

Incongruent phylogenetic hypotheses and character conflicts in morphology: The root and early branches of the hexapodan tree

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Abstract: Angestoßen durch molekulare Untersuchungen sind die Verwandtschaftsverhältnisse der Großgruppen der Arthropoden in der letzten Dekade ein Hotspot der phylogenetischen Diskussion geworden. Ein heiß diskutiertes Thema ist die Frage nach der Monophylie der Insekten. Die in diesem Zusammenhang besonders interessanten „Ur-Insekten“ bilden eine morphologisch sehr heterogene Gruppe von primär flügellosen Insekten. Während die Annahme verwandtschaftlicher Beziehungen der Archaeognatha und Zygentoma zu den Pterygota (Ectognatha) breite Unterstützung finden, gibt es widersprüchliche Hypothesen hinsichtlich der phylogenetischen Stellung der Collembola, Protura und Diplura. Zu heftigen Diskussionen haben die Analysen mitochondrialer Gene durch NARDI et al. (2003) geführt. In den rekonstruierten Bäumen sind die Ectognatha näher verwandt mit Crustaceen als mit Collembolen, wodurch die Hexapoden eine paraphyletische Gruppierung werden. Dieser Befund hat eine Flut von neuen molekularen Untersuchungen ausgelöst und nahezu alle denkbaren Kombinationen der Verwandtschaftsbeziehungen zwischen Collembola, Protura, Diplura und Ectognatha haben Unterstützung gefunden. Kann die Morphologie zur Klärung dieser phylogenetischen Konflikte entscheidend beitragen? Sind die Hexapoden wirklich ein Monophylum oder ist die Hexapodie mehrfach unabhängig voneinander entstanden? Nach Diskussion ausgewählter Konflikte zur phylogenetischen Stellung der verschiedenen „Ur-Insekten“ werden anhand eigener Untersuchungen exemplarisch einige Probleme morphologischer Datensätze in der modernen stammesgeschichtlichen Forschung aufgezeigt und entsprechende Lösungsmöglichkeiten diskutiert.

Key words: Hexapoda, phylogeny, character concept, Apterygota, congruence

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Before the turn of the millenium the investigation of phylogenetic relationships was revolutionized by two major inputs, the use of molecular sequence data for phylogenetic reconstruction, paralleled by the sophistication of computer aided reconstruction methods. The ever growing number of data however did not only result in clarifications of open questions, but brought forth a number of new conflicting phylogenetic hypotheses. Sometimes they are wrongly referred to as conflicts between morphological and molecular approaches, which sporadically even culminated in the rejection of the usefulness of one of the two approaches (e.g. SCOTLAND et al 2003). These scientists overlook the great advantage of having two a priori largely independent data sets (WÄGELE 2001) which in a synthetic way enable the greatest progress in phylogenetic research. However, solely putting data together will not suffice to choose among conflicting hypotheses. The increasing number of conflicts necessitates approaches that go beyond mere data congruence, but searching for the possible reasons of conflicts.

In the present paper, problems in the reconstruction of the phylogenetic origin of Hexapoda, as well as of the early branchings within the Hexapoda, will exemplify approaches of critical re-evaluation and testing of data used in morphological data matrices for phylogenetic analyses. The early cladogenetic events of hexapods are especially suited for such a discussion for several reasons. The hexapods, as the most species-rich group of organisms, look back at a long and multi-faceted history of taxonomic and phylogenetic studies, culminating in a number of conflicting hypotheses. Triggered by incongruences with morphological analyses the reconstruction of the hexapodan roots likewise became a hot-spot of molecular research activities during

the last two decades. Furthermore the phylogenetic positions of the oldest lineages branching off within the hexapodan clade, the Diplura, Protura and Collembola, are in particular very difficult to reconstruct. While at least the latter two are well defined by morphological autapomorphies their phylogenetic position could not be reconstructed unambiguously, since their morphology seems highly derived with respect to the hexapodan ground pattern.

1. From typological to phylogenetic reasoning

In LINNÉ's *Systema Naturae* (1758) groups of organisms were comprised into taxa based on typological reasoning. The aim in these early periods of taxonomy was to find unchangeable characteristics that reflect the hierarchically organized correspondence observable in nature. For example LINNÉ's *Insecta*, one of six classes of animals, comprise all animals with articulated limbs, roughly matching modern Arthropoda. One of the seven orders among *Insecta*, the *Aptera*, are characterized by the lack of wings. This order thus comprehended primarily wingless insects (basal hexapods), secondarily wingless insects, crustaceans, chelicerates and myriapods. Taxa thus form a system of hierarchically organized, graduated categories, differentiated by unchangeable observable properties.

A century later DARWIN (1859) set up the hypothesis that common descent is the cause for certain correspondences and consequently taxa were defined as groups of organisms that share a common ancestor. Since every character of an organism may change, it was recognized that a number of characters has to be studied to constitute taxa, nowadays known as the principle of character congruence. Following these insights BRAUER (1885) was able to distinguish primarily wingless insects, his *Apterygogenea*, from secondarily wingless insects, which he recognized as subgroups of his *Pterygogenea*. Taxa already are meant to constitute groups of common ancestry. However, dichotomous reasoning allows both modes of a character to support a taxon each. For example the taxon *Pterygogenea* is legitimated by the possession of wings, while in parallel the lack of wings legitimates the taxon *Apterygogenea*.

