

1 **100 years Zoraptera – a phantom in insect evolution and the** 2 3 **history of its investigation** 4

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43 18 Zoraptera are a cryptic and enigmatic group of insects. The species diversity is
4445 19 lower than in almost all other groups of Hexapoda, but may be distinctly higher
4647 20 than presently known. Several new species were described from different regions
4849
50 21 recently. The systematic placement was discussed controversially since the group
5152 22 was discovered 100 years ago. Affinities with Isoptera and Psocoptera were
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55 23 discussed in earlier studies. A sistergroup relationship with Acercaria (Psocodea,
5657 24 Thysanoptera, Hemiptera) was proposed by W. Hennig, for the first time based on
5859 25 a strictly phylogenetic argumentation. More recent studies consistently suggest a
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1 placement among the “lower neopteran orders” (Polyneoptera). Close affinities to
2 Dictyoptera were proposed and alternatively a sistergroup relationship with
3 Embioptera or with Embioptera + Phasmatodea (Eukinolabia), respectively. The
4 precise placement is still controversial and the intraordinal relationships are
5 largely unclear. Recent transcriptome analyses tentatively suggest a clade
6 Zoraptera + Dermaptera as sistergroup of all other polyneopteran orders.
7 The oldest fossils are from Cretaceous amber. An extinct genus from this era may
8 be the sistergroup of all the remaining zorapterans. The knowledge of the
9 morphology, development and features related to the reproductive system greatly
10 increased in recent years. The general body morphology is very uniform, whereas
11 the genitalia differ strongly between species. This is likely due to different kinds
12 of selection, i.e. sexual selection in the case of the genital organs. The mating
13 pattern also differs profoundly within the order. A unique external sperm transfer
14 occurs in *Zorotypus impolitus*. A species-level phylogeny and more investigations
15 of the reproductive system should have high priority.

18 **Introduction**

19 Zoraptera, also known as groundlice or angel insects (e.g., Grimaldi & Engel 2005), are a
20 cryptic, inconspicuous and still enigmatic group of insects. Weidner (1969) pessimistically
21 pointed out that they are in an evolutionary dead end and can only lead a wretched life
22 (“kümmerliches Dasein”) in a very limited habitat. The systematic placement has been
23 controversial since the group was introduced as an order by Silvestri (1913) (e.g., Trautwein
24 et al. 2012) (see Table 1). Consequently the term “the Zoraptera problem” was coined by
25 Beutel & Weide (2005).

1 Zorapterans were characterized by Silvestri (1913) as „Insecta terrestria, parva, aptera,
2 agila, praedantia“, which means „living in earth, small, apterous, agile and predacious
3 insects” (Weidner 1969). The scientific name given to the order („purely apterous ones”,
4 Greek: zoros = pure, strong; aptera = apterous) is a misnomer as zorapterans are primarily
5 winged (Caudell 1920) and small and poorly sclerotized besides. The wing dimorphism is one
6 of few autapomorphies of the order, correlated with the presence or absence of compound
7 eyes and ocelli, and the presence or absence of a distinct pigmentation, with distinctly darker
8 alate specimens.

9 Zoraptera currently comprise 39 extant species and nine species are known as fossils
10 (Engel 2008; Mashimo et al. 2013). They were considered as the least known insect order by
11 Kristensen (1995). The fact that the situation has changed profoundly in recent years (see Fig.
12 1) stimulated us to present this review. The aims are to summarize the current knowledge, to
13 point out remaining problems, and to illuminate the history of the investigation of this small
14 group of cryptic insects.

16 **1. Distribution, diversity and taxonomy**

17 Zoraptera are largely restricted to tropical and subtropical regions. They live under bark or
18 inside galleries made in rotting wood by other insects. Only *Zorotypus hubbardi* has expanded
19 its range as far north as Indiana, Iowa and Illinois. This species might survive in colder
20 regions by hiding in sawdust (Riegel 1963). Zoraptera have not been recorded from Australia,
21 but *Zorotypus novobritannicus* was recently described from New Britain (Terry & Whiting
22 2012) and the group was also recorded from New Zealand and Easter Island (Weidner 1969;
23 Choe 1989, 1992; Grimaldi & Engel 2005).

24 Silvestri (1913) introduced the order and described the genus and three species (in
25 Latin). He collected the type species *Zorotypus guineensis* himself in Ghana (“Costa d’Oro”),
26 and specimens of *Zorotypus ceylonicus* (Sri Lanka) and *Zorotypus javanicus* (Java) were

1 provided by other entomologists. Eight new species from different parts of the world were
2 described in the next 15 years (e.g., Karny 1922, 1927), and four species including the North
3 American *Z. hubbardi* were introduced in a study also containing a key and a discussion of
4 possible relationships of the order (Caudell 1918). A catalog of the Order published by
5 Hubbard (1990) contained 29 extant species and one from the Eocene. Two new species were
6 described by Chao & Chen (2000) and Engel (2000). A distributional checklist of zorapteran
7 species was published in Engel & Grimaldi (2002) and an updated checklist of “World
8 Zoraptera” by Rafael & Engel (2006). In the latter, 34 extant and six fossil species were listed,
9 and the authors provided information on sexes, winged forms, and nymphs of each species.
10 The most recent account is given in Mashimo et al. (2013). This study contains the
11 descriptions of three new species from Peninsular Malaysia, bringing the number of extant
12 zorapterans to 39. Zoraptera have been regarded as rare and one of the least diverse group of
13 hexapods. However, apparently their diversity remains underexplored (Rafael & Engel 2006;
14 Mashimo et al. 2013).

15 In taxonomic studies the shape of the basal antennomeres, the chaetotaxy of the ventral
16 metafemoral surface, the shape of the cerci, and the male genitalia are traditionally recognized
17 as useful to define species. The male genitalia are highly variable and potentially suitable for
18 classifying the group. It has been noted that closely related species with very similar external
19 features can be clearly discriminated based on male genital structures (Paulian 1949, 1951;
20 Hwang 1974, 1976; New, 1978; Rafael & Engel 2006; Rafael et al. 2008; Mashimo et al.
21 2013). Some species were described based solely on immature or female specimens (e.g.,
22 Silvestri 1913; Caudell 1923, 1927; New 1995), or the information on the male genitalia was
23 insufficient (e.g., Choe 1989; Zompro 2005; Rafael & Engel 2006; Terry & Whiting 2012). In
24 future taxonomic studies, a detailed investigation of both sexes or at least the male genitalia
25 should be obligatory for an unambiguous circumscription of species (Mashimo et al. 2013).

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2. Morphology

The illustrations provided by Silvestri (1913) were of high standard, displaying external and internal features, including the setation, the mouthparts, endoskeletal and pretarsal structures, and also internal organs such as the central nervous system, digestive tract, tracheal system and excretory organs (six free Malpighian tubules). A series of comparative studies were carried out by G.C. Crampton. They covered head structures, the neck region, the thorax, the wings, and wing base sclerites of Zoraptera and other groups of insects (Crampton 1918, 1920, 1921, 1926, 1927). Gurney (1938) provided more morphological information, mainly based on the North American species *Z. hubbardi*. Like Silvestri (1913) and Crampton (e.g., 1918, 1927) he used simple dissection techniques and light microscopy. He described and illustrated external body parts but also male and female internal genital organs, the digestive tract, Malpighian tubules and even eggs. Delamare-Deboutteville (1947) compared alate and wingless exemplars. The thoracic skeletomuscular system was described for the first time by Rasnitsyn (1998). The availability of only one damaged wingless exemplar and the application of simple preparation techniques led to incomplete and not fully satisfying results. Weidner (1970) summarized the available information in the "Handbuch der Zoologie" series.

