[REVIEW]

The Phylogeny of Hexapoda (Arthropoda) and the Evolution of Megadiversity

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Abstract

   Phylogenetic analyses of single genes and transcriptomes confirm that Hexapoda are a subgroup of Pancrustacea, arguably most closely related with the specialized cave-dwelling Remipedia. The earliest evolutionary history in the marine environment remains unknown. The monophyly of Hexapoda is clearly supported by molecular evidence, by the specific tagmosis, and by morphological apomorphies implied by the Pancrustacea concept. The basal branching pattern, *i.e.*, the interrelationships of the entognathous orders remain ambivalent. The monophyly of Insecta (= Ectognatha), Zygentoma (incl. the "living fossil" *Tricholepidion*), Dicondylia (= Zygentoma + pterygote insects), and Pterygota is confirmed. The acquisition of wings was arguably the most important single (and unique) innovation in hexapod evolution, providing an efficient escape mechanism, drastically improving the dispersal ability, and making three-dimensional space easily accessible. The basal branching pattern in Pterygota remains ambiguous (referred to as "Palaeoptera problem"). The long disputed monophyly of Polyneoptera is confirmed by morphological and developmental features, and also by transcriptomic data. The controversial Zoraptera is placed in this lineage, which is mostly characterized by plesiomorphic morphological features. The branching pattern within Polyneoptera remains ambiguous even though a large clade comprising Xenonomia (= Grylloblattodea + Mantophasmatodea), Eukinolabia (= Embioptera + Phasmatodea) and Dictyoptera (= Mantodea + Blattodea incl. termites) is suggested by analyses of transcriptomic data, with the species-rich Orthoptera as its sister taxon. Acercaria (= Paraneoptera excl. Zoraptera) and Holometabola form a clade Eumetabola, even though this is weakly supported by morphological data if at all. The monophyly of Acercaria remains ambiguous with respect to Psocodea (Psocoptera + Phthiraptera). Thysanoptera form a clade Condylognatha with Hemiptera. The great diversity of the latter group is likely linked with mouthparts specialized on piercing and sucking fluids, and with a close connection to plants. Holometabola, by far the most species rich group of insects, comprise three large clades Hymenoptera, Neuropteroidea [= Neuropterida + (Strepsiptera + Coleoptera)], and Mecopterida (Amphiesmenoptera + Antliophora). The successful evolutionary interaction between angiosperm plants emerging in the Cretaceous and holometabolous groups has triggered unparalleled diversifications.

   About 1,000,000 extant insect species are named and described while estimates with different approaches suggest a total of at least 5 million (Stork *et al.*, 2015). With these enormous numbers, Hexapoda (insects in the widest sense, *i.e.*, including the entognathous orders) very clearly surpass the diversity of all other known groups of organisms (*e.g.*, Grimaldi & Engel, 2005: fig. 1.3). The extreme species-richness is only one of many factors that made insects a highly attractive and interesting group. Aside from studies related to

the tremendous medical and economic importance of insects, the morphology, embryology, phylogeny and evolutionary biology have attracted numerous researchers since a long time (*e.g.*, Grimaldi & Engel, 2005; Friedrich *et al.*, 2014; Kjer *et al.*, 2016). A milestone in insect systematics was laid by the German dipterist Willi Hennig with his "Stammesgeschichte der Insekten" (Hennig, 1969) in the late 1960s (Fig. 1), by that time exclusively based on morphological characters, but with a revolutionized method of phylogenetic evaluation

("Phylogenetische Systematik"; Hennig, 1950, 1966). In the last two decades, molecular data became more and more available and dominant in the exploration of phylogenetic relationships of insects, with data sets of rapidly increasing size (*e.g.*, Trautwein *et al.*, 2012; Yeates *et al.,* 2016; Kjer *et al.*, 2016). This tendency culminated in a recent study based on 1478 orthologous genes with taxon sampling of 144 terminals covering all insect orders plus outgroups, and including rare phylogenetic key taxa such as for instance *Tricholepidion gertschi* (Zygentoma?) or *Nannochorista* (Mecoptera?) (Misof *et al.*, 2014).

The present study summarizes the present state of insect phylogenetics, covering recent studies based on innovative anatomical techniques and large and well-documented morphological character sets, investigations of embryological features, and evaluations of extensive molecular data, transcriptomes or genomes. Possible factors leading to strong diversification are discussed as well as future perspectives of insect phylogenetics and evolutionary biology.

The position of Hexapoda

A critical issue is the phylogenetic origin of Hexapoda. Traditionally the group is placed in a monophylum Tracheata (=Antennata or Atelocerata) together with Myriapoda (*e.g.*, Kraus and Kraus, 1994). It was long accepted that a common ancestor of this lineage acquired terrestrial habits in the Silurian, and that specific adaptations such as the tracheal system and Malpighian tubules have evolved only once, as autapomorphies of this unit.

Today it is widely accepted that insects are not the closest relatives of myriapods but of aquatic crustaceans (*e.g.*, Richter, 2002). The monophyly of a clade Pancrustacea (= Tetraconata) was consistently supported by analyses of molecular data. This started with a study based only on few hundred base pairs of ribosomal genes and a very limited taxon sampling (Friedrich and Tautz, 1995), continued with evaluations of an increased number of genes plus morphological characters (*e.g.*, Giribet *et al.*, 2001, 2005), and was finally also confirmed by analyses of multi-gene data sets (Regier *et al.*, 2008, 2010) and transcriptomic data (v. Reumont *et al.*, 2009, 2012; Meusemann *et al.*, 2010; Misof *et al.*, 2014). The precise position of Hexapoda in the clade Pancrustacea is not fully clarified yet. However, it is evident that insects are "terrestrial crustaceans", and probably closely related with the highly specialized cave-dwelling Remipedia (Fanenbruck *et al.*, 2004; v. Reumont *et al.*, 2012; Misof *et al.*, 2014), a group with only *ca*. 20 known free-swimming, cave-dwelling and eyeless species.

The Pancrustacea hypothesis implies that hexapods have acquired terrestrial habits independently, probably already in the Ordovician (Misof *et al.*, 2014). Consequently, the tracheal system, Malpighian tubules, sperm transfer *via* a spermatophore, and other features considered as autapomorphies of "Tracheata" have evolved independently in

hexapods and myriapods. That these features evolved several times (also in Arachnida) can be explained by a strong and similar selective pressure related to the change to the terrestrial environment.

The example of Tracheata shows, that seemingly plausible evolutionary scenarios are only as good as the underlying phylogenetic hypothesis. The picture can change very distinctly when a long accepted systematic concept turns out to be wrong.

The earliest evolution of Hexapoda and potential stem group fossils

Following the Pancrustacea concept it is evident that Hexapoda must have had an origin in the marine environment. However, no ancestral aquatic fossils are known so far. A Devonian fossil described as *Devonohexapodus bocksbergensis* was interpreted as an aquatic stem group of hexapods (Haas *et al.*, 2003). This would have had a strong impact on the interpretation of the earliest evolution of the group. However, it was shown by Kühl and Rust (2009) that the single (and somewhat deformed) specimen belongs to a species already described, *Wingertshellicus backesi*. The systematic affinities remain rather unclear. However, *Wingertshellicus* is not closely related to Hexapoda, but probably ancestral within Euarthropoda (Arthropoda excl. Onychophora and Tardigrada), close to the root of this large monophylum (Kühl and Rust, 2009). Consequently, the earliest evolutionary history of insects remains in the dark. The example of "*Devonohexapodus*" shows that fossils, especially poorly preserved ones and single specimens, should be treated very carefully and critically. Wrong identifications can lead to serious evolutionary misinterpretations.