The final step toward strict phylogenetic reasoning was performed by HENNIG (1950), by realizing that not corresponding features, but transformation series (what we nowadays call "character") are necessary to reconstruct phylogeny. Only transformations from a plesiomorphic to an apomorphic state, when assigned to a stem lineage of a taxon, are able to support the monophyly of this taxon. Among Hexapoda the primary winglessness was recognized to constitute a symplesiomorphy, a correspondence irrelevant for phylogenetic reconstruction (HENNIG 1953). Consequently BRAUER's *Apterygogenea*, the *Apterygota* of LANG (1888), were recognized as an artificial group. Among hexapods HENNIG (1953) differentiates between *Entognatha* (Collembola, Protura, Diplura) and *Ectognatha*. The latter apart from the pterygote orders, comprises *Archaeognatha* and *Zygentoma*, two primarily wingless orders. *Zygentoma* are reconstructed as the sister group of *Pterygota* constituting the taxon *Dicondylia*.

2. Characters as transformation series

To be useful for phylogenetic purposes in terms of Hennigian reasoning, data has to meet some basic demands: (i) Variation has to be present in steps, so that states can be formulated which are parts of the same transformation series (i.e. a character). (ii) One state of a character is hypothesized to be the one from which every other state of the same character is eventually derived by transformations during evolution. (iii) Pairs of character states are hypothesized to be related by transformations that have occurred during evolution. (iv) Each pattern used to describe a character state has to be hypothesized homologous among all organisms assigned to the respective state. This pattern has to correspond and thus be observable among all organisms assigned to this state. The homology hypothesis regarding the pattern, used to describe the character state, supposes that after a single evolutionary origin in a common ancestor of all the united organisms, the pattern was maintained along diverging genealogical lineages by robust pattern transmission (SZUCSICH & WIRKNER, 2007). Note that HENNIG used the term *transformation series* for what we today call *character*, and *character* for what we now call *character state*. We will follow the modern terminology since it is widely used, although the terminology of HENNIG somewhat better reflects underlying hypotheses.

The above mentioned demands can be well exemplified on a character of HENNIG (1953). For the *mandible*, he distinguished two states *monocondylic* and *dicondylic* (for discussions whether this coding strategy reflects observable variations see e.g. STANICZEK 2000, FÜRST VON LIEVEN 2000 and KOCH 2001). The variation is

described in discrete steps since the states refer to the number of articulation points of the mandible with the head capsule. Hennig hypothesized monocondylic mandibles to be plesiomorphic, thus dicondylic mandibles were derived from monocondylic mandibles by an evolutionary transformation.

The patterns describing the character states use variations of the connections of the mandibles to the head capsule. The homology hypotheses underlying character state delineation in this character thus are as follows. In monocondylic mandibles a single posterior articulation point is built up by a ball and socket joint between a mandibular projection and a socket of the head capsule. This connection pattern is hypothesized to have originated in a common ancestor, maybe of all mandibulate arthropods. Dicondylic mandibles possess an additional anterior articulation point with a small condylic protrusion at the clypeus that is jointed to either a socket or ridges of the mandible. This pattern is hypothesized to have originated in the last common ancestor of Zygentoma and Pterygota by an evolutionary transformation from the monocondylous state.

That these two states are really parts of a single transformation series is supported by the frame homology of the mandibles themselves (for the term “frame homology” see RIEDL 1978; SCHOLTZ 2005; RICHTER 2005). Mandibles are the appendages of the second postantennary head-segment and homologous among all hexapodan (and mandibulate) orders. The homology of this character frame increases the plausibility of the hypothesized transformation and can be seen as a test of transformation hypotheses.

3. Computer aided reconstruction tools and the construction of data matrices

The introduction of computer-software that performs phylogenetic analyses has some effects on both character conceptualization and interpretation of character evolution. Data matrices are constructed that contain an ever increasing number of characters, i.e. independent hypotheses of evolutionary transformation series.

Since trees are reconstructed using congruence among an ever increasing number of characters, the risk of character dependence likewise increased since the dawn of computer-aided analyses. This is an often neglected reason for increased demands in proper character choice.

The polarization of characters, i.e. the interpretation which state is plesiomorphic and which is apomorphic, is mostly not made a priori as practised by HENNIG, but in modern analysis is one of the outcomes of rooting the tree. The latter is a process enabled by the inclusion of an outgroup.

As long as the ingroup is monophyletic with respect to the outgroup every outgroup taxon can be chosen to root a tree. However, to allow for unambiguous character reconstruction it is most preferable to take the sister group of the most narrow monophylum that includes all the ingroup taxa (e.g. STEWART 1993).

4. Conflicting hypotheses

As mentioned before, the history of phylogenetic analyses of deep hexapodan relationships resulted in a high number of conflicting trees, of which only some can be shortly reviewed here.

4.1. The origin of Hexapoda within Arthropoda

Before the dawn of molecular phylogenetics most scientists agreed that among Arthropoda myriapods are more closely related to hexapods than either crustaceans or chelicerates. The consequence is a monophylum comprising all Hexapoda and Myriapoda (*Atelocerata*- or *Tracheata*-hypothesis). However conflicting hypotheses exist regarding the relationships among myriapods and hexapods. Some authors prefer a sistergroup-relationship between Hexapoda and a monophyletic Myriapoda (BOUDREAUX 1979, 1987; AX 1999), while others believed the latter to be a paraphyletic assemblage. Mostly either Symphyla (e.g. HENNIG 1953; SHAROV 1966) or Progoneata, i.e. Symphyla + Pauropoda + Diplopoda (DOHLE 1965; WILLMANN 2005) then were proposed to be the sister group of Hexapoda.

The results of nearly all analyses that use molecular datasets are incongruent with morphological trees in that they reveal crustaceans as more closely related to Hexapoda than both myriapods and chelicerates (Pancrustacea- or Tetraconata-hypothesis). Mostly the analyses find crustaceans to be paraphyletic with respect to Hexapoda (e.g. MALLAT et al 2004; REGIER et al. 2005; GIRIBET et al 2005; MALLAT & GIRIBET 2006). The most promising candidates for the hexapodan sister group within crustaceans are either Branchiopoda or Malacostraca.

