The morphology of the eggs of three species of Zoraptera (Insecta)

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A B S T R A C T

The egg structure of Zorotypus magnicaudelli, Zorotypus hubbardi and Zorotypus impolitus was examined and described in detail. Major characteristics of zorapteran eggs previously reported were confirmed in these species, with the partial exception of $Z$. impolitus: 1) a pair of micropyles at the equator of the egg's ventral side, 2) a honeycomb pattern on the egg surface, 3) a two-layered chorion, 4) micropylar canals running laterally, 5) a flap covering the inner opening of the micropylar canal and 6) no region specialized for hatching. These features are probably part of the groundplan of the order. Three groups (A–C) and two subgroups (A1 and A2) of Zoraptera can be distinguished based on characters of the reproductive apparatus including eggs. However, information for more species is needed for a reliable interpretation of the complex and apparently fast evolving character system. The egg of $Z$. impolitus presumably shows apomorphic characteristics not occurring in other species, a chorion without layered construction and polygonal surface compartments with different sculptures on the dorsal and ventral sides of the egg. Another feature found in this species, distinct enlargement of the micropyles, is also found in $Z$. hubbardi. The increased micropylar size is likely correlated with the giant spermatozoa produced by males of these two species. These two features combined with the large size of the spermatheca are arguably a complex synapomorphy of $Z$. hubbardi and $Z$. impolitus. The phylogenetic placement of Zoraptera is discussed based on the egg structure. A clade of Zoraptera + Eukinolabia appears most plausible, but the issue remains an open question.

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1. Introduction

Insects are the most diverse and successful multicellular organisms in terrestrial ecosystems (cf. Engel and Grimaldi, 2005; Beutel et al., 2014; Misof et al., 2014). They were the first to acquire wings, and have expanded into new niches rapidly, thus increasing their diversity at a remarkable rate. Neoptera, which contains ca. 98% of all known species of Hexapoda, is composed of three major groups, Polyneoptera, Acercaria (= Paraneoptera excl. Zoraptera) and Holometabola (= Endopterygota).

Zoraptera is probably the most enigmatic neopteran lineage in terms of its phylogenetic placement (cf. Mashimo et al., 2014c). The systematic position of the order has remained controversial since the group was discovered by Silvestri in 1913 and the issue was referred to as the “Zoraptera problem” (cf. Beutel and Weide, 2005). The Paraneoptera (= Acercaria + Zoraptera) concept was widely accepted for a long time (e.g., Hennig, 1969; Kristensen, 1975; Beutel and Weide, 2005), but recently, morphological and molecular evidence has increasingly suggested a placement in monophyletic Polyneoptera (Yoshizawa and Johnson, 2005; Yoshizawa, 2007, 2011; Ishiwata et al., 2011; Mashimo et al., 2011, 2014a; Misof et al., 2014; Wipfler and Pass, 2014; Matsumura et al., 2015; Wipfler et al., 2015). However, the affinity of Zoraptera within this large lineage remains unresolved. A basal placement in Polyneoptera with Dermaptera as a sister taxon in a transcriptomic study by Misof et al. (2014) was insufficiently supported. Presently, the “Zoraptera problem” remains as one of the last unsolved enigmas in insect phylogenetics.

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In the last ten years, investigation of the morphology of Zoraptera has remarkably accelerated (head structure: Beutel and Weide, 2005; Wipfler and Pass, 2014; Matsumura et al., 2015; thorax and wing base structure: Yoshizawa, 2007, 2011; Friedrich and Beutel, 2008; postabdomen: Hünefeld, 2007; reproductive systems: Dallai et al., 2011, 2012a, 2012b, 2014a, 2014b, 2015; Matsumura et al., 2014). Embryological studies of the order have also been reported, including the egg structure (Mashimo et al., 2011) and the embryonic (Mashimo et al., 2014a) and post-embryonic development (Mashimo et al., 2014b). However, these studies were largely restricted to one species, Zorotypus caudelli Karny, 1927. Obviously, information on a broader spectrum of species is required for a reliable interpretation with respect to the supra- and subordinal relationships of the order.