The monophyly of Hexapoda

The monophyly of Hexapoda was never seriously questioned in the morphological era of insect phylogenetics (*e.g.*, Hennig, 1969), even though Kristensen (*e.g.*, 1975) noted that the morphological support is weak, with basically only one apomorphic character complex, the tagmosis with a relatively short 3-segmented thorax and a distinctly longer abdomen, with 11 segments in most groups (groundplan of Insecta and probably also Hexapoda). A crucial evolutionary innovation linked with this feature is the far-reaching division of labor within the postcephalic body. The locomotor organs with their strongly developed musculature are concentrated in the thorax, three pairs of legs in all hexapods and two pairs of wings in most groups of pterygote insects. The abdomen contains the largest section of the digestive tract, the excretory organs, the main part of the fat body, and the genital organs.

That this unique and complex apomorphy has greatly contributed to the diversification in the early evolution of hexapods is unlikely. Four of five apterygote orders have a low diversity, with only several hundred species each. However, it is likely that a compact tagma equipped with strong
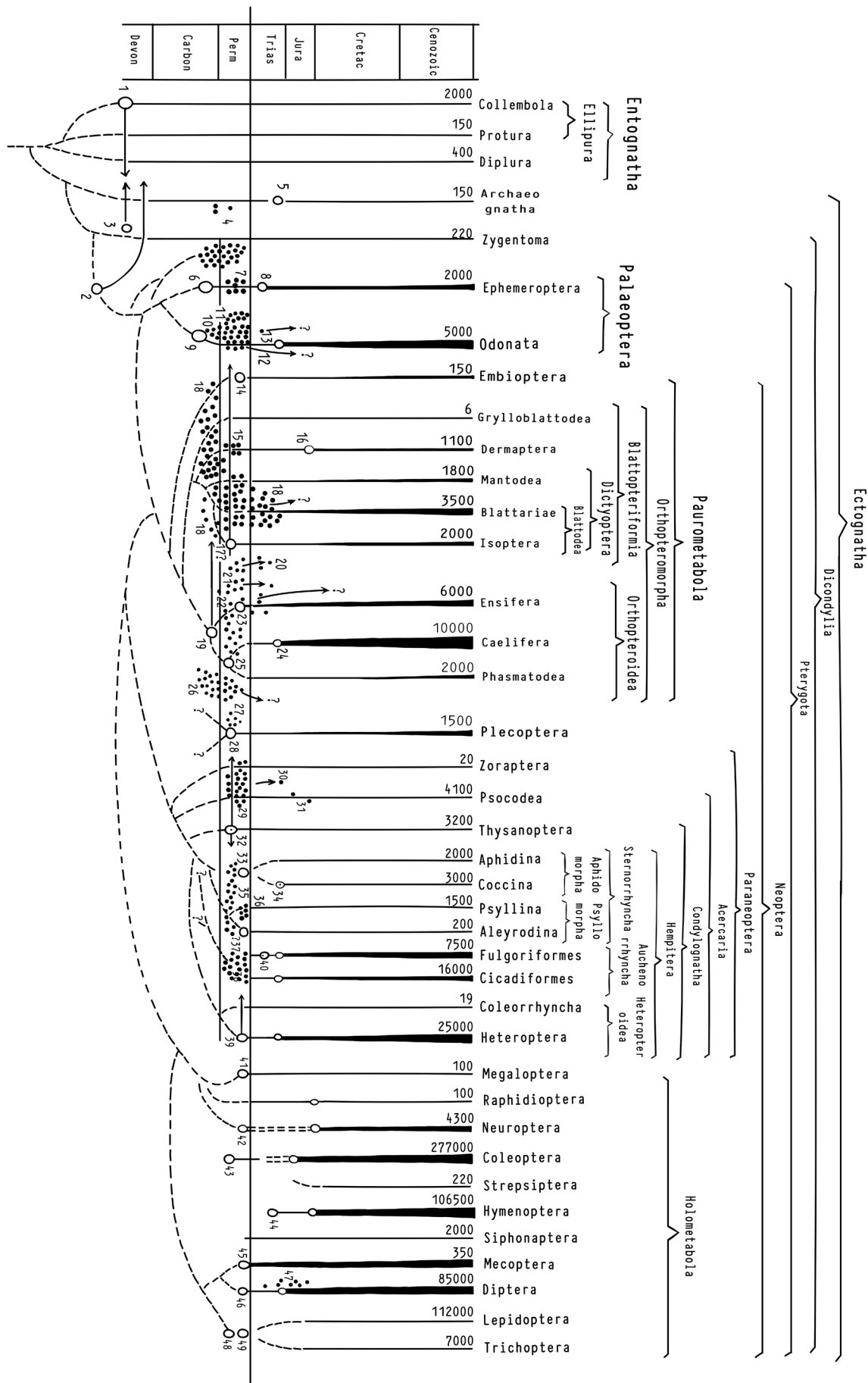
Fig. 1 Phylogeny of Hexapoda, compiled from Die Stammesgeschichte der Insekten (Hennig, 1969), from Kjer *et al.* (2016) (Royal Society Interface).

musculature was a precondition for the formation of the flight apparatus, which evolved early as a key feature of pterygote insects. The large and rather compact fat body, mainly located in the abdomen, enables insects to mobilize energy-rich stored substances very efficiently. This was probably also a precondition for the evolution of flight.

The monophyletic origin of insects in the widest sense was challenged by a relatively early molecular study based on mitochondrial genomes (Nardi *et al.*, 2003), with Collembola (but also the honeybee and a louse) placed among some crustacean taxa. The analysis in this study had already been questioned methodologically by Delsuc *et al.* (2003). Moreover, Hexapoda was fully confirmed as a clade by studies based on single genes (*e.g.*, Sasaki *et al.*, 2013), multi-gene analyses (Regier *et al.*, 2010) and transcriptomic studies (*e.g.*, Meusemann *et al.*, 2010; v. Reumont *et al.*, 2012; Misof *et al.*, 2014). Moreover, the Pancrustacea concept adds new strong morphological evidence for a monophyletic origin. It implies that an entire series of characters are additional autapomorphies of Hexapoda. This includes Malpighian tubules, the tracheal system, sperm transfer *via* spermatophores, the formation of a massive fat body, and also the loss of several organs or structural complexes. The second antennae, the ventral food rim, nephridial organs, and the mid gut glands are completely reduced (Beutel *et al.*, 2014). A conspicuous developmental feature obviously linked with terrestrial habits is the loss of the planctonic nauplius larva (Fig. 2: metanauplius of *Triops cancriformis* [Notostraca]), which is probably a groundplan apomorphy of Pancrustacea. An anamorphic development with a free-swimming larval form with a highly specialized feeding apparatus with three appendages is replaced with an epimorphic development (full number of segments after hatching from the egg) and larvae adapted to a moist but terrestrial environment (Fig. 3).