After the first period of excitement the mentioned incongruence stimulated morphologists to re-evaluate different character complexes. Thus characters were (re-)described that support the Pancrustacea-hypothesis

e.g. in the central nervous system (Harzsch 2004), and the ultrastructure of the compound eyes (DOHLE 2001, RICHTER 2002, PAULUS 2003). Correspondences in the structure of the ommatidia are recorded in the naming *Tetraconata* as a synonym for Pancrustacea.

Conflicts thus are able (and we think even necessary) to stimulate further scientific progress.

4.2. The monophyly of Diplura

A comparative analysis of ovarian structures among basal hexapods prompted STYS et al. (1993) to question the monophyly of Diplura. The metameric arrangement of ovarioles in Japygina and Ectognatha was recommended as a potential synapomorphy of these groups. Since Campodeina, like Collembola and Protura have non-metameric, saclike ovaries Diplura were supposed to be paraphyletic with respect to Ectognatha, if not polyphyletic (fig. 1).

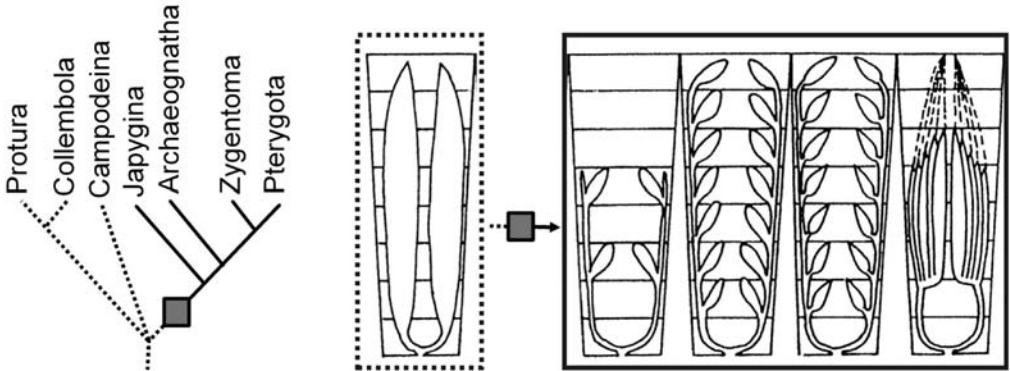


Fig. 1: Incongruences of a character tree (insect ovaries) with an established tree. Metameric ovaries as putative autapomorphies of Japygina and Ectognatha (modified after STYS et al. 1993)

So, like in many other cases, the addition of new data led to a new hypothesis, incongruent with traditional phylogenetic trees. The monophyly of Diplura was questioned seriously, since morphological characters supporting monophyly were scarce. However, the conflict again stimulated other authors to make comparative analyses of the two dipluran subgroups, resulting in additional support for dipluran monophyly (KOCH 1997, KRISTENSEN 1997).

4.3. The monophyly of Entognatha and the position of Diplura

Conflicting with the monophyly of Entognatha, some phylogeneticists proposed a sister group relationship of Diplura and Ectognatha (e.g. KUKALOVA-PECK 1987; KOCH 1997; KRAUS 1997; BEUTEL & GORB 2006). Such a clade is supported by possible apomorphies like the evolutionary origin of paired pretarsal claws and paired cerci. Both alternative trees can be obtained by rooting the same topology at different branches (fig. 2). As a consequence the polarization of certain character transformations differ between the trees. In Hennig's tree a transformation from ectognathy to entognathy is reconstructed as a possible synapomorphy of Diplura and Ellipura. Most authors that prefer a sister group relationship between Diplura and Ectognatha propose a convergent evolution of entognathy in Diplura and Ellipura (e.g. KOCH 1997). This however is not a necessary conclusion. A more parsimonious interpretation would be entognathous mouthparts as the plesiomorphic condition and a transformation from entognathy to ectognathy as a synapomorphy of Archaeognatha and Dicondylia (i.e. in the stem-lineage of Ectognatha). The main point of this example is the fact that some conflicts are not the result of an incongruent phylogenetic signal inherent in different character sets (i.e. different data matrices), but can be e.g. explained by re-interpretation of hypotheses of character polarity. It should be noted however, that the alternative trees result in different conflicts regarding homology hypotheses of single character states.

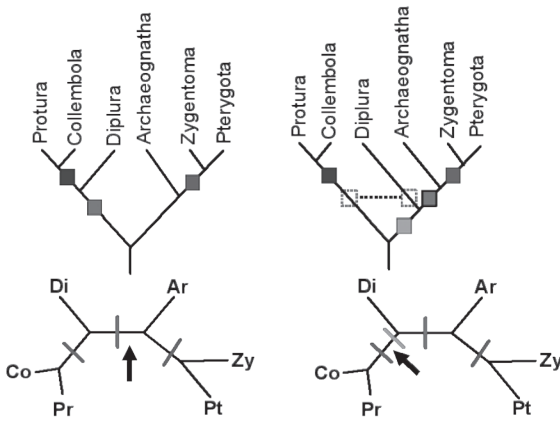


Fig.2: Competing trees due to differential rooting, of the same topology (see arrow), resulting in different hypotheses of character polarization. Trees may be based on the same data matrix. left: e.g. HENNIG (1953), AX (1999) right: e.g. KUKALOVÁ-PECK (1987), KOCH (1997), KRAUS (1997), BEUTEL & GORB (2006)

4.4. The monophyly of Ellipura

Analyses using nuclear ribosomal genes as markers result in a sister group relationship of Diplura and Protura (e.g. GIRIBET et al 2004; KJER 2004; LUAN et al 2005; MALLAT & GIRIBET 2006; DELL’AMPIO et al subm.), mostly even with a very high statistical support. This result is in conflict with the Ellipura–hypothesis. Since highly preferred in analyses based on ribosomal gene sequences, both hypotheses should be evaluated with as many independent datasets as possible. Especially incongruences with traditional hypotheses that are supported by only few apomorphies, or where supporting apomorphies represent mainly reductions and losses should induce comparative morphological re-investigations to test among the competing hypotheses.

