscle, llm: lateral longitudinal muscle, olm: oblique longitudinal muscle, vlm: ventral longitudinal muscle.

that gm1 serves as gill adductor, whereas gm2 functions as gill abductor. Additionally there is another muscle that serves as an indirect gill adductor. The dvm3 has a common origin with the posterior portion of the dvm2. From there it runs in a dorsolateral direction to its point of attachment. It inserts not at the gill base itself, but medially of it at the intersegmental membrane.

In *Baetis rhodani* gm1 and gm2 take a corresponding course, but gm1 does not insert at the gill base itself (Fig. 5B). It runs to a small, falciform sclerite that is easily overlooked, as it is situated medially of the gill and is embedded in the intersegmental membrane. This gill sclerite is connected with its lower end to the gill. At its upper end a delicate chitinous bond connects the sclerite with a sclerotised processus at the end of the segment.

Internal reproductive system and male genitalia

In *Siphlaenigma* the gonads of both male and female are located dorsally of the digestive

tract. In older female larvae the ovaries extend from the mesothorax to the VIth abdominal segment. The ovaries are medially contiguous in the thorax. They appear to be bilaterally extremely flattened, because each ovary bears only two longitudinal rows of ovarioles. The ovarioles are orientated slightly obliquely to the longitudinal axis of the oviduct. In older male larvae the cylindrical testes are straight and extend from abdominal segment I to VI. In the abdominal segment VIII each vas deferens suddenly dilates to form an apparent vesicula seminalis that extends up to segment IX.

In the male imago the well separated vasa deferentia abruptly bend down beside the colon at the border between the VIIIth and IXth abdominal segment. The vasa deferentia proceed ventrally from the digestive tract up to the posterior half of the IXth segment. At the border between the IXth and Xth segment both vasa deferentia are fused medially (Fig. 6A). The gonoducts remain fused at a length of approximately 100 µm, and are again well separated immediately anterior to the proximal part of the penes (Fig. 6B). The vasa deferentia continue to the penis lobes. They change to the ductus ejaculatorii at about the middle of the penis lobes. Anterior to their caudal junction the vasa deferentia each are surrounded by a thin muscle layer. Additionally, there are three muscles that are attached to the penes (pm1 - pm3). The pm1 is a ventral longitudinal muscle, that arises in the first third of the IXth sternum. Its origin is located just ventrally of the dvm2. It runs in a ventromedial direction towards its insertion at the penial bar (Fig. 6B). The pm2 originates dorsally in relation to the dvm2 close to the caudal end of segment IX. From there it runs almost dorsoventrally in a medioventral direction. It inserts laterally of the pm1 at the penial bar. The intrinsic penis muscle (pm3) arises from the proximal ventral part of the penes and diverges to the lateral part of the penis lobe (Fig. 6C). It inserts at the lateral part of the penis lobe at about half of its length. The penes themselves are well developed (Fig. 6D) and sclerotised, but are covered ventrally to a great extent by a medial projection of the styliger plate. In their basal half the penes are entirely fused medially.

The forceps consist of three segments. The styliger plate laterally bears the gonocoxal

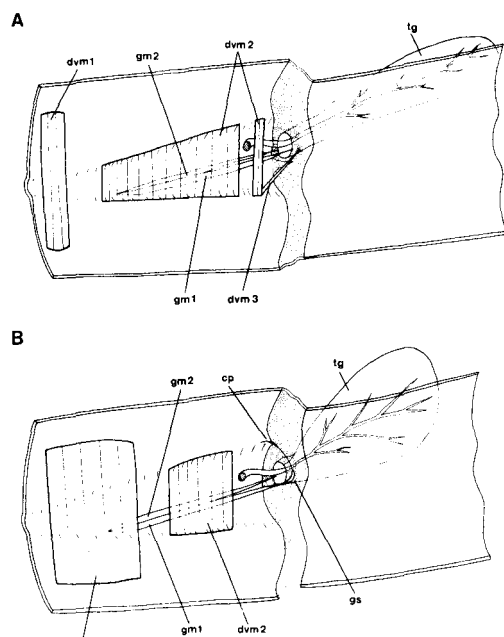


Fig. 5. Medial view onto the lateral region of a gill-bearing segment of (A) *Siphlaenigma janae* and (B) *Baetis rhodani*. The ventral and dorsal borders have been drawn asunder to enable a view onto the gill base. Abbreviations: cp: cuticular process, dvm: dorsoventral muscle, gm: gill muscle, gs: gill sclerite, tg: tracheal gill.