Basal branching events: the entognathous orders Collembola, Protura and Diplura

In contrast to the monophyletic origin, the basal branching events in Hexapoda are still a controversial issue. Hennig (1969) tentatively suggested a monophylum Entognatha comprising Collembola, Protura and Diplura, characterized by mouthparts more or less completely enclosed by extensions of the genae, and also by partly or completely reduced compound eyes and short or missing Malpighian tubules. Kukalová-Peck (1991) suggested a clade Cercophora for the first time (Fig. 4), with Diplura as the sister group of Insecta (= Ectognatha), implying the paraphyly of Entognatha. This hypothesis was based on the presence of cerci, a sperm axoneme pattern $9 + 9x2 + 2$, and paired claws, and is also strongly supported by the formation of an amnion (Ikeda and Machida, 1998, 2001; Machida, 2006; Sekiya and Machida, 2009). As a third alternative, some molecular studies suggested a clade Nonoculata, combining the eyeless Protura and Diplura (*e.g.*, Luan *et al.*, 2005). However, recently

published embryological evidence is incompatible with this option (Tomizuka and Machida, 2015). The presently available morphological evidence is clearly ambivalent, supporting either Entognatha and Ellipura (= Collembola + Protura) (Hennig, 1969), or alternatively Cercophora. Interestingly, analyses of transcriptomic data do not yield a well-supported solution (compare Meusemann *et al.*, 2010; v. Reumont *et al.*, 2012; Dell'Ampio *et al.*, 2014; Misof *et al.*, 2014; current 1KITE transcriptomic analyses). The crucial position of Diplura varies depending on the taxa sampled for this apparent key taxon. Moreover, heterogeneity along the tree considering early hexapod lineages might cause confounding signal within datasets.

Collembola - the springtails - are by far the most species rich group of the apterygote hexapods, with more than 7,000
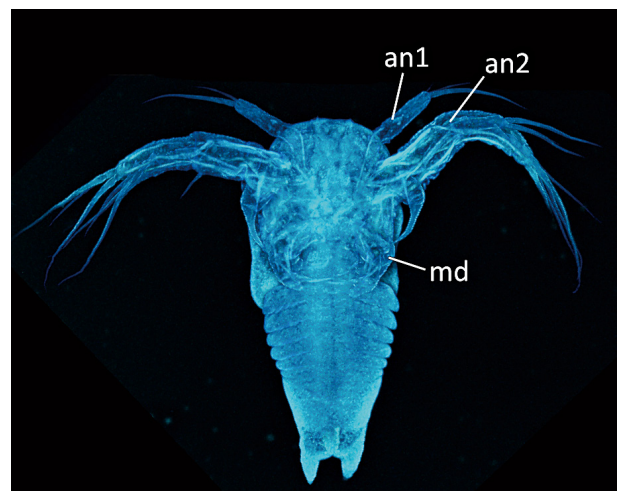

Fig. 2   Metanauplius larva of *Triops cancriformis* (Branchiopoda, Notostraca), ventral view. Courtesy Dr. Martin Fritsch (Museum für Naturkunde Berlin) and Prof. Dr. Stefan Richter (Universität Rostock).
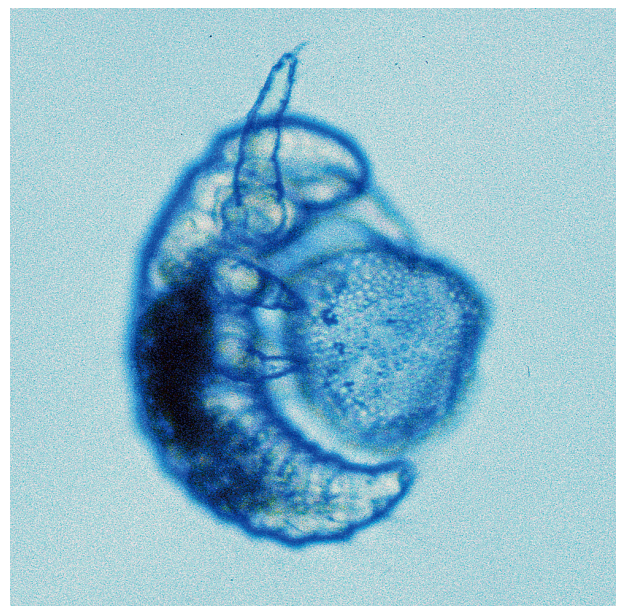an1: antennule, an2: antenna, md: mandible

Fig. 3   *Baculentulus densus*. Prelarva shortly after hatching (with its chorion).

species. One conspicuous autapomorphy of the small and weakly sclerotized insects is the abdominal jumping device (furcula). Considering the high degree of morphological specialization, the ecological versatility of the group is surprising. Whereas the other primarily wingless insects are ground oriented and usually live in leaf litter or similar environments, Collembola occupied very different niches, such as for instance tree canopies, marine tide pools, glaciers,
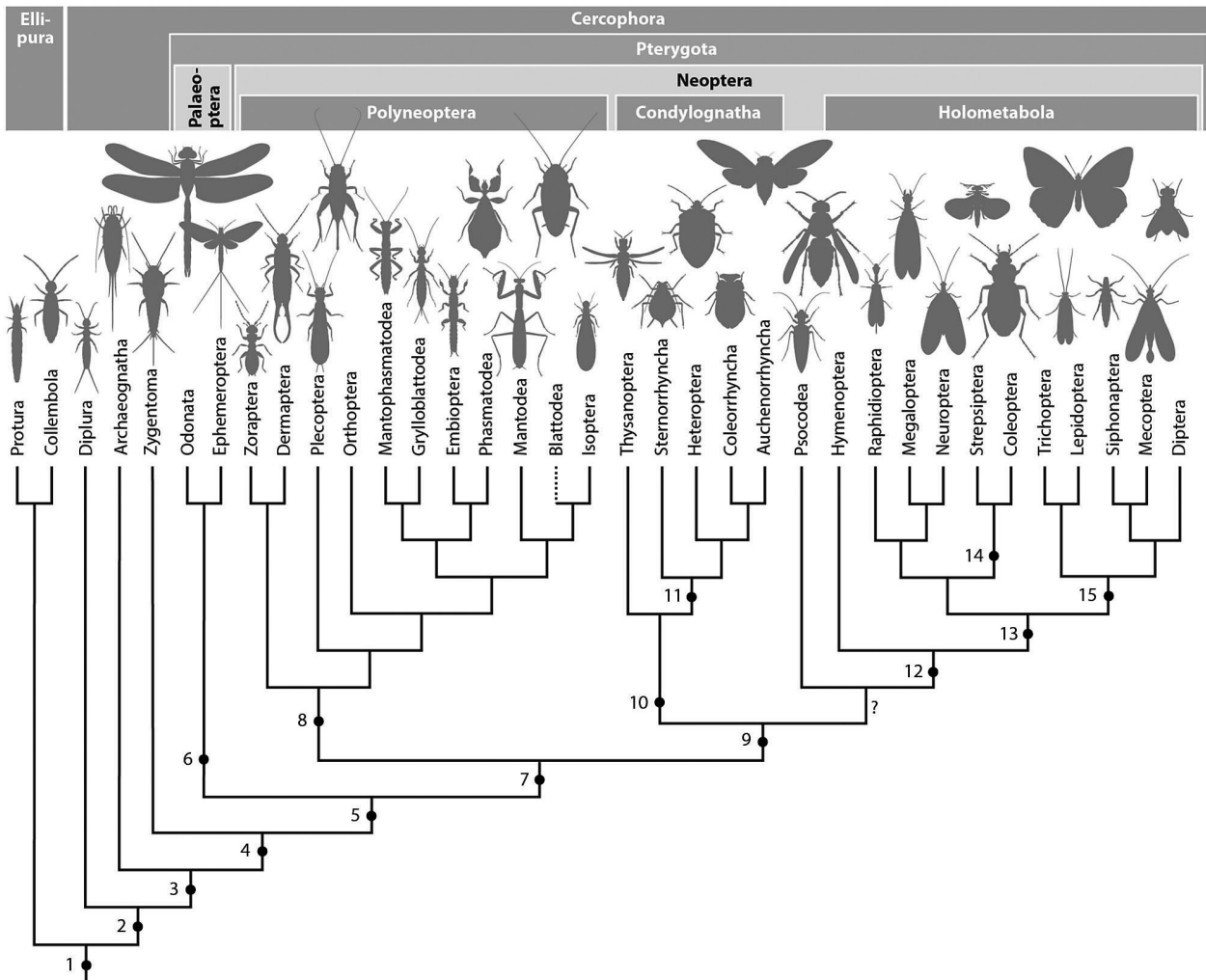