Zoraptera is a very small group containing only 40 extant and nine fossil species (Yin et al., 2015). Kukalova-Peck and Peck (1993) proposed a multi-generic classification with six genera based on wing venation, and Chao and Chen (2000) suggested a new genus, Formosozorosoros, based on its long basal tarsomerese and elongated cerci lacking a terminal spine. The generic characteristics of the wing venation proposed by Kukalova-Peck and Peck (1993) are not stable enough to justify the proposed subdivision of Zoraptera (cf. Engel and Grimaldi, 2000). Likewise, the status of Formosozorosoros as a separate genus lacks a sufficient phylogenetic basis, that is, lacking evidence that the single species is not nested within Zorotypus. The elongation of the cerci is shared with Zorotypus longigeractus Caudell, 1927, while the long first tarsomere is unique within Zoraptera and probably just an autapomorphy. Consequently, Engel and Grimaldi (2000) argued that “Until a system can be developed and taxa diagnosed on a variety of character systems… it is most prudent not to present a new, formal classification”, and synonymized all genera proposed by Kukalová-Peck and Peck (1993) and Chao and Chen (2000) under the genus Zorotypus Silvestri. Aiming at an intraordinal classification, morphological surveys for taxonomically useful characters are clearly required. The reproductive system of zorapteran species, which has recently been revealed to show striking variability in spite of the uniformity of the general morphology, is likely to be of taxonomic and phylogenetic value (Dallai et al., 2014a, 2014b, 2015). This includes the egg structure, which was recently examined in one species of Zoraptera (Mashimo et al., 2011). Features of the eggs have already turned out to be useful characters in the polynopteran orders Phasmatoidea and Orthoptera (so-called “ototaxonomy”: e.g., Hinton, 1981; Mazzini, 1987; Mazzini et al., 1993; Scali and Milani, 2009).

In the present study, we observe and describe the egg structures of three zorapteran species, which were already used by Dallai et al. (2012b, 2014a, 2014b) for detailed investigations of the reproductive system and spermatozoa, namely, Zorotypus magnicaudelli Mashimo et al., 2013, Zorotypus impolitus Mashimo et al., 2013 and Zorotypus hubbardi Caudell, 1918. The documented characters of the egg are discussed with respect to the systematic placement of the order, but mainly concerning their potential for an intraordinal classification, and also in the context of other features of the reproductive system investigated in previous studies (Dallai et al., 2012b, 2014a, 2014b).

2. Materials and methods

2.1. Materials

Adults and nymphs of Z. magnicaudelli and Z. impolitus were collected under the bark of decaying logs in Ulu Gombak, Selangor, Peninsular Malaysia, in the spring of 2011. Adults and nymphs of Z. hubbardi were laboratory-reared from specimens originally collected in wooded areas in and around Gainesville, Florida, USA, in the spring of 2011. The insects were kept in small containers with a moist soil bottom at 24 °C and fed on dry yeast and powdered dried Bombyx pupae (commercially sold fishing bait).

2.2. Fixation

Thirty eggs of each species were cleaned with an ultrasonic cleaner, soaked in Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde 0.1 M HCl-sodium cacodylate buffer solution, pH 7.2 [SCB]) for 5–6 min, punctured with a fine needle and further fixed with the same mixture at 4 °C for 24 h. Fixed eggs were stored in SCB at 4 °C.

2.3. Light microscopy (LM)

To observe the micropyles in detail, the egg membranes were mounted in a polyvinyl-lactophenol medium (Heinz liquid), examined under a Nikon Optiphot-2 biological microscope equipped with Nikon apochromatic lenses and photographed using a Nikon DS-Fi2 camera. Light micrographs taken at different focus levels were processed using the image stacking software Combine ZP (Hadley, 2010) to produce a single image with all sections of the specimen in focus.