In the 21st century the study of Zoraptera accelerated. Beutel & Weide (2005) provided the first complete study of head structures (*Z. hubbardi*) using semithin microtome sections and SEM. Friedrich & Beutel (2008) described the thoracic anatomy of alate and wingless specimens of the same species and emphasized the highly conserved condition of the skeletomuscular apparatus, presumably close to the neopteran groundplan. Based on the results of this study the authors proposed a new consistent and extendable nomenclature for the thoracic muscle system of neopteran insects (Friedrich & Beutel 2008). Wing base structures of *Z. caudelli* were investigated by Yoshizawa (2007, 2011a). A computer-based 3D reconstruction of the male postabdomen of *Z. hubbardi* was presented by Hünefeld

1 (2007), based on microtome sections. Structures of the male postabdomen were described by
2 Bolívar y Pieltain (1940), Hwang (1974), New (1978), Rafael & Engel (2006) and others.
3 They are extremely variable, probably related to different mating patterns (e.g., Dallai et al.
4 2013).

5 Recently different aspects of Zoraptera were investigated by a collaborative group
6 including entomologists from Japan (University of Tsukuba), Italy (University of Siena), and
7 Germany (Friedrich-Schiller-Universität Jena), mainly specialized on development (R.
8 Machida), ultrastructure (R. Dallai) and the skeletomuscular system (R.G. Beutel),
9 respectively. Combined with collecting efforts in Malaysia (R. Machida and coworkers) and
10 Ecuador (Y. Matsumura) (Fig. 2), several studies focused on the genital region were
11 presented, using a broad array of techniques including transmission electron microscopy
12 (TEM), confocal laser scanning microscopy (CLSM), and micro computed tomography (μ CT)
13 (Fig. 3). One result was the marked discrepancy between a far-reaching uniformity of the
14 group in the general body morphology and conspicuous differences in the male genital
15 structures (Dallai et al. 2011, 2012a, b; Y. Matsumura, pers. comm.).

16 Zorapteran eggs were described in earlier studies for several species (Caudell 1920;
17 Gurney 1938; Silvestri 1946). SEM micrographs of eggs of *Zorotypus gurneyi* and *Zorotypus*
18 *barberi* (Panama) were shown in Choe (1989). Mashimo et al. (2011) described eggs of *Z.*
19 *caudelli* (Malaysia) with different approaches including TEM. The whitish egg is 0.6 mm
20 long and 0.3 mm wide, the two-layered chorion shows a honeycomb pattern, and an
21 operculum or a hatching line are missing. A pair of micropyles is present at the equator on the
22 dorsal side, similar to the condition found in eggs of *Timema* (Phasmatodea) (Mashimo et al.
23 2011).

24 Spermatozoa were already observed by Silvestri (1913). The spermatogenesis and
25 sperm ultrastructure was investigated by Dallai et al. (2011) using transmission electron
26 microscopy. A common feature of Zoraptera is the great length of the spermatozoa. An

1 unusual feature of *Z. caudelli* is a disconnection of sub-tubules A and B at the level of
2 microtubule doublets 1 and 6 of the mature sperm cells (Dallai et al. 2011), whereas
3 disproportionately large mitochondrial derivatives are characteristic for *Z. hubbardi* (Dallai et
4 al. 2012b). Character combinations found in different species suggest that spermatozoa do not
5 evolve as a unit, but that like in other body regions components can be modified
6 independently from each other. A derived feature, dense laminae radiating in a cartwheel
7 array between neighboring centriolar triplets, is shared with Phasmatodea and Embioptera. An
8 apomorphy shared with Phasmatodea is the presence of 17 protofilaments in the tubular wall
9 of the outer accessory microtubules (Dallai et al. 2011, 2012b; Gottardo et al. 2012).

11 **3. The phylogenetic position of Zoraptera (Figs 4, 5)**

12 **3.1. Pre-Hennigian approaches**

13 When Silvestri (1913) described the first zorapteran species and introduced the family
14 Zorotypidae he assumed that they must be close relatives (“collocare vicino”) of roaches
15 (“Blattoidei”) and Isoptera. He listed several differences separating Zoraptera from these
16 polyneopteran groups, such as for instance the presence of a bundle of setae on the left
17 mandible. He also mentioned similarities with Dermaptera, but explicitly referred to them as
18 superficial. After Silvestri, affinities (not necessarily in a phylogenetic sense) with Isoptera
19 were emphasized by Caudell (1918) and the “distinguished albeit eccentric” G.C. Crampton
20 (1920) (Grimaldi & Engel 2005). Potential arguments for a closer relationship included a
21 general resemblance in the habitus, certain structural details of the thorax and abdomen,
22 colonial habits, and dehiscent wings. While recognizing these similarities, Crampton (1920)
23 clearly pointed out affinities with the acercarian orders, for which he hypothesized an origin
24 from “Prothorthopteroid ancestors in the common Prothorthopteran-Protoblattid stem”. He
25 explicitly suggested a very close relationship with Psocoptera (“Corrodentia”). A possible

1 relationship with Psocoptera was also discussed in Gurney (1938), concluding that “affinities
2 with Corrodentia are more noticeable than those with orthopteroid insects”.

3 Weidner (1969) suggested “strong thoracic synapomorphies” of Zoraptera + Isoptera,
4 but did not uphold this view in his Handbuch der Zoologie volume (Weidner 1970) where he
5 explicitly rejected superficial arguments for such a hypothesis and emphasized the difficulty
6 of placing Zoraptera. He rather vaguely referred to the order as a specialized, “today obsolete
7 (“verkümmert”) branch of Blattodea” (including roaches and termites).

9 **3.2. Hennig’s interpretation and follow up studies**

10 W. Hennig revolutionized insect systematics with his classical work “Die Stammesgeschichte
11 der Insekten” (Hennig 1969). In an earlier study he rather vaguely suggested monophyletic
12 Paraneoptera¹ (Zoraptera + Acercaria [= Psocodea, Thysanoptera and Hemiptera]) with
13 Zoraptera basal and definitely outside of Psocoptera (Hennig 1953). A similar hypothesis was
14 proposed in a little-known study by Wille (1960: Fig. 1), who addressed Zoraptera as “the
15 most primitive group of the hemipteroids [= Acercaria], at the base of their evolutionary
16 branch and close to the orthopteroids”. Hennig (1969) indicated a single “certain derived
17 feature” supporting Paraneoptera, the greatly condensed condition of the abdominal
18 ganglionic chain, with two separate ganglionic masses in zorapterans, and only one in the
19 remaining groups (Hennig 1953, 1969). He considered the reduced number of three
20 tarsomeres (groundplan) as an additional potential synapomorphy, but it is apparent that
21 losses of tarsal segments occurred in many groups. The hypotheses suggested by Hennig
22 (1953, 1969) and Wille (1960), were tentatively followed by Kristensen (e.g., 1975),
23 Willmann (2005), and Beutel & Weide (2005). The presence of only six Malpighian tubules
24 (four in Acercaria), a cercus composed of only one segment or entirely missing (Acercaria), a

58 ¹ The term Paraneoptera is presently often used for a group that does not include Zoraptera (e.g., Grimaldi &
59 Engel 2005)

1 fork of the cubitus posterior (areola postica) (Willmann 2005), a strengthened cibarial sucking
2 pump, and a blade-like lacinia lacking mesally directed spines (Beutel & Weide 2005) were
3 considered as additional arguments. The last feature is likely an autapomorphy of *Zorotypus*
4 *hubbardi*, the species examined by Beutel & Weide (2005). A largely unmodified lacinia is
5 present in other species (e.g., Silvestri 1913). The strongly developed cibarial dilator is a
6 rather unspecific feature which has also developed in other groups of insects (e.g.,
7 Antliophora; Beutel & Baum 2008). In his later review studies, Kristensen (e.g., 1995)
8 questioned Hennig's Paraneoptera concept and treated acercarian and polyneopteran affinities
9 of Zoraptera as competing working hypotheses.