Fig. 4 Phylogeny of Hexapoda from the 1KITE project (www.1kite.org/; from Misof *et al.* 2014, modified, courtesy of Hans Pohl). Presumptive morphological and developmental apomorphies mapped on transcriptomic tree. 1. Hexapoda: tagmosis with head, 3-segmented thorax and abdomen; Malpighian tubules (?, missing in Collembola); tracheal system; loss of 2nd antenna; loss of ventral food rim; loss of nephridial organs; loss of mid gut glands; sperm transfer *via* spermatophore. 2. Cercophora (= Diplura + Insecta): cerci (?); double claws; amnion; sperm axoneme with $9 + 9x2 + 2$ microtubuli pattern. 3. Insecta (= Ectognatha): flagellar antenna; Johnston's organ; tentorial bridge (?); tarsus subdivided; pretarsus distinctly reduced; ovipositor; terminal filament. 4. Dicondylia: secondary mandibular joint (?, possibly groundplan of Insecta); tracheal system with anastomoses; closed amniotic cavity. 5. Pterygota: wings and associated structures; ligamentous endoskeleton reduced; legs with ligamentous diapghragm; abdominal coxal vesicles absent; epicuticular wax layer; internal fertilization (?, probably not groundplan Pterygota). 6. Palaeoptera: shortened, bristle-like antenna; loss of antennal circulatory organs; dentisetae of lacinia; larval galea and lacinia fused; aquatic immatures. 7. Neoptera: ability to fold back wings and associated characters of the wing base (?, possibly groundplan of Pterygota), arolium (?), 3rd valvulae form sheath for 1st and 2nd valvulae (groundplan?). 8. Polyneoptera: embryo formation by the fusion of paired regions with higher cellular density; blastokinesis accompanied by full elongation of embryo on egg surface; forewings modified as tegmina (groundplan? absent in Zoraptera, Embioptera, termites and wingless groups); enlarged anal field of hind wing (groundplan? absent in Zoraptera, Embioptera, termites and wingless groups), pad-like tarsal euplantulae (groundplan?, absent in Zoraptera and some other groups). 9. Eumetabola: ocelli absent in immature stages. 10. Condylognatha: mandible(s) modified as stylet; labrum narrowed; maxillary palps absent; dorsal shift of anterior tentorial pits. 11. Hemiptera: four-segmented labial rostrum; labial endite lobes and palps absent; buccal pump; brain and suboesophageal complex form compact unit. 12. Holometabola (= Endopterygota): holomatobolous development with complete metamorphosis; pupa as non-feeding immature stage (groundplan?); endopterygotism (wing buds internalized); larva with reduced number of antennomeres, simplified mouthparts and undivided tarsi. 13. Aparaglossata (= Holometabola excl. Hymenoptera): ovipositor distinctly modified or reduced; reduced number of Malpighian tubules (maximum 8); paraglossae and their muscles reduced; stemmata as simplified larval eyes. 14. Coleopterida (= Strepsiptera + Coleoptera): median labral retractor absent; salivarium absent; number of antennomeres reduced (13 or less); posteromotorism; cerci of adults absent. 15. Mecopterida: orthopteroid ovipositor reduced; telescoping female postabdomen; ventral metasternal process elongated; larval stipes divided into basistipes and dististipes; larval M. craniodististipitalis present. See text and also Beutel *et al.* (2014).

and even caves.

What may have contributed to the evolutionary success is the jumping capacity, an efficient escape mechanism. Why Collembola were able to occupy a much greater variety of habitats than related groups remains unclear, but this has likely also contributed to the diversification. Another factor is the exceptionally high reproduction rate, often linked with parthenogenesis and a short generation cycle.

The rise of Insecta (= Ectognatha) and early splitting events

A strongly supported lineage is Insecta (= Ectognatha), comprising the small apterygote orders Archaeognatha and Zygentoma, and the extremely species-rich winged insects (Fig. 4). A convincing synapomorphy is the flagellate antenna, with muscles only in the basal scapus and a chordotonal organ (Johnston's organ) in the following pedicellus. An important evolutionary novelty is the ovipositor with specific appendages (gonocoxae and gonapophyses) of the female genital segments VIII and IX. The obvious advantage is that eggs can be deposited in narrow crevices or plant tissue. Another important apomorphy is the subdivision of the tarsus. This is a precondition for the development of tarsal attachment devices, which later evolved in pterygote insects and enabled them to walk efficiently on plant surfaces (*e.g.*, Beutel and Gorb, 2001, 2006).

Archaeognatha and Zygentoma are very similar in their habitus and lifestyle and share numerous plesiomorphic features. Nevertheless, there is no doubt that Zygentoma form a clade Dicondylia together with Pterygota (Fig. 4), a concept unambiguously supported by morphological (*e.g.*, Hennig, 1969; Kristensen, 1975, 1995), developmental (Machida, 2006, 2009; Masumoto and Machida, 2006) and molecular evidence (*e.g.*, Misof *et al.*, 2014). The main traditional argument is the dicondylic condition, the presence of a second mandibular joint, but this has possibly evolved earlier and may belong to the groundplan of Insecta (Blanke *et al.*, 2015). Besides that, an additional joint reduces the degrees of freedom at the mandibular base and allows more powerful biting movements. The obvious advantage is that a broader spectrum of food can be processed, including solid plant tissues. The apterygotes largely rely on fungal hyphae and spores, algae, and soft decaying materials. However, it is important in this context that the secondary mandibular joint was still a rather flexible gliding device in Zygentoma and mayfly immatures (Staniczek, 2000).