4.5. The monophyly of Hexapoda

In 2003 NARDI et al published phylogenetic analyses of mitochondrial sequence data that for the first time included basal hexapodan representatives (i.e. collembolan sequences). The analyses failed to reveal the monophyly of Hexapoda, but instead proposed a sister group relationship of Ectognatha and Crustacea. The Collembola remain members of a monophyletic Pancrustacea, but their branch was revealed as the earliest split within this clade. Most following analyses of mitochondrial genes likewise failed to recover hexapodan monophyly (CAMERON et al 2004; CARAPELLI et al 2005; COOK et al 2005; HASSANIN 2006; but see DELSUC et al 2003).

Many of these studies rightfully point at the weak morphological support for monophyletic Hexapoda (problems reviewed in KLASS & KRISTENSEN 2001). The most prominent putative apomorphy of Hexapoda is the evolutionary origin of the tripartite body organization, comprising the head, a three-segmented thorax bearing the three pairs of locomotory limbs, and an abdomen that lack walking legs (e.g. HENNIG 1953; KRISTENSEN 1981, 1991; BITSCH et al 2004). The correspondences in leg morphology go beyond the mere occurrence in triplicate in that the legs of nearly all hexapods are subdivided into six podomeres (coxa, trochanter, femur, tibia, tarsus and pretarsus). However, in Collembola only two podomeres occur distal to the femur, called tibiotarsus and pretarsus. If six podomeres are regarded an apomorphy of Hexapoda, this has to be interpreted as a secondary change in the number of podomeres of Collembola.

Another possible apomorphy of the Hexapoda is the evolutionary origin of two primary pigment cells in their eyes (e.g. PAULUS 1979). Remarkably again in the orders which most probably have originated from the earliest splits among Hexapoda this character is problematic. All representatives of both Diplura and Protura lack eyes and thus have to be coded inapplicable in this character. Moreover, two primary pigment cells may have already been present in the pancrustacean ground pattern (RICHTER 2002).

AX (1999) following JAMIESON (1987) mentioned the evolutionary origin of a ring of 9 accessory tubules in the sperm axonema as a possible apomorphy of Hexapoda. Such sperm axonemes of the 9+9+2 type, however, can be observed only in Diplura and Ectognatha. Among Protura and Collembola either highly derived states occur (BACCETTI & DALLAI 1973), or the axoneme structure exhibits the 9+2 type, which since very common

among Metazoa is most probably the plesiomorphic state (JAMIESON 1987; DALLAI et al. 2004).

Thus we see that most characters that present possible apomorphies are either not applicable or show (maybe secondary) variations in some of the primarily wingless hexapod orders. We can hope that congruence among characters still suffices to reveal hexapodan monophyly, however we have to check whether alternative hypotheses really result in a significant increase of single-character conflicts. The arbitrariness of some hypotheses is worsened since many of the characters lack unambiguous reconstructions, due to the unresolved hexapodan sister group problem.

But are these problems sufficient to reject the monophyly of Hexapoda due to the results obtained on mitochondrial sequence data? What we have to bear in mind is that the alternative hypothesis to monophyletic Hexapoda is not “hexapods not monophyletic”. The analyses of mitochondrial genes by Nardi et al. proposed that Ectognatha are more closely related to Crustacea than to Collembola. This alternative phylogenetic hypothesis needs support from independent datasets. So far no possible morphological synapomorphy is known to the authors that supports a Crustacea + Ectognatha clade (fig. 3). This sister group relationship likewise lacks support from independent molecular datasets. Even analyses of mitochondrial gene sequences mostly do not reveal the same clade, but conform only in the point that they do not reveal hexapodan monophyly (COOK et al. 2005; HASSANIN 2006). On the other hand the hypothesis of monophyletic Hexapoda already obtained support from molecular analyses (REGIER et al. 2005; MALLAT & GIRIBET 2006).

Thus despite of conflicts monophyly of Hexapoda still seems to be the best corroborated among incongruent phylogenetic hypotheses. However, the incongruent results obtained by analyses of mitochondrial sequence data are important to stimulate re-investigations of the early steps in hexapodan phylogeny and the search for causal explanations of incongruences.

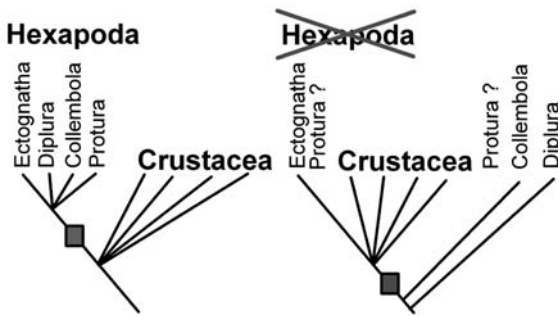


Fig.3: Monophyly of Hexapoda?

left: The hypothesis of hexapodan monophyly is dependent on supporting synapomorphies for Ectognatha, Diplura, Collembola and Protura
 right: The alternative hypothesis proposed by CARAPPELLI et al. 2005 based on mitochondrial genes is dependent on supporting synapomorphies of Crustacea + Ectognatha (since no data on proturan mitochondrial genes exist so far, the position of Protura is unclear)

5. Case studies: causes for conflicts and incongruences

Currently we seek in our research to shed further light on these early phylogenetic events, employing both morphological and molecular data sets. First results of our studies are presented to exemplify search strategies for causal explanations of conflicts both within and among data sets.