10 Similarities of the antennae of Zoraptera and Isoptera (chemoreceptors, tactile setae,
11 Johnston's organ) were described by Slifer & Sekhon (1978). However, it is evident that these
12 features have evolved independently given the strong support for a subordinate placement of
13 Isoptera within Blattodea (e.g., Deitz et al. 2003; Lo 2003; Klass 2009). A sistergroup
14 relationship with Embioptera was first proposed by Minet & Bourgoïn (1986), who suggested
15 an entire series of potential synapomorphies including a reduced wing venation (groundplan),
16 a hypertrophied metafemur and metathoracic tibial depressor, moniliform antennae, fusion of
17 tarsomeres, loss of the arolium, reduction of the ovipositor, and gregarious habits. Even
18 though some of these features are rather unspecific modifications or reductions, a close
19 relationship with Embioptera gained further support by Engel & Grimaldi (2000) and
20 Grimaldi (2001), like in Minet & Bourgoïn (1986) based on informal character evaluations.

21 In a study on the zorapteran wing venation Kukulová-Peck & Peck (1993) addressed the
22 intraordinal relationships and also the placement of the order within neopteran insects. Based
23 on an informal analysis of wing characters they suggested that Zoraptera "probably diverged
24 from the Blattoneoptera (= Grylloblattodea, †Protelytroptera, Dermaptera and Dictyoptera),
25 almost certainly before the (†Protelytroptera + Dermaptera) line, and much before the
26 (Isoptera + (Blattodea + Mantodea)) line". The hypothesis was illustrated with a phylogenetic

1 tree (Kukalová-Peck & Peck 1993: Fig. 23) showing Zoraptera as the second branch of
2 Blattoneoptera after Grylloblattodea.

3 A sistergroup relationship with Holometabola (“Scarabaeiformes”) was suggested by
4 Rasnitsyn (1998) based on the alleged presence of a precursor of the medial mesocoxal
5 articulation in *Z. hubbardi*. As pointed out above (2. Morphology), the morphological data
6 were insufficient. Moreover, the phylogenetic argumentation was based on an ad hoc
7 interpretation of a single vague character. Rasnitsyn (1998) provisionally placed Zoraptera in
8 an otherwise extinct superorder Caloneurida, supposedly subordinate to a “Cohors
9 Cimicoformes” (= Acercaria). He hypothesized Caloneurida “to be ancestral to other
10 cimicoform superorders as well as to the Cohors Scarabaeiformia” (= Holometabola). This
11 taxonomic treatment of Zoraptera is not compatible with the suggested phylogenetic position
12 as sistergroup of Holometabola.

13 Kusnetsova et al. (2002) examined the karyotype ($2n = 38, 36 + \text{neo-XY}$) and genital
14 structures of *Z. hubbardi*. Based on the presence of panoistic ovaries and holokinetic
15 chromosomes they rather vaguely suggested that Zoraptera may “represent a group of
16 Polyneoptera nearest to the origin of Paraneoptera”.

17 Zompro (2005) suggested a position of Zoraptera in “Orthopteromorpha”, supposedly
18 comprising the polyneopteran orders excluding Plecoptera, Embioptera and Phasmatodea.
19 This placement was based on elongate coxae and eggs without a strongly sclerotized egg
20 capsule and also lacking an operculum. However, a stringent character discussion and
21 analytical approach were lacking. Furthermore, the coxae of Zoraptera are not elongated (e.g.,
22 Friedrich & Beutel 2008) and the condition of the egg is obviously plesiomorphic (Mashimo
23 et al. 2011).

24 A character system previously not explored with respect to the position of Zoraptera is
25 the antennal heart and associated structures. The hitherto unknown antennal pulsatile organ of
26 Zoraptera is characterized by a t-shaped configuration of muscles (pers. obs. B. Wipfler), with

1 a fairly broad transverse muscle attached to the head capsule on both sides (assigned as
2 "Mxy" in Beutel & Weide 2005) and a narrower vertical muscle. Such a configuration occurs
3 in several groups of Polyneoptera but is completely unknown in Acercaria. This feature
4 clearly supports a placement of Zoraptera among the polyneopteran lineages.

5 Recent detailed morphological studies related to the reproduction suggested a
6 sistergroup relationship of Zoraptera with Embioptera or Eukinolabia (Embioptera +
7 Phasmatodea), by Mashimo et al. (2011, in press) based on the egg structure and development
8 of egg tooth and by Dallai et al. (2011, 2012b) based on the spermatozoan structures, i.e. 17
9 protofilaments comprising accessory tubules of axonemes and L-shaped electron-dense
10 lamellae accompanying microtubular triplets in the centriole adjunct.

12 **3.3. Cladistic approaches**

13 The first morphology-based formal cladistics analysis including all hexapod orders were
14 presented by Wheeler et al. (2001) and Beutel & Gorb (2001). Wheeler et al. (2001) was
15 primarily based on molecular data but also presented an independent morphological analysis
16 based on a matrix with 275 characters. The analysis of the morphological data yielded a
17 sistergroup relationship between Acercaria and Zoraptera in agreement with Hennig (1969).
18 Zoraptera were placed as sistergroup of Eumetabola (Acercaria + Holometabola) in the study
19 of Beutel & Gorb (2001), which had a main focus on the evolution of attachment structures.
20 The same result was obtained in a series of studies on the head morphology (Wipfler et al.
21 2011; Blanke et al. 2012; 2013; Wipfler 2012). However, it was pointed out by the authors
22 that the unit Zoraptera + Eumetabola was not supported by convincing synapomorphies. In a
23 follow-up study Beutel & Gorb (2006; expanded morphological data set, Mantophasmatodea
24 included) retrieved Zoraptera as sister to Acercaria, essentially supported by the potential
25 synapomorphies listed above (e.g., blade-like lacinia, condensed abdominal ganglionic chain).

1 Cladistic analyses of characters of the wing articulation (Yoshizawa 2007, 2011a)
2 yielded a sistergroup relationship Zoraptera + Embioptera, like in Minet & Bourgoïn (1986).
3 This was also supported when additional characters from Beutel & Gorb (2001, 2006) were
4 added to the matrix. The fusion between the first axillary sclerite and the posterior notal wing
5 process was emphasized as a potential synapomorphy, as this modification also involves a
6 modification of the wing hinge, which is extremely conservative throughout the winged
7 insects (Yoshizawa & Ninomiya 2007; Ninomiya & Yoshizawa 2009).

9 **3.4. Molecular phylogenetic and phylogenomic approach**

10 Wheeler et al. (2001; 18S, 28S, morphology) and Terry & Whiting (2005; 18S, 28S, Histone
11 3, morphology) analyzed the relationships of the entire Hexapoda or of the polyneopteran
12 orders, respectively. Very different placements of Zoraptera order resulted from separate
13 analyses of 18S and 28S rRNA in Wheeler et al. (2001: Figs 13, 14) (see Table 1), and
14 analyses of both genes combined suggested an unlikely unit comprising Zoraptera,
15 Trichoptera and Lepidoptera. Terry & Whiting (2005) placed Zoraptera as sistergroup of
16 Dermaptera and referred to this possible clade as Haplocecata. The analytical methods used in
17 both studies (POY) have been criticized (Kjer et al. 2007; Ogden & Rosenberg 2007;
18 Yoshizawa 2010). A contaminant of a dermapteran sequence in the zorapteran dataset
19 (Yoshizawa 2010, 2011b) suggests that the clade Zoraptera + Dermaptera may be artificial.