Pterygota: insects get airborne

There is little doubt that the most important single innovation in insect evolution was the development of wings. Pterygote insects comprise *ca*. 99% of all species. That wings evolved more than once can be ruled out with certainty considering the complexity of the pterothoracic flight apparatus, even though many different variations of this character system evolved (*e.g.*, Brodsky, 1994).

One obvious advantage of active flight is the increased ability to escape predators. What is probably more important is the enormously increased dispersal capacity compared to apterygote insects. This applies also to other successful non-related group–spiders and spider mites (*e.g.*, Bell *et al.*, 2005). Juvenile spiders can fly passively using silk rafts. Using this mechanism, they can disperse over distances of 100 km and more. That flight and an improved dispersal capacity have played a very important role is also underlined by the fact that all secondary wingless groups of insects have a very low diversity, such as for instance Zoraptera or Grylloblattodea (*e.g.*, Beutel *et al.*, 2014).

In the Carboniferous–apparently linked with the evolution of wings–the first great wave of diversification took place. Within a geologically very short time span almost all orders of hemimetabolous groups appeared, and towards the end of the period also Holometabola (*e.g.*, Kukalová-Peck, 1991).

Another important evolutionary novelty of pterygote insects is internal sperm transfer. Apterygote hexapods, with the possible exception of Protura (M. Fukui; unpublished observations) and some collembolan species (*e.g.*, Schaller, 1971; Proctor, 1998), externally deposit a spermatophore, which quite often does not lead to successful fertilization, for instance when males of other species or females eat or destroy the sperm package (*e.g.*, Dallai *et al.*, 2009). Moreover, indirect sperm transfer is usually restricted to very moist habitats, especially soil, and it often requires the production of stalk or silk thread material (*e.g.*, Schaller, 1971; Proctor, 1998). The reproductive efficiency and economy is increased in pterygotes with direct sperm transfer with a postabdominal intromittent organ, the aedeagus. This has arguably also contributed to the evolutionary success. Again, this also applies to the successful Araneae. Male spiders transmit spermatophores into the female genital tract with their modified pedipalps (Alberti and Michalik, 2004). Different modes of internal sperm transfer have also evolved in Opiliones (with a penis), Ricinulei, and within the orders Acaria (in some cases with a penis) and Solifugae (Alberti and Michalik, 2004).

Even though the switch to internal fertilization, usually with a spermatophore, was a major evolutionary innovation in insects, this feature is likely not an autapomorphy of Pterygota. The pre-abdominal secondary copulatory organ of Odonata has obviously evolved independently and it was suggested that stem-group dragonflies had an external sperm transfer (Bechly *et al.*, 2001: *Namurotypus*).

In different groups of pterygote insects, male and female genitalia fit together like lock and key, a mechanism that prevents interspecific copulation (see *e.g.*, Shapiro and Porter, 1989). It is conceivable that this has contributed to the extreme diversity of the genitalia and also to the diversification of insects. However, more recent investigations suggest that this is only a marginal phenomenon in insects (*e.g.*, Shapiro and Porter, 1989). What apparently plays the major role in the

evolution of highly diverse genitalia is sexual selection, female cryptic choice (Eberhardt, 1985, 1996). The females develop variations of the genitalia to prevent fertilization or to allow it only selectively, whereas the male genitalia vary to overcome the obstacles. This is one example of an evolutionary arms race in insects.

The "Palaeoptera problem"

A deep-rooted and strongly disputed problem in systematic entomology is the basal branching pattern in Pterygota (*e.g.*, Kristensen, 1991). Hennig (1969) placed Ephemeroptera and Odonata in a clade Palaeoptera, supported for instance by aquatic immatures and strongly shortened, bristle-like antennae. Boudreaux (1979) suggested a clade Chiastomyaria including Ephemeroptera and Neoptera. Potential synapomorphies are the indirect flight muscles and the direct sperm transfer with a postabdominal aedeagus. Staniczek (2000) suggested Metapterygota comprising Odonata and Neoptera, based on an entire series of apomorphies linked with the mandibles. Additional arguments for this option are abdominal spiracles with a closing mechanism, additional connections in the tracheal system (*e.g.*, Kristensen, 1991), and the loss of the subimago, a winged immature stage of mayflies that moults before the adult stage is reached. More detailed morphological investigations showed that Palaeoptera may indeed form a clade (Blanke *et al.*, 2012a, b). Mandibular features studied by Staniczek (2000) are closely correlated and not independent characters, leading to artefacts caused by concerted convergence (see Blanke *et al.*, 2012b). Recent analyses of transcriptomes also tentatively support the Palaeoptera concept (Fig. 4; Misof *et al.*, 2014). However, the results are still ambiguous. Like the issue of the entognathous orders, the "Palaeoptera problem" remains unresolved at present.

Neoptera

Another key feature which evolved early in pterygote insects characterizes Neoptera (= Pterygota excl. Ephemeroptera and Odonata), the ability to fold back the wings over the abdomen. An obvious advantage is that winged adults can now hide in relatively narrow spaces. This means better protection and also reduced water loss in moist microhabitats. The neopterous conditions is linked with specific features of the wing base, a subdivided median plate, flexion lines in the wing base, longitudinal veins detached from the axillary sclerites, and a pleural muscle attached to the 3rd axillary (*e.g.*, Wootton, 1979; Beutel *et al.*, 2014). The ability to fold back the wings was generally accepted as an autapomorphy of Neoptera (*e.g.*, Kristensen, 1975, 1995; Beutel and Gorb, 2001). However, it was pointed out by Willkommen and Hörnschemeyer (2007) that this condition may be part of the pterygote groundplan, and that the
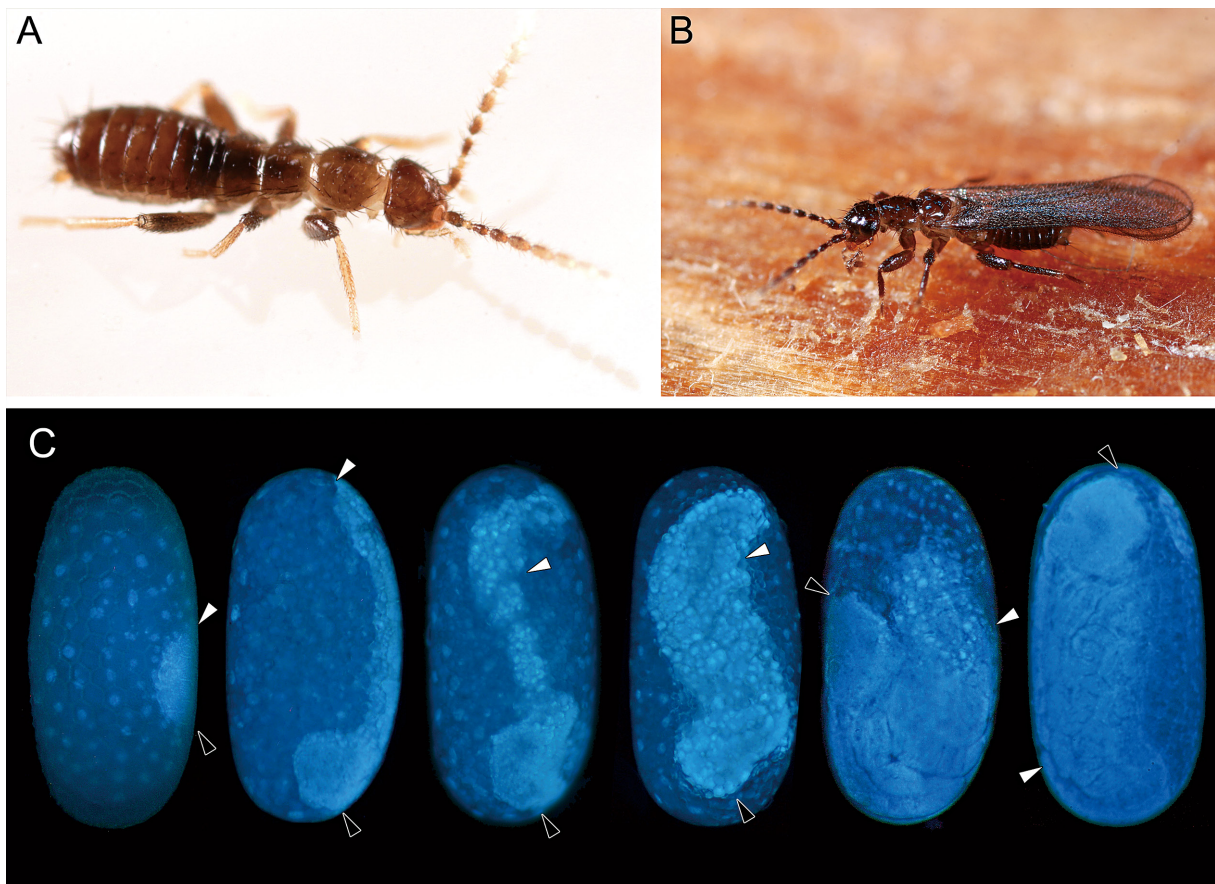