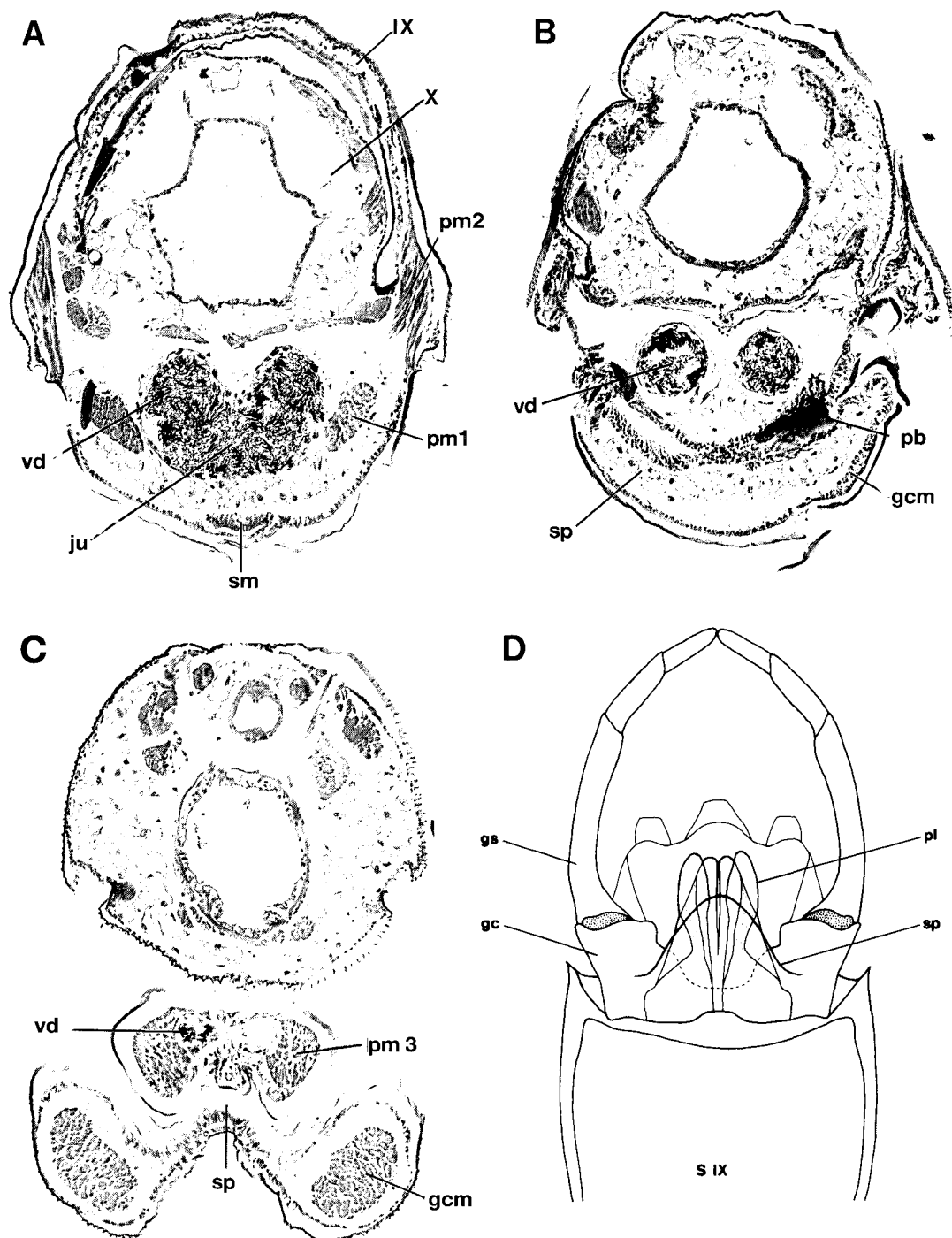


Fig. 6. Male genitalia of *Siphlaenigma janae*: (A-C) cross section through the insertion of penes at the border between abdominal segment IX and X (D) genitalia in ventral view. Abbreviations: gc: gonocoxa, gcm: conocoxal muscle, gs: gonostylus, ju: junction of vasa deferentia, pb: penial bar, pl: penis lobe, pm: penis muscle, sm: styliiger muscle, sp: styliiger plate, vd: vas deferens.

muscle (gcm) that originates on the lateral ventral side of the styliger plate. It inserts at the proximal base of the first segment of the forceps. The unpaired styliger muscle (sm) runs from its medial origin at the proximal half of the ninth sternum in a longitudinal direction to its medial insertion at the anterior ventral part of the styliger plate.

As there were no female imagines available, nothing can be stated about the anatomy of the female genital opening.

Eggs

The entire egg of the last instar larva of *Siphlaenigma* is covered by an exochorionic

layer of fiber-coils (Fig. 7A), that strongly resembles the condition in *Siphonurus* (DEGRANGE, 1960; KOSS & EDMUNDS, 1974). In contrast to the latter there are no terminal fiber clusters present. There are also neither knob-terminated coiled threads nor polar caps developed. The fiber-layer is interrupted by one or two micropyles, that appear as oval breaks in the layer of fiber-coils (Fig. 7B). These openings enable a view onto the inner chorionic layer, that seems to be tuberculate.