20 Yoshizawa & Johnson (2005; 18S rRNA) aligned their data using a secondary
21 structure model. Maximum likelihood and Bayesian analyses retrieved Zoraptera as sister to
22 Dictyoptera. However, very unusual molecular evolutionary trends observed in Zoraptera
23 (e.g., extremely accelerated substitution rates and modifications of secondary structures) made
24 the placement of the order highly unstable. Wang et al. (2013; 28S rRNA) also employed
25 secondary structure-based alignments and obtained the same result for Zoraptera. However,
26 for both 18S and 28S rRNA an extreme acceleration of the substitution rate and modification

1 of secondary structures is evident in Zoraptera, making the results unstable. Wang et al.
2 (2013) identified unique features of the secondary structure of 28S rRNA shared by Zoraptera
3 and Dictyoptera.

4 Misof et al. (2007; 18S rRNA) specifically addressed problems caused by character
5 interdependence by employing secondary structure information and a Bayesian approach. A
6 partly robust tree was obtained based on a hexapod consensus secondary structure model and
7 mixed DNA/RNA substitution models. Zoraptera were placed as sistergroup of Dermaptera +
8 Plecoptera, but with very low Bayesian support (0.45). This underlines that the frequently
9 used 18S rRNA is apparently insufficient to fully resolve supraordinal insect
10 interrelationships.

11 Ishiwata et al. (2011; DPD1, RPB1, RPB2) retrieved Polyneoptera incl. Zoraptera with
12 good support, and a sistergroup relationship Zoraptera + Dictyoptera in some of the trees with
13 low support. Consequently, Zoraptera were shown in an unresolved polyneopteran polytomy
14 in a summary tree (Ishiwata et al. 2011).

15 Simon et al. (2012) placed Zoraptera as sister to all other polyneopteran terminals in a
16 preliminary phylogenomic study. The support values were extremely low and several orders
17 (Embioptera, Phasmatodea, Mantophasmatodea, Grylloblattodea) were not included. In a
18 follow-up study (Letsch & Simon 2013) Embioptera and Phasmatodea were added. The
19 analyses also supported a sistergroup relationship between Zoraptera and the remaining
20 polyneopteran lineages. Recent unpublished results from the 1KITE project (ca. 100 hexapod
21 terminals representing all orders) suggest a sistergroup relationship between a unit Zoraptera
22 + Dermaptera (confirming the Haplocercata s. Terry & Whiting 2005) and a clade comprising
23 all other polyneopteran orders. However, this pattern is not strongly supported statistically.

24 **4. Intraordinal phylogeny**

1 Kukalová-Peck & Peck (1993) suggested a classification based on an informal evaluation of
2 wing characters. They recognized two families of Zoraptera and several genera. A new genus
3 *Formosozoros* (from Taiwan) was described by Chao & Chen (2000). However, like the
4 genera introduced by Kukalová-Peck & Peck (1993) it was not considered as valid in recent
5 studies, and all extant species are currently classified into a single genus *Zorotypus* (e.g.,
6 Engel & Grimaldi 2002; Grimaldi & Engel 2005).

7 No molecular approach explicitly addressing the intraordinal phylogeny has been made
8 to date. Yoshizawa & Johnson (2005; 18S rRNA) included four representatives of Zoraptera
9 in their study (Old World and New World samples) and Yoshizawa (2010, 2011b; 18S rRNA)
10 six. A deep divergence between the Old World and New World species was demonstrated.
11 This is seemingly in conflict with evidence from male genitalia. The New World *Zorotypus*
12 *snyderi* shares an elongated and coiled intromittent organ with the Old World species (Gurney
13 1938; Dallai et al. 2011; Mashimo et al. 2013), but molecular data suggest that it is closely
14 related with *Z. hubbardi* (also New World) which lacks this unusual structure. Therefore it is
15 conceivable that the coil was present in the groundplan of Zoraptera (or a large subgroup) and
16 was secondarily lost in the majority of species. It cannot be fully excluded that it was acquired
17 independently in several lineages, but independent gain of such a complex structure appears
18 less likely.

20 **5. Fossils**

21 Grimaldi & Engel (2005) suggested a possible origin of Zoraptera in the lower Mesozoic. The
22 first described extinct species was an apterous female of †*Zorotypus palaeus* from Dominican
23 amber (Middle Miocene; Poinar 1988; Iturralde-Vinent & MacPhee 1996). The first winged
24 specimen was described by Engel & Grimaldi (2000) from the same formation. Four species
25 were identified in Burmese Cretaceous amber (Engel & Grimaldi, 2002). Three of them were
26 very similar to extant zorapterans and consequently assigned to the genus *Zorotypus*. A basal

1 position of two of them (†*Zorotypus anathothorax*, †*Zorotypus nascimbenei*) was tentatively
2 suggested by Engel (2003), and both were placed in a subgenus †*Octozoros* based on a
3 reduced number of eight antennomeres and the presence of jugate setae along a part of the
4 posterior forewing border (Engel, 2003). One of the four species was placed in a separate
5 genus †*Xenozorotypus* (Engel & Grimaldi, 2002) and considered as sister to all other
6 zorapterans. †*Palaeospinosus hudaie* was described from Jordanian amber by Kaddumi (2005),
7 but the genus has been synonymized with *Zorotypus* (Engel 2008).

9 **6. General biology**

10 Zorapterans are omnivorous scavengers and feed on fungal hyphae and spores and on very
11 small arthropods like mites and collembolans (Choe 1992; Grimaldi & Engel 2005). They are
12 usually found in colonies under bark of decaying logs. As social behavior has not been
13 reported yet and is probably missing in all species, zorapterans should be referred to as
14 gregarious. The individuals spend much time grooming various body parts (Valentin 1986). In
15 *Zorotypus barberi* some parts that cannot be reached are groomed by conspecifics (Choe
16 1992). Some species emit a slight but characteristic odor, reminiscent of a fragrance produced
17 by the osmeterium of some swallowtail butterfly (Shetlar 1978; pers. obs. Y. Mashimo). Little
18 is known about the life cycle of Zoraptera. The length of the nymphal period is about 1-2
19 months and adult lifespan is a few months, as reported by Gurney (1938) and Shetlar (1978).
20 While Shetlar (1974) estimated five nymphal instars by measuring morphological features,
21 Riegel & Eytalis (1974) suggested four instars based on different head widths. However,
22 these descriptions are insufficient and the conclusions largely speculative. The specific wing
23 dimorphism, a potential autapomorphy of the order, is also insufficiently investigated.
24 According to observations mentioned in a review by Choe (1992) crowding and heredity
25 seem to affect the production of the winged morphs. However, relevant details are unknown.
26 Further intensive observations and investigations of the biology of Zoraptera are required.

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7. Embryonic development

The embryonic development of Zoraptera was described recently by Mashimo et al, (in press). The embryo is formed by a fusion of paired blastoderm regions with higher cellular density extends along the egg surface. After reaching its full length, it migrates into the yolk and finally moves to take its position on the ventral surface of the egg accompanied by a reversion of its anteroposterior axis. These embryological features are widely known in Polyneoptera, and strongly suggest a placement of Zoraptera in this lineage (Mashimo et al. in press).

8. Mating behavior

Several studies on the mating behaviour were carried out by Choe (1994a, b, 1995, 1997), focused on the precopulatory courtship and copulation of *Z. gurneyi* and *Z. barberi* from Panama. The two sympatric species display a distinctly different mating behaviour. In *Z. gurneyi* a linear dominance hierarchy is established by mutual antennation and females were fertilized only once with very few exceptions. Dominant males performed 72% of the copulations, while the others were able to mate only occasionally. *Z. barberi* males lack this hierarchy. They are characterized by a highly unusual courtship ritual including nuptial feeding on a secretion of a cephalic gland (Choe 1997).