For observation of the micropylar canals, four fixed eggs of each species were dehydrated in a graded ethanol series and embedded in Külzer Technovit 7100 methacrylate resin, according to Machida et al. (1994a, 1994b). Semithin sections (2 μm) were cut with a Meiva Superhard Knife (tungsten carbide steel knife) set on a Bio-Rad H-1500 microtome. Stained with 0.1% Delafield's hematoxylin for 12 h, 0.5% eosin G for 1 h and 0.5% fast green FCF 100% ethanol solution for 1 min, and observed under a Nikon Optiphot-2 biological microscope.

2.4. Electron microscopy (SEM and TEM)

For scanning electron microscopy (SEM) observation, five eggs of each species were post-fixed with 1% osmium tetroxide for 1 h, dehydrated in a graded ethanol series and dried with a tosimplis Samdri-PVT-3D critical point dryer. The specimens were coated with gold using a JEOL JFC-1100 ion sputter coater and observed under a TOPCON SM-300 scanning electron microscope at an accelerating voltage of 15 kV.

For transmission electron microscopy (TEM) observation, three eggs of each species were post-fixed with 1% osmium tetroxide for 1 h, dehydrated in a graded acetone series, and embedded in Nissin EM Quetol 651 or Agar Scientific “Agar Low Viscosity Resin” kit. Ultrathin sections (100 nm) were cut with a diamond knife set on an RMC MT-XL ultramicrotome, and then observed with a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 80 kV.

3. Results

Following Mashimo et al. (2014a), we designate the orientation of eggs as follows: the side of the egg facing the substrate is dorsal, the opposite side with the micropyles is ventral, the slightly narrowed end is anterior and the slightly broadened end is posterior.

3.1. Z. magnicaudelli

Eggs of Z. magnicaudelli are pale to yellowish in color (Fig. 1A and B) and elliptic with a length of about 800 μm and a width of about 400 μm (Fig. 1A–D). The entire surface of the eggs shows a honeycomb pattern formed by exochorionic ridges (Fig. 1C and D). Each compartment of this pattern has a diameter of about 50 μm, with
Fig. 1. Eggs of Zorotypus magnicaudelli (A–H) and Zorotypus caudelli (I). (A) Egg of Z. magnicaudelli, ventral view, LM, anterior to the top. (B) Egg of Z. magnicaudelli, lateral view, LM, anterior to the top, ventral to the left. (C) Egg of Z. magnicaudelli, ventral view, SEM, anterior to the top. (D) Egg of Z. magnicaudelli, lateral view, SEM, anterior to the top, ventral to the left. (E) Enlargement of ventral egg surface of Z. magnicaudelli, SEM. (F) Micropylar compartment of the egg of Z. magnicaudelli, LM. (G) Flap structure covering the inner opening of the micropylar canal of Z. magnicaudelli, SEM. (H) Fractured egg membrane of Z. magnicaudelli, SEM. (I) Fractured egg membrane of Z. caudelli, SEM. Arrows and arrowheads show a fringe structure and small compartments with the micropyle, respectively. ap: aeropyle, ench: endochorion, exch: exochorion, f: flap, mp: micropyle, mpc: micropylar canal.
Fig. 2. Eggs of Zorotypus hubbardi. (A) Egg, ventral view, anterior to the top, LM. (B) Egg, lateral view, anterior to the top, ventral to the left, LM. (C) Egg, ventral view, anterior to the top, SEM. (D) Egg, lateral view, anterior to the top, ventral to the left, SEM. (E) Enlargement of ventral egg surface, SEM. (F) Fractured egg membrane, SEM. (G) Cross section of egg membrane showing the micropyle, LM. (H) Micropylar compartment, SEM. (I) Micropylar compartment, LM. (J) Flap structure covering the inner opening of the micropylar canal, SEM. Arrows and arrowheads show a fringe and the small compartments with the micropyle, respectively. ap: aeropyle, ench: endochorion, exch: exochorion, f: flap, mp: micropyle, mpc: micropylar canal.
50–60 aeropyles of 1 μm in diameter (Fig. 1E and F). An ill-developed fringe structure formed by an extrinsic substance secreted in the course of oviposition encircles the ventralateral egg surface (Fig. 1C and D). A pair of small compartments of about 15 μm in diameter is found at the equator of the ventral egg surface (Fig. 1A–D, F). Each of them contains about 30 aeropyles and a single micropyle of 2 μm in diameter (Fig. 1F). From each micropyle, a thin microplar canal runs laterally through the chorion. The inner opening of the microplar canal involves a chorionic flap (Fig. 1G). The egg membrane is composed of a two-layered chorion (exochorion and endochorion) and an extremely thin vitelline membrane (Figs. 1H and 4A). The exochorion is about 10 μm thick, penetrated by numerous branched canals (aeropyles) (Fig. 1H). The endochorion is about 1 μm thick with numerous small columnar projections on its outer surface (Figs. 1H and 4A). The vitelline membrane is an extremely thin layer, less than 0.1 μm thick, adhering to the endochorion (Fig. 4A) or to the surface of the serosal cuticle in eggs containing older embryos (Fig. 4B–D).