5.1. Character complexity and danger of homoplasy: clues from claws

Analyses of morphological data do not only differ from molecular analyses, but likewise differ from each other depending on which coding strategy is applied. While in many other characters coding varies between authors, pretarsal claws characters are used very uniformly in phylogenetic analyses. Among Hexapoda the Diplura and Ectognatha are mentioned to possess dorsolaterally articulated pretarsal claws, called ungues. In contrast, the pretarsus of Collembola and Protura itself is a clawlike, fully sclerotized cone tapering toward the tip, lacking paired, dorsolaterally articulated projections (SNODGRASS 1935, BOUDREAUX 1987). This variation was integrated in most of the data matrices for analyses of hexapods as a binary character. The two states of the character pretarsal claws were either “(0) unpaired claws” and “(1) paired claws” (Giribet et al 2005), or “(0) one claw” and “(1) two claws” (BITSCH & BITSCH 2000; BEUTEL & GORB 2001; WHEELER et al 2001; GIRIBET et al 2004). Thus the underlying homology and transformation hypotheses are:

- a) Unpaired claws are homologous among Protura and Collembola, i.e. unpaired pretarsal claws originated in a common ancestor of these groups and the pattern was robustly transmitted from generation to generation.
- b) Paired claws are homologous among Diplura, Archaeognatha, Zygentoma and Pterygota and originated once in a common ancestor of these groups.
- c) In the course of evolution a transformation has taken place changing one state into the other. So either paired claws (plesiomorphic state) were transformed into unpaired claws (apomorphic state) or vice versa.

5.1.1. Conflicts of coding strategy with empirical facts

In all data matrices published so far both Japygina (like Campodeina) and Zygentoma were assigned to the state “(1) paired claws” [or “(1) two claws” respectively]. In both Japygina and Zygentoma, however, a median, unpaired, clawlike structure can be observed in addition to the pair of dorsolateral ungues. Remarkably, this fact was already mentioned by SNODGRASS (1935) and BOUDREAUX (1987) (fig. 4a) but obviously overlooked by the later authors.

Two possible strategies of recoding can be chosen to give consideration to this fact. Either a third character state can be formulated with “(2) three claws”, changing the assignment of taxa to character states, or the characterization of the states can be changed, to retain a binary character with the same assignment of taxa to the states, e.g. in the following way:

Pretarsus (= character frame): “(0) undivided, fully sclerotized cone” (in Protura and Collembola), “(1) a pair of dorsolateral claws separated from the remaining pretarsal sclerites by an unsclerotized region” (in Campodeina, Japygina, Archaeognatha, Zygentoma and Pterygota).
 A second character then may address the observable variation of the pretarsal sclerite itself.

5.1.2. Ambiguous character reconstruction

The reconstruction of this claw character depends on the decision about polarization and was discussed, in two different ways. (i) In analyses supporting monophyletic Entognatha, a pretarsus with paired dorsolateral claws is reconstructed as the plesiomorphic state; consequently the loss of paired dorsolateral claws is a possible synapomorphy of Collembola and Protura. (ii) In analyses supporting a sister group relationship of Diplura and Ectognatha, the reconstruction of the plesiomorphic state is ambiguous. Often, however, an unpaired clawlike pretarsus is discussed to be plesiomorphic, leaving the origin of dorsolateral claws a possible synapomorphy of Diplura + Ectognatha. The best way to chose among these competing hypotheses of character polarity is by outgroup comparison (fig. 4b). The assignment of the hexapodan sister group to one of the states would increase the plausibility that this state is plesiomorphic for Hexapoda.

Among Myriapoda, most Diplopoda possess an undivided clawlike pretarsus, while Symphyla possess paired dorsolateral claws. Among Crustacea, where more probably the sister group of Hexapoda can be found, most species have a clawlike distalmost podomere. However, in some Malacostraca (mainly Amphipoda) paired lateral claws are present. So these outgroups do not help to unambiguously reconstruct the ground pattern of hexapodan claws.

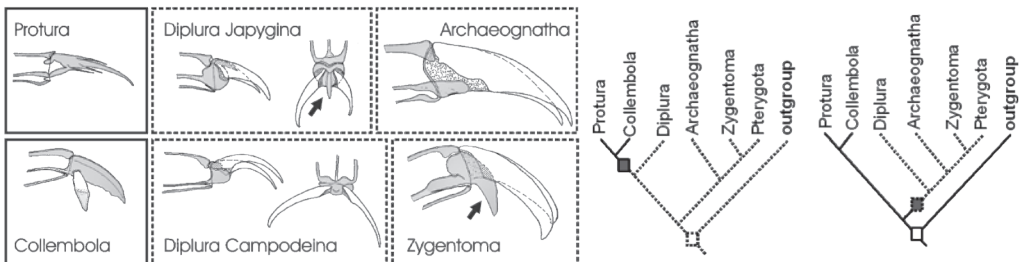


Fig.4: Characters of claws in phylogenetic analyses. a. the presence of a unpaired clawlike structure in Japygina and Zygentoma is in conflict with the conventional coding (modified after BOUDREAUX 1987) b. the polarization of the character can not unambiguously be solved by outgroup comparison

Among the chelicerates, a more distant outgroup, likewise both states can be observed. While most taxa have paired lateral claws, in some taxa the distalmost podomere is an undivided clawlike cone (DUNLOP 2000).

So the apparently high degree of homoplasy among arthropods poses the question, whether a transformation from either state into the other can give a trustworthy support to any node in analyses of deep arthropod phylogeny.