Malpighian tubules

In the last instar larva of *Siphlaenigma* there are about forty Malpighian tubules. Each of the

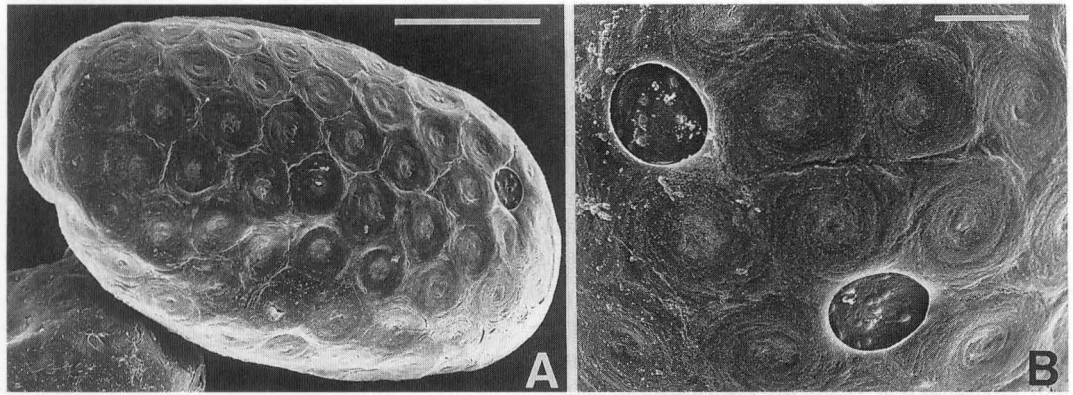


Fig. 7. Egg of *Siphlaenigma janae*: (A) general view (B) micropyles. Scale line A: 40 μ m; B: 10 μ m.

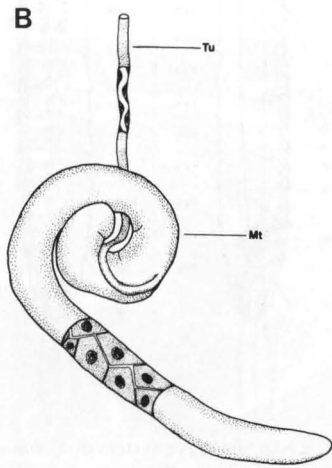
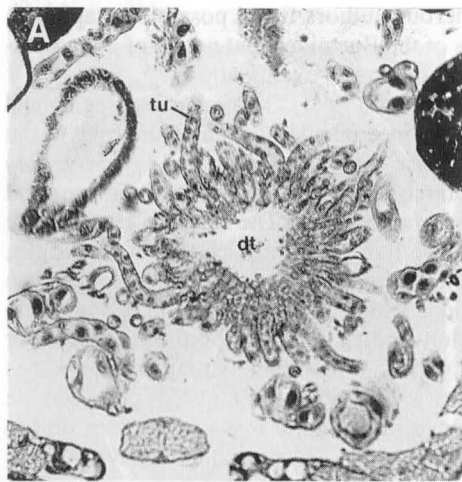


Fig. 8. Malpighian tubules of *Siphlaenigma janae*: (A) cross section through the border between mesenteron and proctodaeum showing the insertion of tubuli uriniferi to the digestive tract (B) general view of a single Malpighian tubule. Both portions also offer a view like that revealed by longitudinal sections. Abbreviations: mt: malpighian tubule, dt: digestive tract, tu: tubulus uriniferus.

Malpighian tubules is divided into a thickened distal part with excretory function, and a smaller proximal part, the tubulus uriniferus. Each tubulus uriniferus singly enters the digestive tract between mesenteron and proctodaeum (Fig. 8A). There are no common ureter trunks. At its distal end the tubulus uriniferus leads to the thickened part of the Malpighian tubule, that is heavily coiled at its proximal tip. In most of the investigated specimens the thickened part had almost no lumen, whereas in the proximal part a lumen was always clearly visible (Fig. 8B). A typical brush border or honeycomb border of the inner lumen has never been observed.

Ventral nerve cord

The ventral nerve cord in *Siphlaenigma* consists of three thoracic and seven abdominal ganglia, that are very well separated (Fig. 9B). In the first abdominal segment there is no ganglion present. The emerging nerves of the metathoracic ganglion reveal that the ganglion of the first abdominal segment has shifted cranially and is fused with the meta-

thoracic ganglion. The abdominal segments II-VIII each bear a distinctive ganglion. The connectives are entirely fused and form a band that connects the ganglia.

Tracheal system

The description of the tracheae of thorax and abdomen follows LANDA (1948) and stresses only characters, that have some importance for phylogenetic considerations. The main lateral trunk (tl) passes the entire thorax and abdomen (Figs 9A, B). It is connected with the future spiracle by the closed trachea arcus lateralis stigmatis (ts) from the metathorax to the eighth abdominal segment. The trachea branchialis (tb) connects the lateral trunk with the tracheal gills in the first seven abdominal segments. Near the branching of the ts also several other tracheae branch off the lateral trunk. Among these are tracheae that supply the nerve cord and the visceral tracheae (tv) that supply the digestive tract. The visceral tracheae are distributed from the metathorax to the eighth abdominal segment (TV2 - TV10). In the ninth segment, a transverse anastomosis of the two main lateral trunks is present [TAV10(IX)].