The egg structure of Z. magnicaudelli mentioned is very similar to that of Z. caudelli (Fig. 1I) (Mashimo et al., 2011), with only minor differences concerning size (long and short diameters of about 0.6 mm and 0.3 mm) and thickness of the exochorion (about 5 μm thick).

3.2. Z. hubbardi

Color very similar to that of eggs of Z. magnicaudelli (Fig. 2A and B). Shape also elliptic, with a length of about 650 μm and a width of about 300 μm (Fig. 2A–D). The honeycomb pattern is less distinct on the dorsal side (Fig. 2C and D). Fifty to 60 aeropyles of about 1 μm in diameter are present in each compartment (Fig. 2E). A pair of small compartments is present at the equator of the ventral egg surface, each of which contains a large micropyle with a diameter of about 6 μm (Fig. 2A–D, H and I). From each micropyle, a thick microplar canal runs laterally through the chorion (Fig. 2G). The inner opening of the microplar canal involves a chorionic flap (Fig. 2G and J). Among 30 eggs observed, one examined egg was found to have five small microplar compartments. Like in Z. caudelli, eggs with more than one pair of micropylar compartments were rarely reported (Mashimo et al., 2011). The egg membrane is composed of a two-layered chorion (exochorion and endochorion) and an extremely thin vitelline membrane (Figs. 2F, G and 4B). The exochorion is 6–7 μm thick, penetrated with numerous branched canals (aeropyles) (Figs. 2F and 4B). The endochorion is about 0.25 μm thick with numerous, small columnar projections on its outer surface (Figs. 2F and 4B). The numerous, small columnar projections on the outer surface of the endochorion in egg shells of Z. magnicaudelli, Z. caudelli and Z. hubbardi comprise the trabecular network important in conveying air to the developing embryo. The vitelline membrane is an extremely thin layer, less than 0.1 μm thick, adhering to the endochorion (Fig. 4B). Around the microplar canal, the boundary between the exochorion and endochorion is obscure (Fig. 2G).

3.3. Z. impolitus

Color and shape similar to those in Z. magnicaudelli and Z. hubbardi, size like that in the latter (Fig. 3A–D). In contrast to the other species considered here, the egg surface shows a remarkable, regional difference in the sculpture of polygonal compartments (Fig. 3C–E). A fringe structure encircles the ventralateral egg surface (Fig. 3C). The compartments on the dorsal side are similar to those of the other species (Fig. 3D and E), simple polygons with several hundred aeropyles of less than 1 μm in diameter evenly scattered (Fig. 3E), sometimes with a projection formed by extrinsic material at their center (white arrowheads in Fig. 3D). In contrast, the compartments on the ventral side have a specific pattern resembling hieroglyphs, with about 200 aeropyles of less than 1 μm in diameter (Fig. 3C, E and F). A pair of small compartments of about 15 μm in diameter is present at the equator of the ventral egg surface (Fig. 3A–D, H and I). A single large micropyle of about 8 μm in diameter in each compartment is usually plugged by secretion (Fig. 3G–I), as demonstrated as a brown spot in the LM images (Fig. 4A and B). From each micropyle, a thick microplar canal runs laterally through the chorion (Fig. 3G). The inner opening of the microplar canal involves a chorionic flap (Fig. 3G and J). The egg membrane is composed of a mono-layered chorion of about 5 μm thickness and an extremely thin vitelline membrane of less than 0.1 μm thickness (Fig. 4C and D): the “exochorion” and “endochorion” are indistinguishable with respect to structure and stainability (Figs. 3F and 4C, D). Numerous branching aeropyles perforate the chorion (Figs. 3F and 4C, D).