This character even though not useful in phylogenetic inference, may be interesting to solve general questions of organ evolution. It could be a good model system to search for causes of high degree of homoplasy. Maybe only a few changes during development may be sufficient to change from undivided clawlike distalmost podomeres to podomeres with a pair of lateral claws.

5.2. Is extraction of literature data sufficient to construct a data matrix? Leg characters of Diplura

Over centuries morphologists have accumulated a vast amount of comparative data in many character complexes. Here we want to point out some reasons why nevertheless we think that mere extraction of literature data usually does not suffice to construct morphological data matrices for phylogenetic reconstructions. This will be exemplified on re-evaluations of characters of the dipluran leg, which up till now were extracted from literature if used in phylogenetical analyses.

In comparative studies on the arthropod leg MANTON (1977) described correspondences between the two suborders of Diplura, Japygina and Campodeina. She mentioned both the unique rotator muscles inserting to the trochanter–femur joint region, and a unique pivot of the femur–tibia joint region to be unique among Hexapoda. Since other possible morphological synapomorphies are rare, KRISTENSEN (1991) stated that the monophyly of Diplura is “seemingly justified by the unique leg features noted by Manton”.

MANTON compared only representatives of the dipluran subgroups Campodeoidea and Japygoidea. Therefore the aim of our own study in the first place was to include a representative of Projapygoidea into the comparison. Our analyses are mainly based on SEM and 3D-reconstruction of semi-thin section series.

5.2.1. Trochanter–femur joint region

Manton (1977) described the trochanter–femur joint in Diplura as monocondylic, represented by a conspicuous anterior articulation, while lacking a posterior articulation. Regarding musculature, she described a single muscle inserting at the femur and called it the femoral rotator. Combining the monocondylic nature and the femoral muscle she concluded that the trochanter–femur joint functions as a rotating pivot. Manton likewise compared this joint region among hexapodan orders (fig. 5). Only in Protura she likewise described a monocondylic joint. However, she concluded that in all non-dipluran hexapods the joint can be flexed only in a two dimensional moving plane. The more complex movement of the trochanter–femur joint in Diplura she referred to as a *twisting* or *rocking* movement.

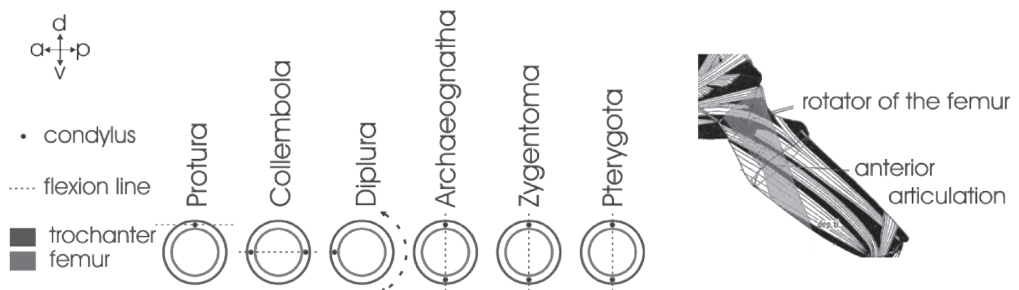


Fig.5: Trochanter-femur joint region as described by MANTON (1977). A rocking movement of the monocondylic joint in Diplura is achieved by the rotator of the femur, the only muscle inserting at the region (modified after MANTON 1977)

Three analyses up till now included Manton’s data into their data matrices. WHEELER et al. (2001) and GIRIBET et al. (2004) delineated two states distinguishing “(0) thoracic leg musculature without trochanter–femur twisting muscle” from a state “(1) leg musculature with such muscles”. GIRIBET et al (2005) likewise referring to Manton, applied an absent-present coding with “Unique trochanteral–femur twisting muscle”

“(0) absent” or “(1) present” respectively. Essentially both coding strategies allowed the used algorithms to reveal the possible synapomorphy of Campodeina and Japygina, and thus to support dipluran monophyly. They base on the unique twisting movement, mainly a consequence of the monocondylic nature of the joint. An own re-evaluation in three species of Diplura revealed the presence not only of a conspicuous anterior articulation, but also of a somewhat smaller posterior trochanter–femur articulation. Thus what we found is an anterior-posteroventral dicondylic trochanter–femur joint with a similar position of the articulations as in Collembola (Fig. 6), which allows for no twisting movement of this joint.

The analysis of the muscles inserting at the femoral base revealed a highly corresponding pattern in all investigated dipluran species. One muscle originates at the coxa and inserts to the dorsoanterior femoral base. A second muscle originates at the ventral trochanteral base and inserts to the ventral femur. A third muscle originates at the dorsal trochanteral surface and obliquely runs toward the ventral femoral surface. Whether a fourth muscle should be addressed to as a separate muscle or a bundle of the third can not be decided unambiguously.

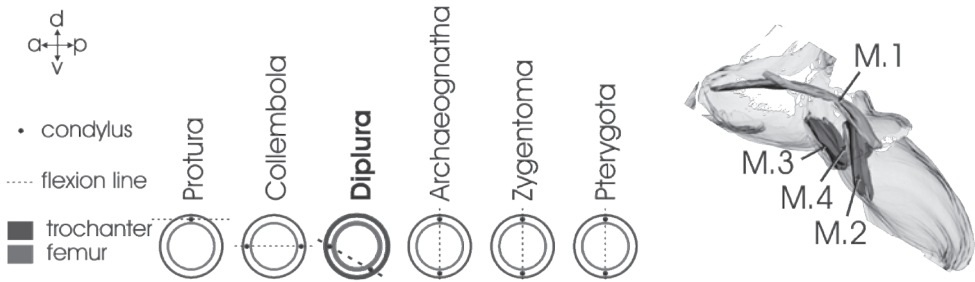


Fig. 6: Re-evaluation of the trochanter-femur joint region. Three to four muscles insert at the region of the dicondylic joint in Diplura. right: 3D-Reconstruction of the trochanter–femur joint region of *Campodea augens*

What counts however is the insight that it is hard to state which of these muscles is the one observed by MANTON (1977). We think that Manton, since two muscle portions are depicted in her figures, united all muscles inserting at the ventral femur in her femoral rotator, but failed to observe the muscle of coxal origin. Regardless of this speculation a comparison with other taxa is necessary to conclude whether this muscular pattern may be used in character coding. The coding as used by previous authors, however, is not in accordance with the empirical facts. The observed correspondence among all investigated dipluran species however in future may reveal some possible synapomorphy of Campodeina and Japygina.