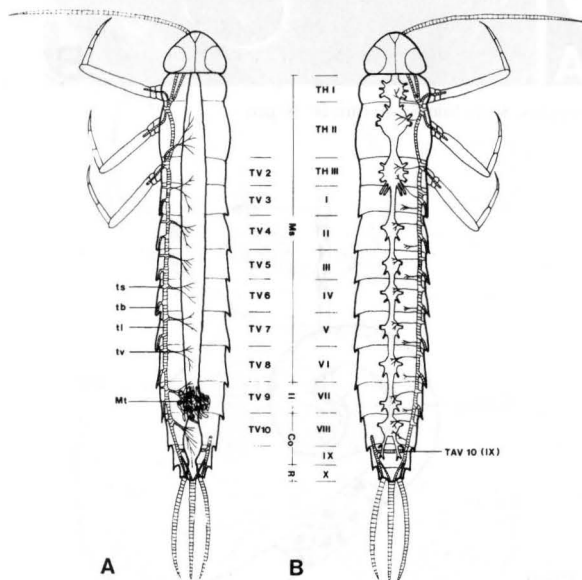


Fig. 9. Tracheal and nerve system of *Siphlaenigma janae*: (A) visceral tracheae (B) ventral tracheae, tracheal anastomosis and ventral nerve cord. Abbreviations: tb: trachea branchialis, tl: trachea longitudinalis, ts: trachea stigmatis, tv: trachea visceralis, TAV: trachea anastomotica ventralis.

DISCUSSION

The phylogenetic position of *Siphlaenigma janae*

Numerous authors listed possible autapomorphies of the Baetoidea, but not all of them withstand a critical re-examination:

KLUGE *et al.* (1995) claimed a four-segmented pro- and metatarsus as an autapomorphy of the Baetoidea, but in the imago of *Siphlaenigma* four tarsomeres are present in the meso- and metathoracic legs. The forelegs retained the primitive five-segmented condition (PENNIKET, 1962). The same character states occur in the Baetidae (RIEK, 1973), whereas in the outgroup Siphonuroidea the plesiomorphic character state of five tarsomeres is realised in all legs (KLUGE *et al.*, 1995). RIEK (1973) proposed the presence of a double row of denticles on the larval pretarsus as an autapomorphy of Callibaetinae (Callibaetinae + Cloeoninae *sensu* GILLIES, 1991). However, as PENNIKET (1962) and GILLIES (1991) pointed out, this character is also present in *Siphlaenigma* (Fig. 10). Thus

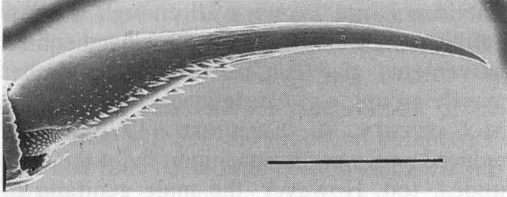


Fig. 10. Middle claw of *Siphlaenigma janae*, lateral view. Scale line 100 μ m.

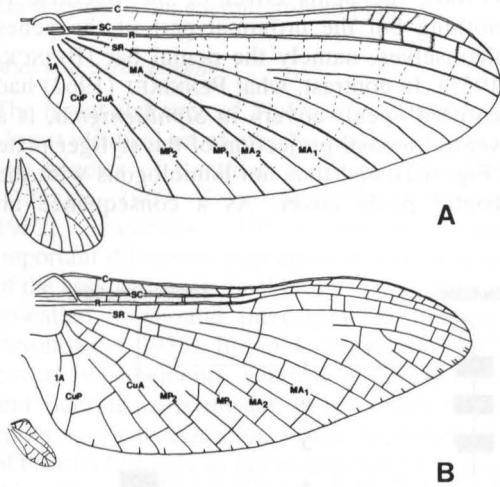


Fig. 11. Wing venation of (A) *Siphlaenigma janae* and (B) *Callibaetis ferrugineus*. Redrawn from PENNIKET (1962) and EATON (1883).

it is highly probable, that an elongated larval pretarsus with a double row of denticles is a ground plan character of the Baetoidea, too.

MCCAFFERTY & EDMUNDS (1979) claimed the detached veins IMA and MA₂ in the forewing as an autapomorphic character of Baetoidea, but in fact the MA fork is still intact in *Siphlaenigma* (Fig. 11). In contrast, the detached IMP and MP₂ are dislocated in both Siphlaenigmatidae and Baetidae (PENNIKET, 1962; DEMOULIN, 1969; EDMUNDS *et al.*, 1976). Only this character can be taken into consideration as an autapomorphy of Baetoidea. Furthermore the reduction of forewing crossveins as well as the size reduction of the hind wings have been treated as autapomorphies of the Baetoidea (PENNIKET, 1962; RIEK, 1973). In fact *Siphlaenigma* has numerous crossveins in the forewing, but, except for a few distal crossveins in the costal and subcostal area, they are extremely weakened. A comparison of the forewing venation in *Siphlaenigma* to the venation in Callibaetinae hardly reveals any differences regarding the amount of crossveins (Fig. 11). Moreover, in Baetidae the crossveins are generally well pronounced. This character distribution implies, that the weakening of crossveins in Siphlaenigmatidae is an autapomorphy of the latter, whereas the actual loss of crossveins obviously is a character that reflects an evolutionary development within the Baetidae. Just the size reduction of the hind