The mating behaviour of several Asian species was documented by Dallai et al. (2013) (Fig. 3). Mating sequences were recorded for many hours and observations were compared to earlier findings of Choe (1994a, b, 1995, 1997). An unusual case of external sperm transfer was described for *Z. impolitus*, a feature unknown in other pterygote insects. The high variability not only of the genital structures but also of the mating behaviour and reproductive strategies was underlined by Dallai et al. (2013).

9. Discussion

1 Despite of their small size, inconspicuous appearance, cryptic habits and very low diversity it
2 would be misleading to consider Zoraptera as “unsuccessful” in their evolution. An origin in
3 the early Mesozoic was suggested by Grimaldi & Engel (2005). Considering a possible
4 placement as sistergroup of a large polyneopteran subunit (possibly together with
5 Dermaptera), an origin in the Carboniferous appears likely. In any case the group has survived
6 for a period of at least 200 Mya. Very small size and cryptic habits, which have prevented
7 discovery by insect collectors until the early 20th century, may have contributed to the long
8 survival. Recently collected and described species from different regions (e.g., Mashimo et al.
9 2013) indicate that the true diversity of the order is likely distinctly higher than known at
10 present.

11 The morphological investigation of the group mainly profited from three factors. The
12 availability of innovative techniques greatly facilitated detailed anatomical and ultrastructural
13 studies (Fig. 1; e.g., Friedrich & Beutel 2008; Dallai et al. 2012a, b). The coordinated effort of
14 researchers with different specialized skills also led to a markedly improved knowledge of the
15 group. Moreover, improved collecting and fixation yielded more and better conserved
16 material for anatomical investigations.

17 Unlike in Strepsiptera (“the Strepsiptera problem”; e.g., Kristensen 1991, 1995; Beutel
18 & Pohl 2006; Beutel et al. 2011) the systematic position of Zoraptera is not obscured by
19 numerous autapomorphies, but rather by many preserved plesiomorphic features, combined
20 with far-reaching reductions in some body regions (e.g., attachment structures, ovipositor).
21 Friedrich & Beutel (2008) showed that the thoracic skeleto-muscular system of winged
22 morphs is probably close to the groundplan of Neoptera. Whereas Strepsiptera have been
23 recently placed as the sistergroup of Coleoptera with strong support (Niehuis et al. 2012; Pohl
24 & Beutel 2013), the “Zoraptera problem” is still not completely resolved.

25 Widely divergent placements were suggested for Zoraptera (Figs 4, 5) and Strepsiptera
26 and in both cases early attempts were impeded by the lack of a sound phylogenetic

1 methodology (Pohl & Beutel 2013). That the spectrum of proposed positions is somewhat
2 narrower in the case of Zoraptera may be partly due to its later discovery, about 120 years
3 after the description of the first strepsipteran species (Rossius 1793; see Pohl & Beutel 2013).
4 The placement of Zoraptera as close relatives of termites (e.g., Weidner 1969) was based on
5 superficial similarities (see Weidner 1970) and an insufficient character evaluation. The
6 structural affinities with Psocoptera emphasized by Crampton (e.g., 1921) and others are
7 either plesiomorphic, superficial, or due to homoplasy. The placement of barklice
8 (paraphyletic with respect to Liposcelididae) within a clade Psocodea is undisputed (e.g.,
9 Yoshizawa & Saigusa 2001; Yoshizawa & Johnson 2006, 2010; Friedemann et al. in press).
10 The sistergroup relationship with Holometabola suggested by Rasnitsyn (1998) was based on
11 an insufficient evaluation of very incomplete morphological data. A clade Zoraptera +
12 Eumetabola (Beutel & Gorb 2001) is an artifact mainly caused by parallel reductions (e.g.,
13 ocelli in immature stages, number of Malpighian tubules).

14 The hypothesized sistergroup relationship between Zoraptera and Acercaria (Hennig
15 1953, 1969; Wille 1960; Kristensen 1975; Willmann 2003, 2005; Beutel & Weide 2005;
16 Beutel & Gorb 2006) is presently largely refuted (Figs 4, 5). Most characters suggesting
17 monophyletic Paraneoptera (incl. Zoraptera) are reductions (e.g., number of tarsomeres,
18 cercomeres, and abdominal ganglia) and polyneopteran affinities (e.g., Yoshizawa 2007,
19 2011a; Ishiwata et al. 2011) imply that these structural modifications evolved independently.

20 A placement of Zoraptera within Polyneoptera is gaining more and more support (Fig.
21 5). However, the monophyly of this unit is not sufficiently supported yet (e.g., Whitfield &
22 Kjer 2008; Trautwein et al. 2012) and the precise placement of Zoraptera is still controversial.
23 A close relationship with Dictyoptera (Boudreaux 1979; Wheeler et al. 2001: p. 148 [“based
24 on the discussion”]; Yoshizawa & Johnson 2005; Wang et al. 2013) is a possible option, but
25 convincing synapomorphies are missing. Boudreaux’ (1979) arguments were not based on a
26 formal character analyses and the features are unspecific or widespread in Insecta (e.g.,

1 “backwardly directed hind coxae”). The conclusion presented by Wheeler et al. (2001) in a
2 summary tree is weakened by conflicting results obtained with the different data sets
3 (morphology, 18S rRNA, 28S rRNA, 18S + 28S rRNA) (see Table 1). It is conceivable that
4 the results based on molecular data were negatively affected by the use of POY (e.g.,
5 Yoshizawa 2010, 2011b). Kukulová-Peck & Peck (1993) provided useful data, but the
6 hypothesized placement of Zoraptera in a clade Blattoneoptera is weakened by the lack of a
7 formal analysis and the exclusive use of wing characters. Wing base characters and
8 morphological features linked to reproduction suggest a placement as sistergroup Embioptera
9 (Yoshizawa 2007, 2011a) or Eukinolabia (Embioptera + Phasmatodea) (Mashimo et al. 2011,
10 in press; egg and embryonic development; Dallai et al. 2011, 2012b; spermatozoa),
11 respectively. Results of single gene analyses of single genes did not yield congruent results
12 yet (e.g., Yoshizawa & Johnson 2005; Misof et al. 2007). Likewise, presently available
13 transcriptome-based studies are not fully convincing. The basal placement within
14 Polyneoptera suggested by Simon et al. (2012) and Letsch & Simon (2013) is weakened by
15 the incomplete ordinal taxon sampling. As the pattern in the recent 1KITE-tree ((Zoraptera +
16 Dermaptera) + (remaining Polyneoptera)) is not strongly supported statistically, the
17 precise placement of Zoraptera remains a challenge.

18 Despite a considerable progress in zorapteran studies it is apparent that important
19 problems remain to be solved, not only concerning the systematic position. To understand the
20 phenomenon of different forms of selective pressure shaping different body parts (natural
21 selection [ecological]/sexual selection) detailed information on genital structures of more
22 species is required. Moreover, a species level phylogeny based on morphological *and*
23 molecular data should have high priority in future studies. A solid intraordinal phylogeny is
24 an essential basis for reconstructing the evolution of the group and to understand evolutionary
25 processes.

1 It is probably safe to assume that zorapterans will remain one of the smallest hexapod
2 orders and only few entomologists would address them as attractive insects. Nevertheless the
3 group is intriguing in different aspects, phylogenetically, as a model case in evolutionary
4 biology, and as cryptic survivors with a hidden diversity still to explore.

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1 Table 1. Systematic placements of Zoraptera with different approaches and character sets.