4. Discussion

4.1. Egg structures, reproductive systems and their diversity in Zoraptera

Mashimo et al. (2011) examined the egg structure of Z. caudelli in detail and compared the observed features with characters found in previous studies on the eggs of other zorapteran species, namely, Zorotypus barbieri Gurney, 1938, Zorotypus brasiliensis Silvestri, 1946, Zorotypus gurneyi Choe, 1989, and Zorotypus neotropicus Silvestri, 1916 (cf. Silvestri, 1946; Choe, 1989). The egg structure of Zoraptera was characterized as follows: 1) a pair of micropyles is present at the equator of the ventral side, 2) a honeycomb pattern is developed on the surface, 3) the chorion is two-layered, 4) with microplar canals running laterally, 5) a chorionic flap covers the inner opening of the microplar canal and 6) a region specialized for hatching is lacking (e.g., hatching line, operculum). These features were confirmed for the eggs of Z. magnicaudelli and Z. hubbardi. Even though these characters are largely preserved in Z. impolitus, this species also displays some unique characteristics: 1) the chorion does not show a two-layered construction, the “exochorion” and “endochorion” being indistinguishable from each other; 2) the egg surface shows a remarkable, regional difference in the sculpture of polygonal compartments; and 3) the micropyle is about 8 μm in diameter, four times larger than in the case of Z. caudelli and Z. magnicaudelli. This is apparently correlated with the size of the spermatozoa of Z. impolitus, which is the largest known in Hexapoda, 3 mm long and 3 μm wide, with a volume of ca. 21,000 μm³ (Dallai et al., 2014a). In addition to the sperm gigantism, Dallai et al. (2013, 2014a) revealed several peculiar features related to reproduction in Z. impolitus: 1) very low ratio axoneme diameter/width of flagellum (1:10–13, cf. 1:2–3 in most groups of insects), 2) a greatly enlarged spermatheca, 3) unique precopulatory behavior
Fig. 4. Egg membranes of three zorapteran species, TEM. (A) Egg membrane of *Zorotypus magnicaudelli*. (B) Egg membrane of *Zorotypus hubbardi*. (C) Egg membrane of the dorsal side of *Zorotypus impolitus*. (D) Egg membrane of the ventral side of *Zorotypus impolitus*. Arrowheads show the vitelline membranes. ap: aeropyle, ch: chorion, ench: endochorion, exch: exochorion, sec: serosal cuticle.
and 4) a unique mode of secondary external sperm transfer. Like the above-mentioned reproductive features, the unique characteristics of the egg structure of this species apparently represent a derived condition within Zoraptera. Dallai et al. (2014a, 2015) pointed out marked similarities between Z. hubbardi and Z. impolitus, including giant spermatheca and very large spermatheca, interpreting these features as potential synapomorphies. In this context, it is noteworthy that Z. hubbardi, which has largely preserved a conventional egg structure, has also extraordinarily large micropylies similar to those of Z. impolitus.

As has been pointed out by Choe (1989), Mashimo et al. (2013) and Dallai et al. (2014b), information on reproductive organs could be crucial for a phylogenetically sound subordinal classification of Zoraptera. Table 1 summarizes several characters related to reproduction as well as the egg features in the zorapteran species in which the information on the egg structures is available. As shown there (Remarks), three groups can be distinguished. Group A is characterized by a chorion with a uniform hexagonal surface pattern, a basal plate formed by a posteriorly bifurcated ventral sclerite of the male genital apparatus and a slender spermathecal duct; small micropylies and spermatzoa of moderate length may be enumerated as the features of group A. This group can be further divided into two subgroups by the presence (A-1: Z. caudelli, Z. magnicaudelli and Z. gurneyi) or absence (A-2: Z. barberi) of a coiled intromittent organ. Group B, which is only represented by Z. hubbardi, is characterized by a chorion with a uniform hexagonal surface pattern, large micropylies, large spermatheca, long spermatzoa, a robust spermathecal duct, and the lack of a basal plate and coiled intromittent organ. Group C represented only by Z. impolitus is characterized by a chorion with a hexagonal pattern regionally differing in sculpture, large micropylies, long spermatzoa, huge spermatheca, a robust spermathecal duct, and the absence of a basal plate and coiled intromittent organ.