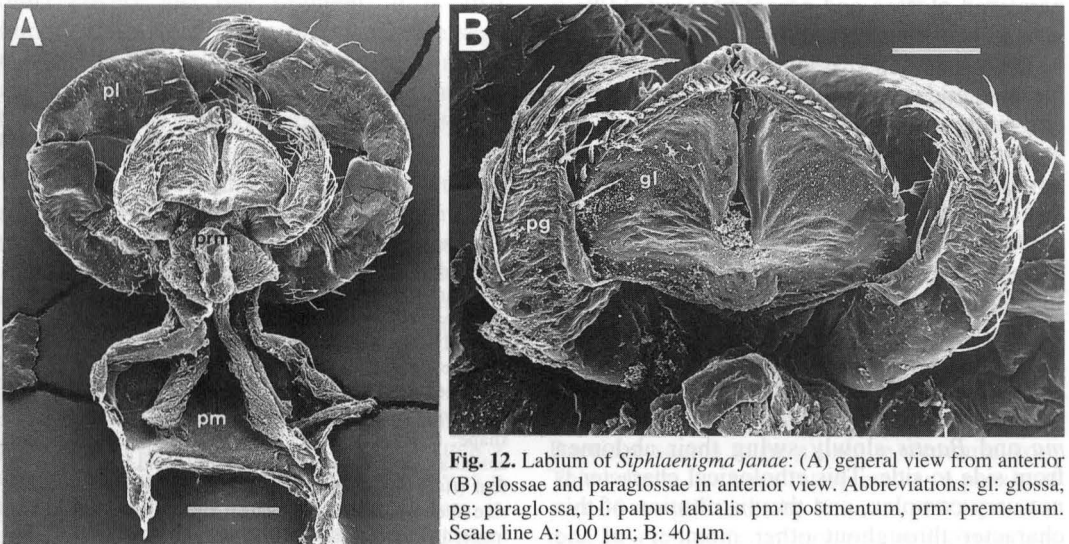


Fig. 12. Labium of *Siphlaenigma janae*: (A) general view from anterior (B) glossae and paraglossae in anterior view. Abbreviations: gl: glossa, pg: paraglossa, pl: palpus labialis pm: postmentum, prm: prementum. Scale line A: 100 μ m; B: 40 μ m.

wing could count as an apomorphic character of the Baetoidea.

TOMKA & ELPERS (1991) proposed the elongation of the larval antenna as an autapomorphy of the Baetoidea. Though this character is not very complex, it could be assessed as a synapomorphic character of Baetidae and Siphlaenigmatidae, as the larvae of other Ephemeroptera generally have shorter antennae.

MCCAFFERTY & EDMUNDS (1979) maintained «narrowed glossae and paraglossae (but less so in *Siphlaenigma*)» as another possible synapomorphy of Baetidae and Siphlaenigmatidae, but in fact *Siphlaenigma* possesses a rather unique labium. The glossae are broadened and of almost triangular shape, the paraglossae are palp-like and slender, but do not appear to be considerably approximated to the glossae (Figs 12A, B). It is most likely, that this peculiar labium represents an autapomorphic character of *Siphlaenigma*. In Baetidae the glossae and paraglossae are narrowed. EDMUNDS (1972, 1973) assumed a closer relationship of Nesameletidae with the Baetoidea, unfortunately without precise arguments. However, in Nesameletidae the glossae and paraglossae are narrowed as in the Baetidae (PHILLIPS, 1930; Staniczek, in prep.). As there is yet no general agreement about the sister group of the Baetoidea, it remains difficult to reconstruct a common baetoid ground plan concerning this character. Supposing a sister group relationship between Nesameletidae and Baetoidea, narrowed glossae and paraglossae would be a ground plan character of the Baetoidea, too. As KLUGE *et al.* (1995) assumed Oniscigastridae + Nesameletidae + Rallidentidae + Ameletopsidae being a monophylum on the basis of the structure of the mesothoracic furcasternum, the development of narrowed glossae in Baetidae and Nesameletidae could also be due to convergence. In this case well separated glossae and paraglossae could be assessed in the ground plan of the Baetoidea. This conflicting evidence awaits further evaluation.

The larval behaviour is regarded as another autapomorphy of the Baetoidea by MCCAFFERTY & EDMUNDS (1979). Larvae of *Siphlaenigma* and *Baetis* slowly swing their abdomen from side to side. This ethological character is not very complex, and the distribution of this character throughout other members of the

Baetidae is not known well enough to draw definite conclusions. On the other hand similar movements also can be observed in other mayfly groups, e.g. Ameletidae.

MCCAFFERTY & EDMUNDS (1979) listed reduced penes as an autapomorphy of the Baetoidea, too. However, the male genitalia of *Siphlaenigma* are well developed (Fig. 6D) and, compared to the small size of the species itself, not reduced at all. PENNIKET (1962) noted a «large penis-cover» in *Siphlaenigma*. As revealed by SNODGRASS (1936) and GRANDI (1960), the penis cover of the Baetidae is nothing but the proximal part of the penes themselves, namely the penial bar (BRINCK, 1957). In contrast, what PENNIKET (1962) had entitled «penis-cover» in *Siphlaenigma*, is a ventral medial projection of the styliger plate (Fig. 6D), and thus not homologous with the baetid penis cover. As a consequence an