Systematic approach/ Study	Characters	Systematic placement
Pre-Hennigian concepts		
Silvestri (1913)	morphology	closely related with roaches and termites (Blattodea)
Caudell (1918)	morphology	affinities with Isoptera
Crampton (1920, 1921, 1926)	different morphological character systems	affinities with Psocoptera (“Corrodentia”)
Weber (1933)	morphology	tentatively in a superorder Blattoidea (Mantodea, Zoraptera, Blattaria, Isoptera)
Gurney (1938)	morphology	affinities with Psocoptera (“Corrodentia”)
Hennigian concepts		
Hennig (1953)	morphological characters	tentatively as basal lineage of Paraneoptera , outside of Psocoptera
Hennig (1969)	morphological characters, abdominal ganglionic chain, number of tarsomeres	sistergroup of Acercaria (Paraneoptera concept)
Weidner (1969)	mainly thoracic characters	tentatively as sistergroup of Isoptera
Weidner (1970)	mainly thoracic characters	obsolete branch of Blattodea
Kristensen (1975)	different morphological	Paraneoptera concept as

	characters	working hypothesis, polyneopteran affinities tentatively refuted
Boudreaux (1979)	wing venation, metacoxae	sistergroup of Dictyoptera („Cursorida = Zorapterida + Blattarida“
Minet et Bourgoïn (1986)	wing venation, thoracic and abdominal morphology	sistergroup of Embioptera
Kristensen (1995)	different morphological characters	either acercarian or polyneopteran affinities
Kukalová-Peck & Peck (1997)	wing base and venation	sistergroup of †Protelytroptera, Dermaptera and Dictyoptera (Blattoneoptera excl. Grylloblattodea)
Kusnetsova et al. (2002)	chromosome structure and genitalia	“in Polyneoptera close to the origin of Paraneoptera”
Zompro (2005)	morphology and egg structure	“Orthopteromorpha” (=Polyneoptera excl. Plecoptera, Embioptera and Phasmatodea)
Dallai et al. (2011)	sperm ultrastructure	In a clade with Embioptera and Phasmatodea
Mashimo et al. (2011, in press)	egg structure and development	In a clade with Embioptera and Phasmatodea

Cladistic approach based on morphology		
Kukalová-Peck & Peck (1993)	wing base and venation	sistergroup of †Protelytroptera, Dermaptera and Dictyoptera (Blattoneoptera)
Wheeler et al. (2001)	morphological matrix with 275 characters, extracted from literature	sistergroup of Acercaria
Beutel & Gorb (2001)	characters of adults and larvae including attachment structures	sistergroup of Eumetabola (Acercaria + Holometabola)
Beutel & Gorb (2006)	characters of adults and larvae including attachment structures, Mantophasmatodea and some developmental features added	sistergroup of Acercaria
Yoshizawa (2011)	wing base	Embioptera
Single gene analyses		
Wheeler et al. (2001)	18S rRNA (analyzed with POY)	Sistergroup of Psocodea
	28S rRNA (POY)	sistergroup of a clade comprising all pterygote

		groups except for Strepsiptera and two ephemeropteran terminals
	18S + 28S rRNA	sistergroup of Amphiesmenoptera (trichopteran and lepidopteran terminals)
	18S, 28S rRNA + morphological data extracted from literature (“total evidence”)	sistergroup of Dictyoptera
Yoshizawa & Johnson (2005)	18S rRNA	Dictyoptera
Misof et al. (2007)	18S rRNA, special focus on character interdependence	sistergroup of Plecoptera + Dermaptera
Ishiwata et al. (2011)	3 nuclear protein-coding genes	In Polyneoptera , possibly sistergroup of Dictyoptera
Wang et al. (2013)	28S rRNA	Dictyoptera
Phylogenomic approach		
Simon et al. (2012)	Transcriptomes, Embioptera, Phasmstodea, Grylloblattodea, and Mantophasmatodea not included	sistergroup of remaining polyneopteran terminals with low support
Letsch & Simon (2013)	Transcriptomes,	sistergroup of remaining

	Grylloblattodea and Mantophasmatodea not included	polyneopteran terminals
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1 Fig. 1. Histogram showing number of publications on Zoraptera under different aspects.

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4 2 Fig. 2. Collecting site of different *Zorotypus* species in Ecuador, Zamora-Chinchipec,
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6 3 Copalinga Lodge Reserve Rainforest, with nymph (lower right).

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10 4 Fig. 3. Copula of *Zorotypus caudelli*, volume render of μ -Ct image stacks.

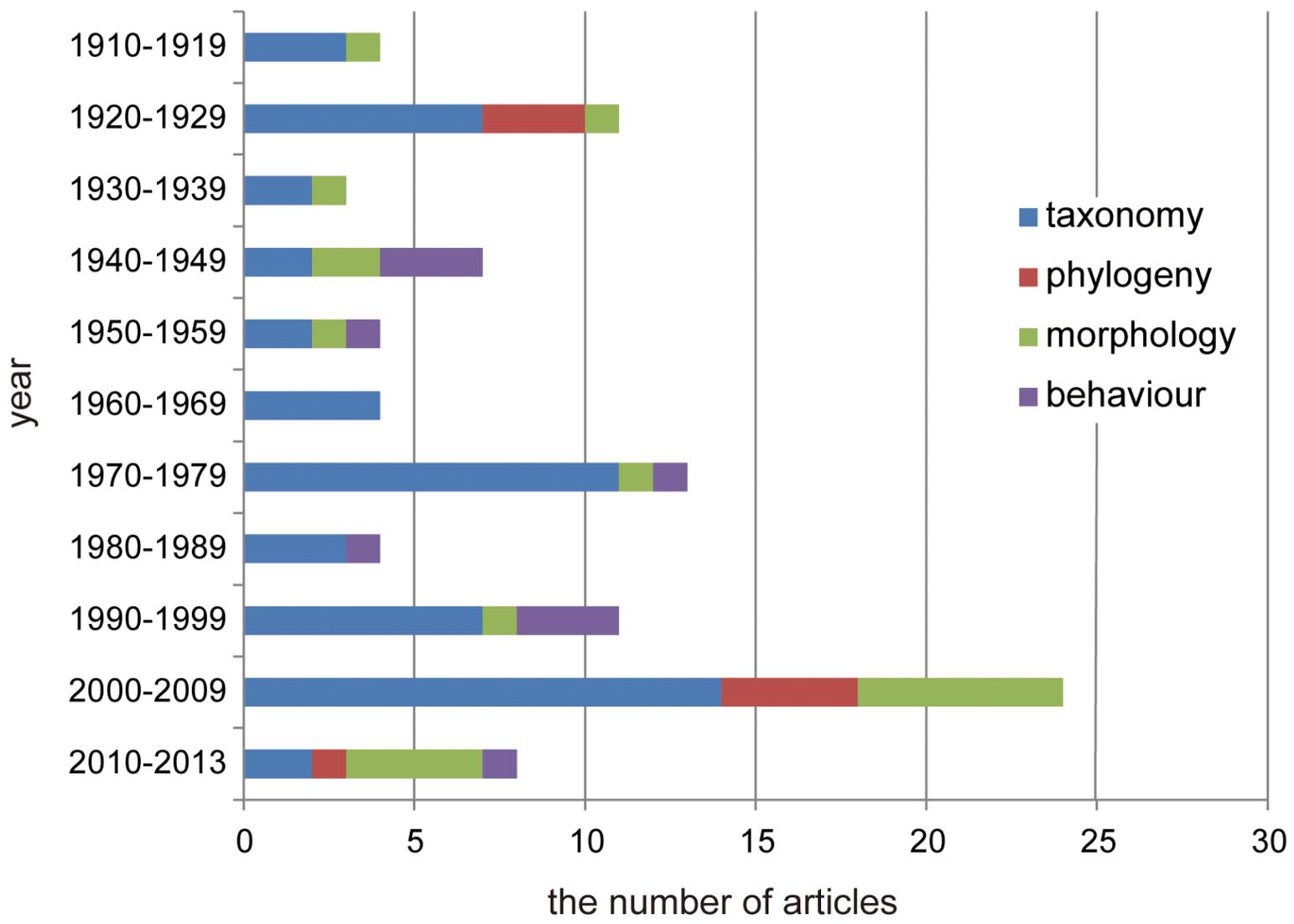
11
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13 5 Fig. 4. Cladograms showing different placements of Zoraptera.