Despite the potential value of characters related to the reproductive system, a reliable phylogenetic interpretation is presently greatly impeded by the lack of data for about half of the species, and additionally the difficulty in identifying suitable outgroup taxa. This situation makes the crucial distinction between plesiomorphic and apomorphic features and the reconstruction of an ordinal groundplan difficult, if not impossible. Important issues are very unclear, especially whether the basal plate and the coiled intromittent organ are ancestral for the order. It appears plausible that a chorion with a uniform hexagonal surface pattern, a slender spermathecal duct, small micropylies and spermatzoa of moderate size are plesiomorphies, but this needs confirmation by a formal character evaluation. The enlargements of the spermatzoa, micropylies and spermatheca are very likely a complex of correlated apomorphic features phylogenetically linking Z. hubbardi and Z. impolitus and possibly other species with presently unknown reproductive features. A unilayered chorion with a regionally differentiated surface pattern is a derived condition presently only known in Z. impolitus.

It is apparent that detailed information on the reproductive system of more zorapteran species is required for a reliable interpretation of the character evolution and the intraordinal relationships. A robust species-level phylogeny based on a suitable molecular data set would also be very helpful for reconstructing the evolutionary pathways of the character system, and both combined will likely lead to a solid phylogeny-based classification of this “enigmatic” order.

4.2. Affinity of Zoraptera within Polyneoptera

The phylogenetic affinity of Zoraptera to either Polyneoptera or Acercaria has been controversial for over half a century (Hennig, 1969; Kristensen, 1975; Boudreaux, 1979; Minet and Bourgoin, 1986; Beutel and Gorb, 2001; cf. Mashimo et al., 2014c). A placement within Polyneoptera has gained strong support recently via studies based on morphological (Yoshizawa, 2007, 2011; Dallai et al., 2011, 2012b; Wipfner and Pass, 2014; Matsumura et al., 2015; Wipfner et al., 2015), embryological (Mashimo et al., 2011, 2014a) and molecular data (Yoshizawa and Johnson, 2005; Ishiwata et al., 2011; Misof et al., 2014).

In the work of Mashimo et al. (2011), the egg structure of Zoraptera was compared with that of sister group candidates, Acercaria, Dermaptera, Dictyoptera and Embioptera. Close affinity of Zoraptera and Eukinolabia (= Embioptera + Phasmatodea) was suggested, based on a peculiar feature, “a pair of micropylies”, as a potential synapomorphy. Subsequently, Shimizu (2013) and Fujita and Machida (2015) re-evaluated the egg structures of Dermaptera and Dictyoptera, respectively. Shimizu (2013), who conducted a comprehensive comparative embryological study of Dermaptera s. str. (Forficulina) (covering all families except for Karschiellidae), concluded that circularly arranged openings around the anterior pole of the egg are micropylies, as described by Heymons (1895), rejecting the interpretation by Chauvin et al. (1991) who interpreted a single opening at the anterior top of the egg as the micropyle. Shimizu’s (2013) re-evaluation of the dermapteran micropylies confirms the profoundly different condition in both orders (Mashimo et al., 2011), as the zorapteran micropylies do not show a circular arrangement around the anterior pole of the egg. Reviewing the egg structure of Dictyoptera, Fujita and Machida (2015) described their micropylies as clustering on the ventral side of the egg, in contrast to Mashimo et al. (2011), who assumed a circular arrangement around the pole of the egg. Like in the previous case, this does not affect the statement of Mashimo et al. (2011) that the eggs of Zoraptera and Dictyoptera differ distinctly and do not share potential synapomorphies.