Fig. 5 *Zorotypus caudelli*. A. Apterous adult. B. Alate adult. C. Embryonic development of *Zorotypus caudelli*. DAPI staining. Black and white arrowheads show cephalic and caudal ends, respectively.

seemingly ancestral conditions found in Ephemeroptera and Odonata differ very distinctly.

Aside from the modified wing base there is not much in support of monophyletic Neoptera. An ovipositor with 3rd valvulae (gonoplacs) forming a sheath for the 1st and 2nd valvulae was suggested as a groundplan apomorphy (see *e.g.*, Kristensen, 1991).

Another possible autapomorphy of Neoptera is the arolium (Beutel and Gorb, 2001, 2006), a median pretarsal attachment lobe. It is the first of several types of attachment devices, which evolved in pterygote insects. An important issue in this context is that tarsal or pretarsal adhesive devices do not just serve as attachment structures. They enable insects to walk efficiently on plant surfaces (Beutel and Gorb, 2001). Plants modify their surfaces to prevent insects from walking and feeding on them, and insects modify their attachment devices to overcome these obstacles. This is another example for an evolutionary arms race, in this case between two very different groups of organisms.

Polyneoptera and the first major diversification

Polyneoptera, one of the three large subdivisions of neopteran insects (*e.g.*, Beutel *et al.*, 2013), emerged in the fossil record in the Carboniferous (*e.g.*, Kukalová-Peck, 1991; Grimaldi and Engel, 2005). The monophyly of this group was strongly debated, especially with respect to Plecoptera (*e.g.*, Kristensen, 1991), and a neutral term "lower Neoptera" was frequently used. The placement of the small and enigmatic order Zoraptera (Mashimo *et al.*, 2014a; Fig. 5A, B) was also very controversial, and a sister group relationship with Acercaria was tentatively suggested by Hennig (1969). Numerous conflicting hypotheses for the internal relationships of Polyneoptera were suggested (*e.g.*, Trautwein *et al.*, 2012; Beutel *et al.*, 2013) and an almost completely unresolved "lower neopteran" phylogenetic pattern (Kristensen, 1991: fig. 5.5) became known as "Kristensen's comb".

The monophyletic origin of Polyneoptera including Zoraptera is now confirmed by different sources of evidence (Fig. 4), especially by embryological characters (Mashimo *et al.*, 2014b; Fig. 5C). The embryonic development in polyneopteran groups differs distinctly from what is found the in acercarian orders, but also in Ephemeroptera and Odonata (Palaeoptera), indicating that the polyneopteran pattern is apomorphic (Fig. 4; Mashimo *et al.*, 2014b). Recent phylogenetic evaluations of morphological data also confirmed the monophyly of Polyneoptera including Zoraptera (Yoshizawa, 2007, 2011; Matsumura *et al.*, 2015; Wipfler *et al.*, 2015), like analyses of single genes (Yoshizawa and Johnson, 2005; Ishiwata *et al.*, 2011; Wang *et al.*, 2013), and finally also transcriptomic data analysed in the 1KITE project (Misof *et al.*, 2014).

Polyneoptera comprise very small orders with distinctly less than 100 described species (Zoraptera: *ca*. 40 spp.; Grylloblattodea: *ca*. 35 spp.; Mantophasmatodea: *ca*. 20 spp.),

some medium sized groups (*e.g.*, Plecoptera: *ca*. 3,500 spp.; Dermaptera: *ca*. 2,000 spp.), and as the only group very successful in terms of species diversity Orthoptera (*ca*. 22,500 spp.). Orthoptera are characterized by jumping hindlegs and different acoustic communication systems have evolved in the group. Caelifera are strictly phytophagous whereas many predacious species occur in Ensifera (*e.g.*, Beutel *et al.*, 2014).

The interordinal relationships are not fully clarified yet but considerable progress has been made. It is generally accepted that termites are a subordinate group of a clade Blattodea (as sister group of Cryptocercidae), which forms the sister taxon of Mantodea (Lo *et al.*, 2000; Deitz *et al.*, 2003; Misof *et al.*, 2014). It is also well established that Grylloblattodea + Mantophasmatodea form a clade (Xenonomia), and also Phasmatodea + Embioptera (Eukinolabia) (Terry, 2004; Terry and Whiting, 2005; Wipfler *et al.*, 2011). Features of the egg and blastokinesis suggesting the monophyly of Xenonomia (= Chimaeraptera) were described by Uchifune and Machida (2005). Fujita and Machida (in press) suggested micropyles clustered on the ventral side of the egg as an embryological autapomorphy of Dictyoptera, and proposed a phylogenetic pattern Mantodea + Blattodea [= Blaberoidea + (Blattoidea + Isoptera)] based on the blastokinesis type. Jintsu *et al.* (2010) supported the monophyly of Eukinolabia based on structural affinities of the eggs of Embioptera and Phasmatodea.