5.2.2. Femur–tibia joint region

According to MANTON the femur–tibia joint, mostly referred to as the knee of Hexapoda, is monocondylic in Diplura and situated at the dorsal side of the leg. The joint region is built up of conspicuous cuticular structures of both podomeres (fig. 7b). The tibia forms a dorsal process Z. Anterior to this process lies the process W of the femur which is linked with the anterior shoulder Y of the tibia.

MANTON additionally described two muscles inserting to this region: one inserting at the dorsal side to the tibial process Z, and a second inserting to the ventral base of the tibia. MANTON again compared this joint region among Hexapoda and found it to be monocondylic in Protura, Collembola and Diplura, opposing a dicondylic joint in Archaeognatha, Zygentoma and Pterygota. However, she stated that the cuticular structures found in Diplura are unique among Hexapoda (fig. 7).



Fig. 7: Femur-tibia joint region as described by MANTON (1977). left: Comparison among hexapods. right: unique cuticular structures of the dipluran knee (modified after MANTON 1977)

MANTON’s data again was extracted into data matrices of three analyses. WHEELER et al. (2001) and GIRIBET et al. (2004) based on MANTON’s comparison delineated two states, distinguishing “(0) monocondylic femur–tibia joints” from “(1) dicondylic joints”. GIRIBET et al 2005 on the other hand in their character “unique femur tibia pivot joint” employed a present-absent coding with Diplura assigned to the state “(1) present”, contrasting all other hexapods assigned to a state “(0) absent”. So while the first two studies hypothesize a transformation between Protura, Collembola and Diplura on the one hand and Archaeognatha, Zygentoma and Pterygota on the other hand. GIRIBET et al (2005) hypothesize a transformation between the remaining Hexapoda and Diplura, allowing the algorithm to obtain the possible synapomorphy of Campodeina and Japygina.

Our re-evaluation of all three investigated species confirms the pattern in cuticular structures as described by MANTON (1977). The analyses of the muscular system again found a high degree of correspondence among all investigated Diplura, however not only a dorsal and a ventral, but additionally an anterior muscle complex could be described.

As mentioned above, Manton stated that the cuticular structures of the dipluran knee are unique among Hexapoda. Thus the delineation of only two states with “(0) monocondylic femur–tibia joints” differentiated from “(1) dicondylic femur–tibia joints” is questionable since Diplura then are assigned to the same character state as Collembola and Protura, where the knee is described as a simple monocondylic pivot joint by MANTON (1977). First results on the currently analyzed knee of *Lepisma sachharina* (Zygentoma) likewise questioned the alternative present-absent coding of GIRIBET et al. (2005). In SEM studies of the silverfish we likewise found an assymmetric femur–tibia joint with a dorsal tibial process Z, again an anterior femoral process W and an anterior shoulder Y which is much more advanced than the posterior shoulder (fig. 8). Preliminary results likewise indicate that three muscle complexes are attached to the tibial base of Zygentoma, at sites corresponding with those of Diplura.

So what are the implications of our results for character coding? Homology hypotheses underlying character state delineation should not be based on functional considerations (like for example in the rotator of the femur). The state „absent“ in present-absent coding should not be used to replace the notion „state not known“ or “state different”. Despite the high importance of literature data in the construction of data matrices, authentic re-evaluations will always be necessary to confirm both plausibility of character state delineations, as well as of homology and transformation hypotheses underlying character conceptualization (Pass et al. 2006). This is even more important since in literature often only the apomorphic character state of a transformation series was specified by a defined pattern, allowing for no proper transformation hypotheses.

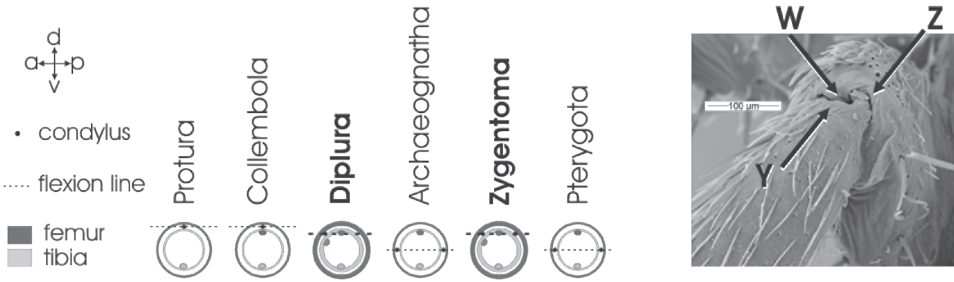


Fig. 8: Re-evaluation of femur-tibia joint region. The knee of *Zygentoma* shows a high degree of correspondence to the dipluran knee, questioning the current coding in phylogenetic analyses.