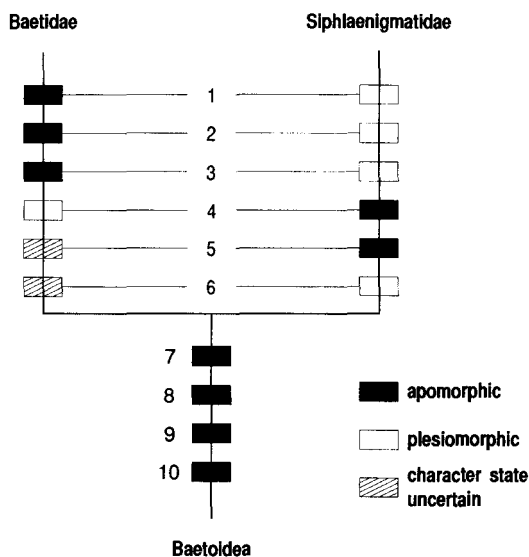


Fig. 13. Scheme of argumentation for the phylogeny of Baetoidea, external characters. Plesiomorphic character states are added in parenthesis: 1. Forewings with (without) short marginal intercalaries; 2. MA fork dislocated (intact); 3. Hind wings with reduction of longitudinal veins (without vein reduction); 4. Weakened crossveins in forewing (crossveins not weakened); 5. Baetidae: narrowed glossae and paraglossae; Siphlaenigmatidae: glossae of triangular shape; 6. Eyes of males divided (undivided); 7. Larval antenna elongated (short); 8. Middle and hind legs with four (five) tarsomeres; 9. Size reduction of hind wing (no size reduction); 10. Detached veins IMP and MP₂ in forewing (not detached).

external penis cover must not be supposed as an apomorphic ground plan character of the Baetoidea.

Concerning the external morphology of the Baetoidea, some of the proposed autapomorphic characters could not be confirmed. Nevertheless there still remain several characters, that altogether confirm the sister group relationship between Baetidae and Siphlaenigmatidae (Fig. 13). Therefore the internal abdominal organisation of *Siphlaenigma* can be compared with the condition in the Baetidae to enlarge our knowledge of the baetoid ground plan.

Muscular arrangement

The larval abdomen of *Siphlaenigma* bears an almost identical set of muscles compared to the few species of Baetidae investigated so far (DÜRKEN, 1907; EASTHAM, 1958; GRANDI, 1962; STANICZEK, 1993). However, an important difference concerns the distribution of the oblique longitudinal muscle 2, which is a so-called compound intersegmental muscle (SNODGRASS, 1935). In *Siphlaenigma* the olm2 covers two segments, whereas in Cloeoninae and Baetinae this muscle even covers three entire segments (GRANDI, 1962). As histology of the olm2 reveals, in *Baetis* and *Siphlaenigma* this compound muscle is likely to be a secondarily modified intrasegmental muscle. In addition, according to GRANDI (1962) in nearly all previously investigated mayfly families the olm2 covers just one segment. It is also important to note, that compound intersegmental muscles only occur in the larval abdomen, whereas these muscles seem to be entirely lost in the adult stages (GRANDI, 1962; GRIMM, 1985).

The olm1 and olm2 are most likely to be responsible for the fast dorsoventral movements of the larvaenymphs while swimming. As the length of a muscle is highly correlated with the velocity of the resulting movement, an elongation of the olm2 would provide an adaptive advantage for a swimming larva.

The assumed character polarity implies the covering of two segments by the olm2 in the ground plan of the Baetoidea. Though such a compound muscle like the olm2 certainly is a derived character within the Ephemeroptera, it can only count as a plesiomorphic ground plan

character of the Baetoidea. GRANDI (1962) recorded the presence of the olm2 in *Siphonurus lacustris*, too. In this species the muscle stretches over two entire segments just as in *Siphlaenigma*. Other families of the Siphonuroidea have not been investigated in this respect so far. It seems possible, that the two-segmented condition of the olm2 could be a common character shared by the Baetoidea together with a part of or even all siphonuroid families. Only further investigations could clarify this intriguing problem.

On the other hand the assumption of an olm2 in the ground plan of the Baetidae, that covers three abdominal segments must be regarded as preliminary, as only taxa of the Cloeoninae and Baetinae have been studied in this respect so far. An investigation of the abdominal muscles in *Callibaetis* thus could prove to be rewarding. Another intriguing problem remains for the different modes of attachment of the gill muscles to the gill base. The presumable ground plan condition of the Ephemeroptera in this respect are two gill muscles that are both attached to the gill base itself. This condition can be observed in Siphonuridae, Nesameletidae, Heptageniidae, Leptophlebiidae, Polymitarcyidae, and other families (DÜRKEN, 1907; GRANDI, 1962; BIRKET-SMITH, 1971; Staniczek, unpublished).

The recruitment of a derivative dorsoventral muscle (dvm3) as an additional gill muscle certainly is an autapomorphic character of *Siphlaenigma*. More than two gill muscles are also reported in *Ephemerella ignita* (Ephemerebellidae), but these additional muscles are a result of the secondary splitting of the gill muscles themselves rather than a result of any involvement of dorsoventral musculature (DÜRKEN, 1907). In any case the dvm3 in *Siphlaenigma* serves as an indirect gill adductor and enhances the acting of the gm1. The secondary reinforcement of the gill muscles by a dorsoventral muscle has obviously been developed as an adaptation to the enhanced demand for oxygen in streams with a low water flow rate.