The major branching events in Polyneoptera ("phylogenetic backbone") are not fully clarified yet. Transcriptome analyses (Misof *et al.*, 2014) suggest a pattern with Zoraptera + Dermaptera as the sister group of the remaining orders, Plecoptera as the next branch, and then Orthoptera as sister group of a large lineage comprising Dictyoptera [Blattodea (incl. termites) + Mantodea], Xenonomia and Eukinolabia (Fig. 4). However, this hypothesis requires further confirmation. The recently described extinct order Alienoptera is probably the sister taxon of Mantodea (Bai *et al.*, 2016), thus representing a "connecting link" between extinct predacious roaches and the praying mantises.

The two completely wing-less orders Grylloblattodea and Mantophasmatodea comprise together only about 50 species. In Grylloblattodea, which also occur in Japan, loss of wings is apparently an adaptation to the preferred cold mountain areas (*e.g.*, Wipfler *et al.*, 2014). The advantage is the reduced risk to be drifted into unsuitable habitats by strong winds. The obvious disadvantage in both taxa is the drastically reduced dispersal ability, which apparently resulted in a very low diversity. Additionally, both groups have only a marginal relationship to plants if at all. It is likely that insect-plant interrelationships have played an important role in the diversification of different insect groups, as for instance in Orthoptera.

Acercaria (= Paraneoptera excl. Zoraptera, hemipteroid assemblage)

Acercaria, the second major neopteran subgroup,

comprises approximately 120,000 described species. The monophyly was generally accepted (*e.g.*, Hennig, 1969; Kristensen, 1975, 1991; Beutel and Gorb, 2001; Wheeler *et al.*, 2001; Friedemann *et al.*, 2014). Potential morphological apomorphies are slender and elongated laciniae detached from the stipes, an enlarged postclypeus and enlarged cibarial dilators, tarsi with three segments or less, completely reduced cerci, at most six Malpighian tubules, and a single abdominal ganglionic mass (*e.g.*, Kristensen, 1991; Yoshizawa and Saigusa, 2001; Friedemann *et al.*, 2014). Even though most of these features are reductions, the morphological support for a monophyletic origin appears strong. Nevertheless, as a surprising outcome, Acercaria were not supported as a clade in Misof *et al.* (2014), with Psocodea placed as sister taxon of Holometabola (Fig. 4). This result, which appears implausible from the morphological perspective, is presently re-investigated with a distinctly extended taxon sampling.

The monophyly of Psocodea was confirmed in Misof *et al.* (2014), and also in earlier studies based either on single gene analyses (Yoshizawa and Johnson, 2003, 2010) or on morphology (Friedemann *et al.*, 2014) Psocoptera was rendered paraphyletic by a sister group relationship between Liposcelididae and the ectoparasitic Phthiraptera. The analyses based on transcriptomic data supported Condylognatha, combining Thysanoptera with the megadiverse monophyletic Hemiptera, as earlier suggested by Yoshizawa and Saigusa (2001), Friedemann *et al.* (2014) and others, in contrast to Micracercaria (= Thysanoptera + Psocodea) suggested by other authors (see Kristensen, 1991).

A key feature of Acercaria is the presence of piercing-sucking mouthparts. However, this condition does not belong to the groundplan (*e.g.*, Spangenberg, 2015). Psocoptera and basal groups of the ectoparasitic lice ("Mallophaga") have maintained biting mouthparts, even though with some specializations (*e.g.*, v. Kéler, 1966; Spangenberg, 2015). Obviously the character complex has evolved two or three times independently, in a very distinctive way in Rhynchophthirina and Anoplura (Tröster, 1990), with a single functional stylet-like mandible in Thysanoptera, and with stylets formed by the mandibles and laciniae in all hemipteran orders (*e.g.*, Spangenberg *et al.*, 2013; Spangenberg, 2015).

It is conceivable that advanced piercing sucking mouthparts and predominantly plant-feeding habits have contributed to a remarkable diversification of Hemiptera, which comprise about 70% of all known acercarian species. The majority of true bugs and thrips, and all species of Sternorrhyncha and Auchenorrhyncha are specialized on sucking fluids of plants (*e.g.*, Beutel *et al.*, 2014). Auchenorrhyncha (cicada, plant hoppers, tree hoppers), which comprise *ca*. 42,000 spp., show evolutionary parallels to Orthoptera, the polyneopteran group with the highest diversity. They also evolved a very good jumping capacity (absent in Cicadoidea) (see Friedemann and Beutel, 2014 and studies cited therein), and acoustic communication systems. This makes it plausible to assume that both character complexes have contributed to the diversification in both non-related lineages.

The major hemipteran diversification started in the Cretaceous, likely correlated with the early radiation of angiosperm plants (Grimaldi and Engel, 2005: fig. 8.31). The strong affinity to plants is also underlined by the fact that hemipteran subgroups have evolved a very broad spectrum of attachment devices (Friedemann *et al.*, 2014). These structures enable them to move very efficiently on different plant surfaces (Beutel and Gorb, 2001).

Eumetabola

A clade comprising Acercaria and Holometabola is supported by molecular data (Fig. 4; Letsch *et al.*, 2012; Misof *et al.*, 2014), even though there is hardly any morphological or developmental evidence. Presently the loss of ocelli in immature stages is the only plausible synapomorphy of the two large lineages (*e.g.*, Beutel *et al.*, 2014).

Holometabola

By far the largest subgroup of Neoptera is Holometabola (= Endopterygota), with an unparalleled diversity of about described 800,000 species, roughly half of all known organisms. In contrast to basal branching events in Hexapoda and the internal phylogeny of Polyneoptera, the relationships of the holometabolan orders seem to be settled in the "age of phylogenomics" (Fig. 4). A large and complex morphological data set (Beutel *et al.*, 2011), single copy nuclear genes (Wiegmann *et al.*, 2009), transcriptomes (Peters *et al.*, 2014; Misof *et al.*, 2014), and genomes (Niehuis *et al.*, 2012) converged upon the same interordinal pattern. Hymenoptera (*ca*. 132,000 described spp.) are placed as the sister group of all remaining orders, in contrast to Hennig (1969), Kristensen (1975, 1995) and Beutel and Gorb (2001), but in agreement with Rasnitsyn and Quicke (2002). Morphological apomorphies of Aparaglossata (= Holometabola excl. Hymenoptera; see Peters *et al.*, 2014) are the loss of the paraglossae and their muscles, the partial reduction of the orthopteroid ovipositor, and a reduced number of Malpighian tubules (Beutel *et al.*, 2011). A large clade Neuropteroidea comprises the three neuropterid orders (Neuropterida) as sister group of a monophylum Coleopterida, combining Coleoptera with the long disputed Strepsiptera. Komatsu and Kobayashi (2012) suggested a knob-like micropylar projection as an apomorphic groundplan feature of Neuropteroidea, even though this structure is not present in most beetles. The sister group relationship between the megadiverse beetles (*ca*. 360,000 described species) and the extremely specialized endoparasitic Strepsiptera ends one of the longest controversies in systematic entomology (*e.g.*, Niehuis *et al.*, 2012; Pohl and Beutel, 2013). The sister group of Neuropteroidea is Mecopterida, a very large monophylum already suggested by Hinton (1958) as "panorpoid orders". This group comprises Amphiesmenoptera, with Trichoptera and the megadiverse Lepidoptera as sister taxa, and

Antliophora containing the small order Mecoptera, the ectoparasitic Siphonaptera, and the extremely species-rich Diptera. Kobayashi and coworkers analyzed embryological characters cladistically to reconstruct the relationships of amphiesmenopteran subgroups (Kobayashi and Ando, 1988; see also Kobayashi *et al.*, 2003). The interrelationships of the three antliophoran orders are presently not completely resolved (Misof *et al.*, 2014), especially with respect to the enigmatic mecopteran subgroup Nannochoristidae, treated as a separate order Nannomecoptera by Hinton (1981).