5.3. Tests for conflicting hypotheses: Ellipura versus Nonoculata

In the molecular part of our project we use the 28S rRNA to reconstruct the early steps in the evolution of Hexapoda. Phylogenetic trees are mainly based on Maximum Likelihood and Bayesian approaches, but likewise include Maximum Parsimony and Minimum Evolution. All methods yielded strong support for the monophyly of Collembola, Diplura, Ectognatha and Dicondylia, as well as for a cluster composed of Diplura and Protura (“Nonoculata-hypothesis”). This last cluster was already obtained by other analyses based on nuclear ribosomal gene sequences (GIRIBET et al 2004; KJER 2004; LUAN et al 2005; MALLAT & GIRIBET 2006).

5.3.1. Data exclusion a posteriori to test for artefacts

A comparison of nucleotide frequencies and branch length support the apprehensions of Luan et al. (2005) that Nonoculata may be an artificial cluster caused by a shared GC bias of the 28S sequences of these two orders, in combination with a long branch effect. An instability of the position of the “Nonoculata” within Pancrustacea dependent on outgroup choice and analytical method further nourished our doubts. Protura and Diplura either form the sister group of Collembola (Entognatha), or cluster with branchiopod crustaceans. Thus we selectively excluded taxa to test for the amount of noise introduced to the analyses by the above mentioned factors. Exclusion of all Nonoculata resulted in an increased support for neighboring nodes. Exclusion of the highly heterogeneous dipluran sequences results in monophyletic Hexapoda, with Protura forming the sister group of Collembola.

5.3.2. Evaluation of Nonoculata- and Ellipura-hypothesis by comparative morphology

We think that the increased rate of sequence evolution and the compositional bias in base frequency is sufficient to explain the clustering of Protura and Diplura in phylogenetic analyses of nuclear ribosomal genes. However, a shared compositional bias may likewise be an indication of common ancestry. Thus we nevertheless recommend a comparative evaluation of the morphological support of the two incongruent phylogenetic hypotheses “Ellipura” and “Nonoculata”.

Ellipura is mainly supported by negative characters, like the reduction of antennae to 4 antennomeres in maximum, the reduction of the tracheal system, the reduction of abdominal segments in newly hatched organisms and the loss of cerci. All these reductive characters are problematic. While Protura have no antennae at all, most collembolan species have four-segmented antennae. Protura hatch with nine abdominal segments and the number increases during postembryonic development to finally 12 segments. Collembola have, like all other hexapods except Protura, an epimeric development, where the number of segments does not change after hatching. Their abdomen is built up of six segments only and thus the formulation of the lack of cerci i.e. appendages of the 11th segment, as an independent character is highly questionable. The tracheal system in Collembola (if present) contains only a stigma in the neck region, while in Protura meso- and metathoracal stigmata are present (in the subgroup Eosentomida only). Thus in all these characters it seems nearly impossible to define a homology hypothesis that legitimates the assignment of Protura and

Collembola to the same character state. The only positive apomorphy of *Ellipura* is the evolutionary origin of the linea ventralis, a cuticular groove at the ventroposterior midline of the head.

When proposing the name “Nonoculata” for a clade uniting Protura and Diplura, LUAN et al. (2005) mentioned the complete loss of eyes as the only morphological synapomorphy known at that time. Two uncertainties remain regarding the homology of completely reduced eyes. To be homologous the eyes have to be lost in a common ancestor of Diplura and Protura. This is in conflict with the controversial fossil *Testajapyx thomasi* which has well developed compound eyes and was assigned to the stemgroup of Diplura by KUKALOVÁ-PECK (1987). This assignment however was questioned by other authors (see GRIMALDI & ENGEL 2005). A second uncertainty lies in the high degree of homoplasy regarding the loss of eyes. Eyes were lost independently in many arthropod groups, e.g. many Collembola.

A second pattern corresponding between Protura and Diplura is the ventral articulation of the coxa with the sternite of the respective body segment (Szucsich pers. obs.). The trustworthiness of such a synapomorphy is dependent on two so far unsettled topics. (i) The conclusive decision whether a ventral articulation is an apomorphic state is dependent on the reconstruction of the hexapodan ground-pattern. This cannot be unambiguously clarified without solving the sistergroup-problem. (ii) The inclusion of additional elements into the homologized pattern of the ventral articulation will increase the plausibility of the homology hypothesis. This can be both a more detailed description of the joint itself, as well as the pattern of muscles inserting at the ventral coxal base. As we see, both the *Ellipura*- and the *Nonoculata*-hypothesis are not well supported from morphology, making a choice among them rather subjective.

6. Conclusions

Data matrices are not hypothesis-free. Causes of conflicts often lie in erroneous assumptions and input-hypotheses necessary for phylogenetic analyses. Among possible causes for trees incongruent with results based on independent datasets are the following:

1. Erroneous homology hypotheses underlying character state delineation. One of the most prominent sources of error is the use of a state “absent” as a melting pot for “different from the other state(s)”, a problem which is not only restricted to character states coded “absent”, but occurs whenever a state is characterized solely by its difference from the alternative state. Such a use is often a consequence of taking over the dichotomous reasoning acceptable in determination, but is at risk to result in phenetic trees instead of phylogenetic ones.
2. High degree of homoplasy resulting in ambiguous character reconstructions
3. Violations of assumptions. Sources of errors in model-based analyses are the assumptions of a steady evolutionary rate through time and along branches.

Incongruences between results obtained by independent data sets are a first guide to localize weaknesses in one of the applied data sets. The data sets then can be further tested to find causal explanations for observed conflicts. So is not the avoidance of conflicts that stimulates scientific progress but its acceptance and deeper analysis. Further development will rely on an integrative approach that consider all different kinds of independent data to reconstruct the succession of phylogenetic events during evolution. Since the weaknesses of data sets are best known to the experts, it will be necessary to focus not only on the strengths but also on the weaknesses of the “own” data set.

With respect to the phylogenetic topic under consideration in this paper probably a number of additional morphological and molecular investigations will be necessary to elucidate the earliest cladogenetic events within Hexapoda.

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