On the other hand, the gill sclerite observed in Baetidae seems to be a unique structure, too. This sclerite has been interpreted as a proximal part of the gill itself by BÖRNER (1908), but it develops in late instar larvae as an independent

sclerotization of the intersegmental membrane (STANICZEK, 1993). Only future studies in different species of the Baetidae will reveal, if it can be established as a ground plan character of the Baetidae, and if it occurs in other mayflies, too.

Internal reproductive system and male genitalia

SOLDÁN (1981) discusses the internal reproductive system of mayflies on a broad comparative basis. In this respect *Siphlaenigma* shares several characters with the Baetidae, namely the dorsal position of testes and ovaries, and their relative position to the body segmentation. This certainly reflects the ground plan condition of the Baetoidea. On the other hand there are mainly differences regarding the arrangement of ovarioles. The unique arrangement of ovarioles in two longitudinal rows most likely represents an autapomorphic character of *Siphlaenigma*, because in Siphonuroidea as well as in the Baetidae cylindrical ovaries are present. Other characters like shape and extension of the vesiculae seminales vary considerably within the order, and parallel developments are most likely to have happened. In the Baetidae each vas deferens dilates gradually to form the vesicula seminalis (SOLDÁN, 1981). In *Siphlaenigma* the dilatation of the vasa deferentia takes places suddenly. SOLDÁN (1981) regards the latter condition as a derived tendency within the Ephemeroptera.

In adults of both Cloeoninae and Baetinae the vasa deferentia are medially fused at a short distance from their very beginning in the VIIth abdominal segment (GRIMM, 1985). GRIMM (op. cit.) refers to this structure as cranial junction of the vasa deferentia. This cranial junction has not been found in any other mayfly family investigated by the author. According to GRIMM (op. cit.), the vasa deferentia are totally separated in *Callibaetis*. The study of *Siphlaenigma* uncovers a caudal junction of the gonoducts directly anterior of the penes. The same character has also been found in several other mayfly families (GRIMM, 1985). On the one hand these results point to the cranial junction of the vasa deferentia as a uniquely derived character of the Cloeoninae + Baetinae, on the other hand the studies of GRIMM (1985) imply that a caudal junction could have evolved

several times within the Ephemeroptera. Thus it is not possible to present a convincing hypothesis regarding the ground plan of the Baetoidea at the moment.

The loss of the intrinsic penis muscle is an autapomorphy of the Baetidae, that is closely linked to the reduced and membranous penis lobes, whereas in the ground plan of the Baetoidea an intrinsic penis muscle has to be assumed.

Eggs

On the basis of comparative egg studies in Ephemeroptera, KOSS & EDMUNDS (1974) rejected a closer relationship between Siphlaenigmatidae and Baetidae. The authors mainly stressed similarities between eggs of Siphlaenigmatidae, Siphonuridae, Nesameletidae, and Oligoneuriidae regarding the uniform layer of fiber-coils, and the absence of derived structures like terminal fiber-clusters or knob-terminated coiled threads in eggs of both Siphlaenigmatidae and Nesameletidae. However, these similarities are merely founded on plesiomorphic characters, and do not prove a closer phylogenetic relationship between *Siphlaenigma* and the above mentioned taxa at all. On the other hand, a closer relationship between Baetidae and another taxon apart from *Siphlaenigma* could only be proven by synapomorphic characters, too. Some Baetidae for example share knob-terminated coiled threads and a large mesh-reticulation of the chorion with several other mayfly families (KOSS & EDMUNDS, 1974; KOPELKE & MÜLLER-LIEBENAU, 1981a; 1981b; 1982). KOSS & EDMUNDS (1974) explicitly stated that knob-terminated coiled threads probably evolved at least seven times convergently in different mayfly groups as parallel adaptations to a lotic habitat. The chorionic sculpturing is, according to KOSS & EDMUNDS (1974), too diverse to be at all of importance in studying familiar relationships. So there are obviously no characters left in egg morphology that could falsify a proposed sister group relationship between Siphlaenigmatidae and Baetidae.

Malpighian tubules

LANDA (1969) and LANDA & SOLDÁN (1985) discussed the Malpighian tubules of Epheme-

roptera on a broad comparative basis. In nearly all mayfly families that have been investigated the proximal end of the thickened portion of the Malpighian tubules is coiled. The Malpighian tubules of *Siphlaenigma* have this peculiar character, too. On the other hand, the distal portion of the Malpighian tubules in the Baetidae is only slightly broader than the tubulus uriniferus, uncoiled, and tube-like. Apart from the Baetidae uncoiled Malpighian tubules just occur in the Rallidentidae and Ameletopsidae. LANDA & SOLDÁN (1985) refer to this condition as the primitive state in Ephemeroptera. Regarding other insect orders, this assumption would seem to be justified. However, the character distribution amongst Ephemeroptera implies another character polarity: Supposing coiled Malpighian tubules in the ground plan of Ephemeroptera, the assumption of a convergent development of straight Malpighian tubules in Baetidae, Rallidentidae and Ameletopsidae is more parsimonious than a convergent evolution of coiled tubules in several different lineages of Ephemeroptera.

Regarding the individual attachment of the Malpighian tubules to the digestive tract *Siphlaenigma* and the Baetidae obviously have retained a plesiomorphic condition.