The most conspicuous feature of Holometabola and arguably a key innovation is the holometabolous development with a complete metamorphosis and a non-feeding and largely or completely immobilized pupal stage. The immobilized and unprotected pupa is apparently a risk-factor in the life cycle, which means evolutionary costs. In contrast to this, the ability of larvae and adults to use different resources and habitats is very likely an advantage, resulting in a decreased intraspecific competition.

Another important feature is the endopterygote condition (*e.g.*, Kristensen, 1991). The wing buds lie below the larval cuticle. This enables the larvae to penetrate very narrow crevices, for instance under bark, or to burrow in plant tissue, even including wood. This means a larval development in an environment providing moisture and inaccessible for most predators.

These factors have likely played a role but do by not sufficiently explain the extreme species richness. It is evident that the major diversification did not take place in the early evolution of the group, but independently in different lineages. The diversity of several groups is low with only few hundred species for instance in Megaloptera (*ca.* 320 spp.) or Mecoptera (*ca.* 550 spp.) (*e.g.*, Grimaldi and Engel, 2005; Beutel *et al.*, 2014). Extremely diverse – each with distinctly more than 100,000 spp. – are Hymenoptera (*ca.* 132,000 described spp.), Coleoptera (*ca.* 360,000 spp.), Lepidoptera (*ca.* 175,000 spp.) and Diptera (*ca.* 154,000 spp.). These four orders – referred to as "big4" (see http://big4-project.eu/) – are not closely related with each other, and each of them includes basal groups with a low diversity, for instance Archostemata with only 40 species, about 0.01% of the total diversity of beetles. Interestingly Archostemata, like basal lepidopteran groups, are associated with gymnosperms, whereas the vast majority of phytophagous beetles is linked with the highly diverse angiosperm plants (*e.g.*, Crowson, 1981).

A distinctly improved flight capacity may have contributed to the diversification in Hymenoptera and Diptera, with functional or anatomical dipterism, respectively. Parasitism has likely played a role in Hymenoptera, probably with an immense hidden diversity still to discover, especially of very small forms. Strong mechanical protection is apparently a key feature of Coleoptera, with ancestral forms adapted to narrow crevices, especially under bark.

A major factor in the "megadiversification" of the "big 4" of Holometabola was likely a successful evolutionary interaction with angiosperm plants, beginning in the early Cretaceous or slightly earlier (*e.g.*, McKenna *et al.*, 2015). New food sources became available for phytophagous insects, and reciprocally different groups of insects have immensely contributed to the dispersal and evolutionary success of angiosperms as pollinators. This connection is well-established in Lepidoptera and subgroups of Hymenoptera (bees *etc.*), beetles, and Diptera (*e.g.*, Beutel *et al.*, 2011). These lineages underwent an explosive radiation in the late Mesozoic. The positive evolutionary interaction resulted in about 200,000 species of angiosperms and the enormous number of *ca.* 800,000 spp. in Holometabola. This is probably the most important example of successful co-evolution.

Perspectives

The investigation of hexapod diversity, morphology, systematics and evolution has a long tradition, going back to the 18th century and even earlier (*e.g.*, Engel and Kristensen, 2013; Friedrich *et al.*, 2014). The work of the German dipterist Willi Hennig in the last century was unquestionably a breakthrough, with a revolutionized phylogenetic methodology (Hennig, 1950, 1966) and a comprehensive work on insect systematics (Fig. 1; Hennig, 1969). In the last two decades, an impressive development of insect phylogenetics took place, with a greatly accelerated acquisition of high quality anatomical data, but also with a breathtaking "evolution" of molecular systematics and analytical methods (Kjer *et al.*, 2016).

Obviously, the anatomy and development of hexapods are not in the mainstream of present day research. Nevertheless, embryology as an essential branch of evolutionary entomology is still carried out on a very high level in some research institutions, for instance the laboratories of Prof. Dr. Ryuichiro Machida (Sugadaira Research Station, Mountain Science Center, University of Tsukuba) (*e.g.*, Mashimo *et al.*, 2014b) and Emer. Prof. Dr. Yukimasa Kobayashi (Tokyo Metropolitan University). Morphological work has gained great momentum since the last turn of the century, mainly due to new anatomical techniques (*e.g.*, Friedrich *et al.*, 2014). They distinctly accelerated the acquisition of high quality morphological data and also greatly improved the documentation. In particular, micro-computed tomography (μ-CT) combined with computer based reconstruction has turned out as highly successful. New techniques like for instance serial block-face scanning electron microscopy (SBFSEM) or nuclear magnetic resonance imaging (NMRI) also allow us to examine extremely small objects (*e.g.*, Knauthe *et al.*, 2016). Ultrastructural features play a minor role in insect systematics. However, insect sperm with characters on the cellular level turned out as phylogenetically informative (*e.g.*, Dallai *et al.*, 2016). Like other limited character sets, it is insufficient to resolve the phylogenetic relationships in a highly diverse group like Hexapoda (Gottardo *et al.*, 2016). However, it provides crucial phylogenetic support for

important branches such as for instance Cercophora (= Diplura + Insecta). Besides this, the evolution of this unicellular character system is amazingly complex and apparently shaped by other mechanisms than other body parts (*e.g.*, Gottardo *et al.*, 2016).

In insect systematics based on morphology in a broad sense, there is a clear tendency to use broader character sets, some of them comprising several hundred well-documented characters (*e.g.*, Beutel *et al.*, 2011). This was made possible by a remarkable renaissance of insect anatomy (*e.g.*, Beutel and Kristensen, 2012; Friedrich *et al.*, 2014). However, even though the size of morphological matrices increased remarkably, they are still dwarfed by recent molecular data sets, as for instance 1,478 orthologous genes sampled for nearly 150 terminal taxa representing all insect orders and outgroup taxa (Misof *et al.*, 2014). Peters *et al.* (2014) presented a two-stage procedure to combine molecular and morphological evidence for Holometabola: the phylogenetic branching pattern is reconstructed using extensive sequence data (in this case transcriptomes) in the first step. In the second step character transformations on the phenotypic level are traced, using the obtained phylogeny (*e.g.*, Mesquite; Maddison and Maddison, 2011).

It is apparent that the future perspective of insect phylogenetics lies in a complex, multifaceted approach, as presently practiced in the 1KITE project (www.1kite.org/): a close and efficient collaboration between specialists in different fields, molecular systematics, bioinformatics, morphology, palaeontology, and last but not least developmental biology. We are confident that this will lead to a new level of insight in the evolution of the extremely successful and fascinating Hexapoda.

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