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17 6 Fig. 5. Cladograms from recent studies showing different placements of Zoraptera.

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Figure



Figure

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Figure
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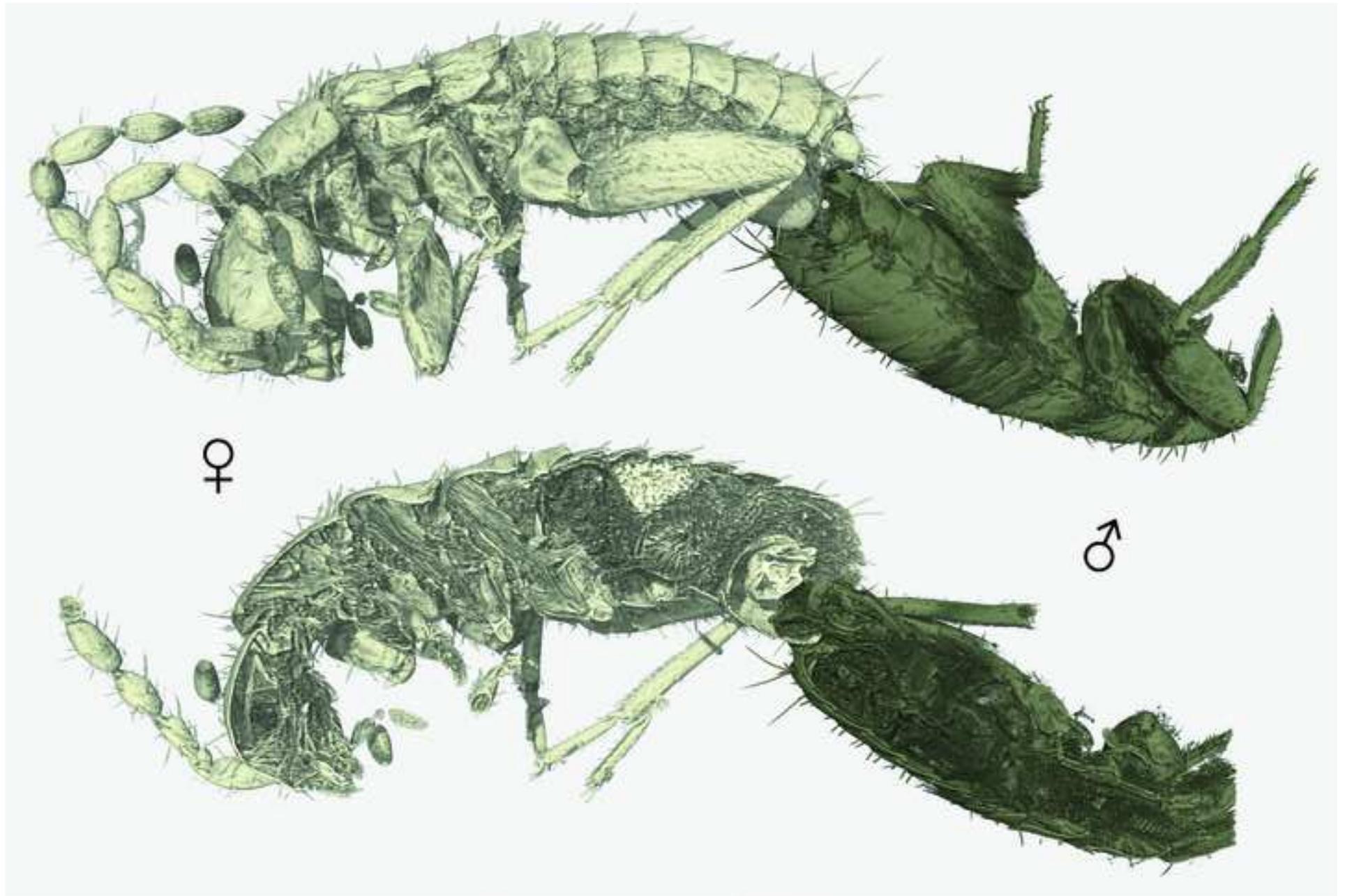
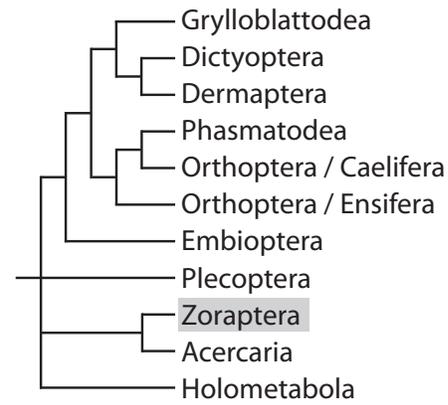
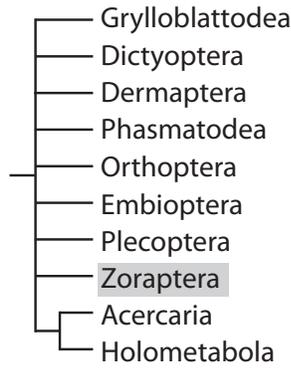


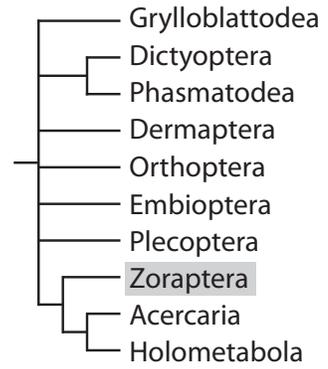
Figure **A Hennig 1969**
Morphology: discussion