Ventral nerve cord

Siphlaenigmatidae and Baetidae both share the fusion of the abdominal connectives to a single band that connects the abdominal ganglia. Though this is a derived character compared to the ground plan of Ephemeroptera, the same character state occurs in several other mayfly families (Rallidentidae, Nesameletidae, Coloburiscidae, Arthropleidae, Behningiidae), too (LANDA, 1969). The character distribution throughout Ephemeroptera implies that this character has evolved at least four times independently. As it is possible that the Baetoidea are more closely related to Rallidentidae + Nesameletidae (EDMUNDS, 1972), this character cannot count for an autapomorphic character of Baetoidea at the moment.

Siphlaenigma has retained a separate ganglion in the eighth abdominal segment, and thus this character has to be assumed for the ground plan of Baetoidea, too. TOMKA & ELPERS (1991) proposed in their higher classification of the

Ephemeroptera a monophylum comprising Ameletopsidae + Acanthametropodidae + Baetoidea + Metretopodidae + Setisura by a single autapomorphic character, namely the shift of the eighth abdominal ganglion to the seventh segment. KLUGE *et al.* (1995) already doubted this proposed apomorphy. The findings in *Siphlaenigma* reveal that such a monophylum indeed is not substantiated.

Tracheal system

In Ephemeroptera the tracheal system has played a major role in phylogenetic considerations. Especially the segmental distribution of visceral tracheae has been used for these purposes (LANDA & SOLDÁN, 1985). The presence of visceral tracheae is generally regarded as a primitive character.

MCCAFFERTY (1991) proposed a monophylum comprising Pisciforma + Setisura by a single autapomorphic character, namely the loss of the visceral trachea of the first abdominal segment (TV3) in these groups. However, the actual character distribution throughout these taxa does not support this hypothesis. LANDA (1969) stated visceral tracheae in each segment from the metathorax to the eighth abdominal segment (TV2-TV10) in many investigated species of Baetidae. Only in *Callibaetis* and in one nearctic species of *Centroptilum* the visceral trachea of the first abdominal segment (TV3) is lost (LANDA, 1969). As the present study reveals, the TV3 is also present in *Siphlaenigma*. Thus it is most parsimonious to assume the presence of the TV3 as a plesiomorphic character in the ground plan of Siphlaenigmatidae, Baetidae, and Baetoidea, too. Moreover, the TV3 is also present in Ametropodidae (LANDA, 1969). There is no reason at all to assume a secondary acquirement of the TV3 in Ametropodidae, Siphlaenigmatidae and partly in Baetidae. It is far more likely that the absence of the TV3 in *Callibaetis* is a derived character state.

As a consequence, a proposed monophyly of Pisciforma + Setisura at present is not substantiated by apomorphic characters.

The ground plan of the Baetoidea

The phylogenetic analysis of the abdominal organisation in Baetidae and Siphlaenigmati-

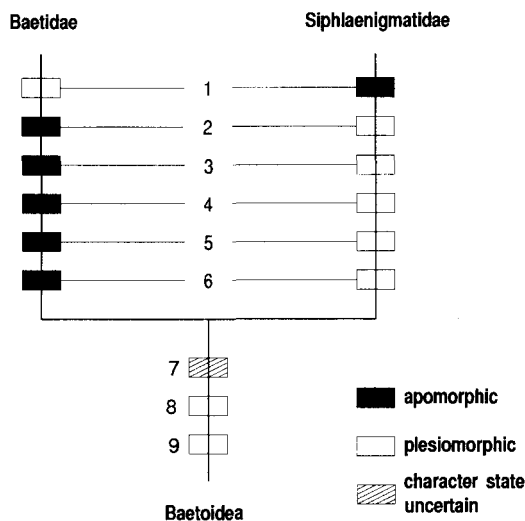


Fig. 14. Scheme of argumentation for the phylogeny of Baetoidea, internal characters. Plesiomorphic character states are added in parenthesis: 1. Dorsoventral muscle 3 acts as an additional gill adductor (two gill muscles); 2. Oblique longitudinal muscle 2 covers three segments (two segments); 3. Penis lobes internal and membranous (external and sclerotised); 4. Loss of intrinsic penis muscle (intrinsic penis muscle preserved); 5. Malpighian tubules straight (coiled); 6. Ganglion of abdominal segment VIII shifted to VII (separate ganglion in segment VIII); 7. Connectives of ventral nerve cord entirely fused; 8. (Visceral trachea present in abdominal segments I-VIII); 9. (Eggs without terminal fiber clusters, knob-terminated coiled threads, or polar caps).

dae leads to the ground plan reconstruction of the Baetoidea that reflects the hypothetical character set of their last common ancestor (Fig. 14). It is obvious that *Siphlaenigma* has retained a lot of plesiomorphic characters, while the Baetidae have evolved numerous derived characters. However, these big phenetic differences might be diminished by a better knowledge of the world fauna of the Baetidae. It is quite possible that some of the characters presently regarded as autapomorphies of the Baetidae have evolved within this group. There is for instance a yet undescribed South American baetid genus whose males have undivided eyes (Dr. Gillies, pers. com.). Further studies of the baetid fauna, especially of the Southern Hemisphere, might contribute to a better understanding of the phylogeny of the Baetidae. The sister group of Baetoidea is not yet recognised. Further comparative studies on the

larval abdominal muscular arrangement in different siphonuroid families might clarify this point, too.

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