B Kristensen 1981, 1991
Morphology: discussion

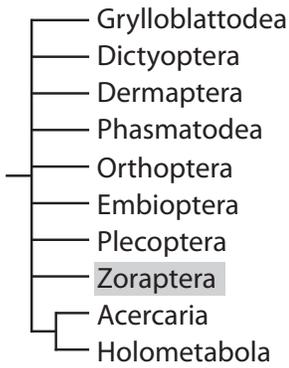


C Beutel & Gorb 2001
Morphology: cladistic analysis



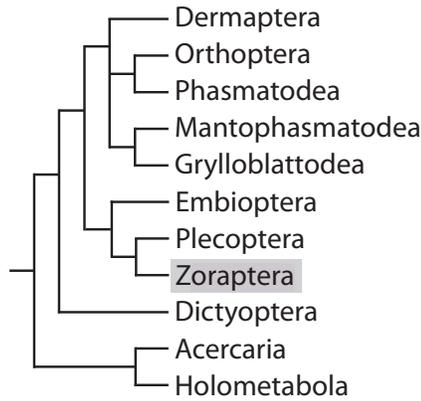
D Wheeler et al. 2001

18S rDNA, 28S rDNA & morphology



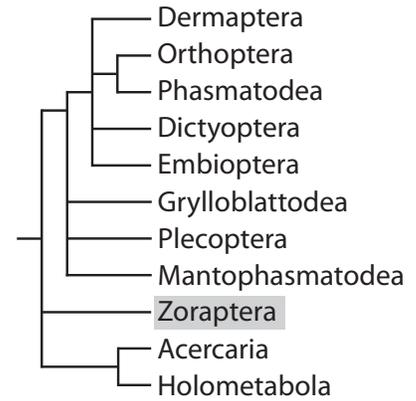
E Grimaldi & Engel 2005

Morphology: discussion



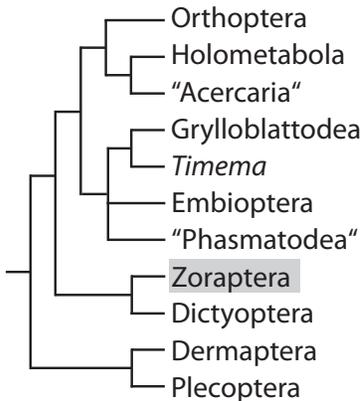
F Willmann 2005

Morphology: discussion



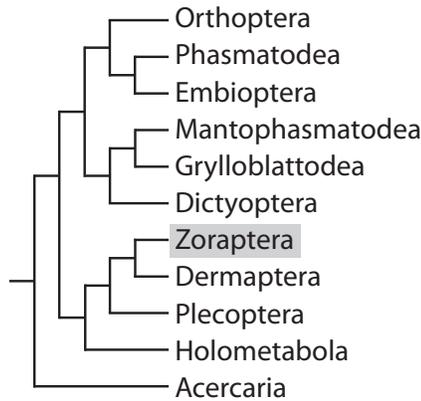
G Yoshizawa & Johnson 2005

18S rDNA



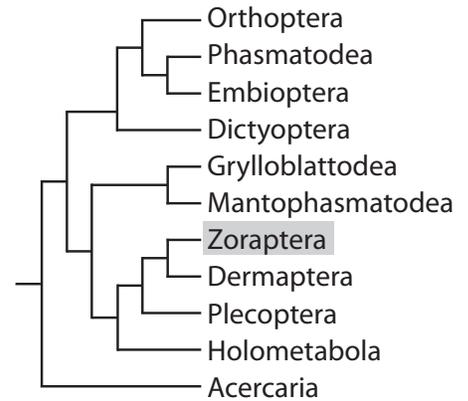
H Terry & Whiting 2005

18S rDNA, 28SrDNA, Histone3
& morphology (PAUP)



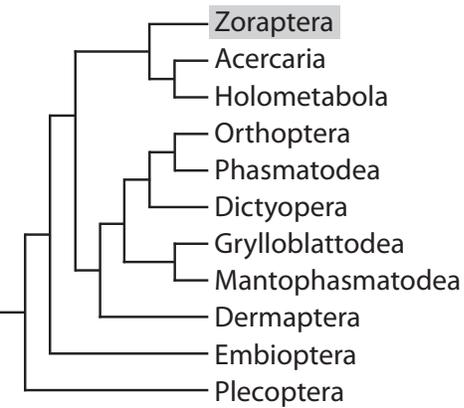
I Terry & Whiting 2005

18S rDNA, 28SrDNA, Histone3
& morphology (ClusterX)



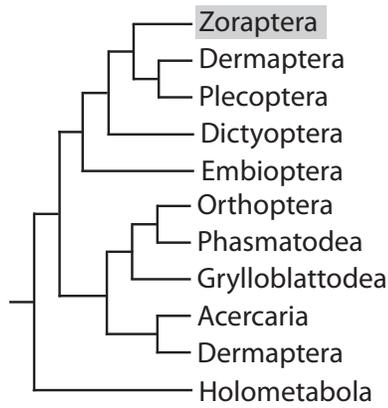
J Beutel & Gorb 2006

Morphology: cladistic analysis



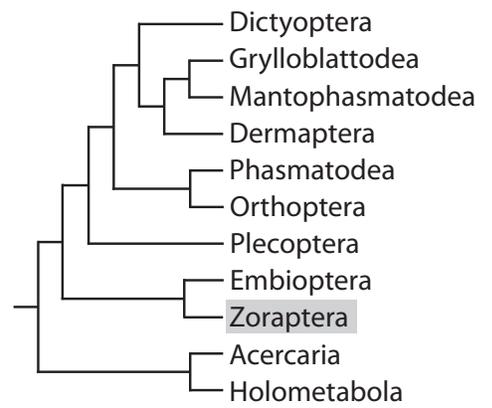
K Misof et al. 2007

18S rDNA



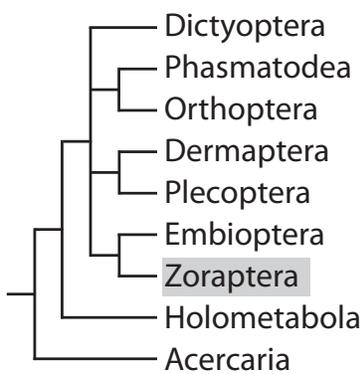
M Yoshizawa 2011

Morphology: cladistic analysis



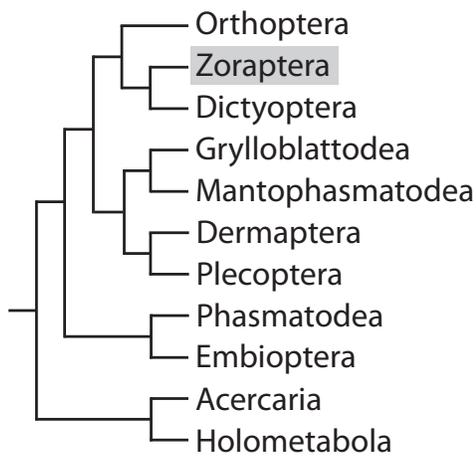
M Yoshizawa 2011

Morphology (wing joint):
cladistic analysis



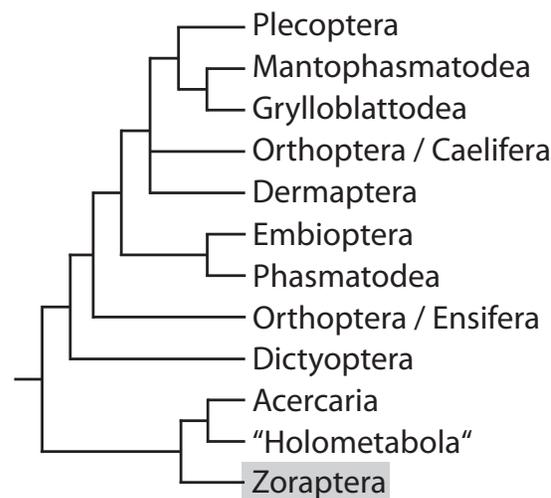
N Ishiwata et al. 2011

DPD1, RPB1, RPB2



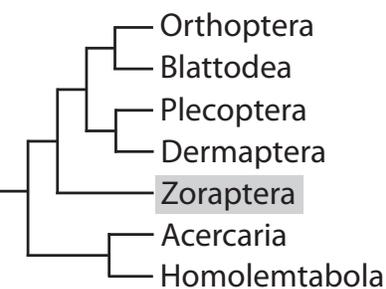
O Wipfler 2012

Cephalic morphology:
cladistic analysis



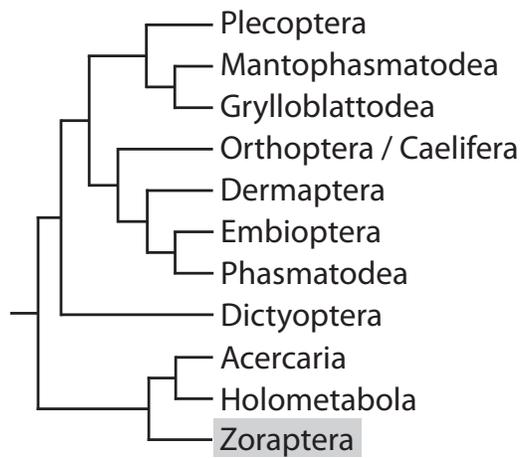
P Simon et al. 2012

Transcriptomic data



Q Blanke et al. 2012

Cephalic morphology:
cladistic analysis



R Letsch & Simon in press

Transcriptomic data