A pair of micropylies on the ventral side, a potential groundplan apomorphy of Zoraptera + Eukinolabia (Mashimo et al., 2011), was found in all three species examined in the present study. Although both Eukinolabia and Mystroptera (= Zoraptera + Embioptera) were suggested based on morphological and ecological evidence (Minet and Bourgoin, 1986; Engel and Grimaldi, 2000, 2002; Yoshizawa, 2007, 2011; Dallai et al., 2011, 2012b; Mashimo et al., 2011, 2014a; Wipfner et al., 2011; Friedemann et al., 2012), only the former has support from molecular phylogenetics (e.g., Ishiwata et al., 2011; Misof et al., 2014). In a recent phylogenetic analysis based on mitochondrial genomes, Ma et al. (2014) suggested an assemblage of Phasmatodea + Mystroptera. However, both Zoraptera and Embioptera exhibited very long branches. Apparently, more extensive taxon sampling is required to avoid artifacts caused by this phenomenon. Moreover, the reliability of mitochondrial genomes in high-level (interordinal) phylogenetic analyses has been questioned (e.g., Cameron et al., 2004).

As the case stands, a close relationship between Zoraptera, Embioptera and Phasmatodea is one of the most likely working hypotheses concerning the “Zoraptera problem”. A clade of Zoraptera + Dermaptera remains an alternative option, albeit not supported by structural features of the reproductive system (including eggs) or other morphological characters. This concept was first proposed by Terry and Whiting (2005) in a total evidence analysis with morphological and molecular data. However, an 18S rRNA sequence was erroneously assigned to Zoraptera as a result of contamination, as shown by a BLAST analysis conducted later (Yoshizawa, 2010). The clade of Zoraptera + Dermaptera was revived in the first contribution from an extensive transcriptomic project (1KITE: http://www.1KITE.org/). However, this arrangement was only moderately supported in an analysis of 1478 orthologous
### Table 1
Comparison of available information on egg structure and reproductive systems.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Surface structure</th>
<th>Chorionic layer</th>
<th>Micropylar size</th>
<th>Basal plate</th>
<th>Coiled intromittent organ</th>
<th>Sperm size</th>
<th>Spermathecal size</th>
<th>Spermathecal duct</th>
<th>Remarks</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Z. caudelli</em></td>
<td>uniform hexagonal pattern</td>
<td>two-layered</td>
<td>2 μm</td>
<td>present</td>
<td>present</td>
<td>ca. 800 μm × 0.7 μm</td>
<td>small (100 μm in diameter)</td>
<td>2 mm × 2–20 μm</td>
<td>group A-1</td>
<td>Indonesia, Malay Peninsula</td>
</tr>
<tr>
<td><em>Z. magnicaudelli</em></td>
<td>uniform hexagonal pattern</td>
<td>two-layered</td>
<td>2 μm</td>
<td>present</td>
<td>present</td>
<td>moderate length, ca. 0.8 μm in thickness</td>
<td>small (100–120 × 60 μm)</td>
<td>group A-1</td>
<td>Malay Peninsula</td>
<td></td>
</tr>
<tr>
<td><em>Z. gurneyi</em></td>
<td>uniform hexagonal pattern</td>
<td>—</td>
<td>small?</td>
<td>present</td>
<td>present</td>
<td>—</td>
<td>small (not exactly measured)</td>
<td>slender, long</td>
<td>group A-1</td>
<td>Panama, Costa Rica</td>
</tr>
<tr>
<td><em>Z. snyderi</em></td>
<td>uniform hexagonal pattern</td>
<td>—</td>
<td>—</td>
<td>present</td>
<td>present</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>group A-1</td>
<td>Jamaica, United States (Florida)</td>
</tr>
<tr>
<td><em>Z. barberi</em></td>
<td>uniform hexagonal pattern</td>
<td>—</td>
<td>small?</td>
<td>present</td>
<td>absent</td>
<td>—</td>
<td>small (not exactly measured)</td>
<td>slender, long</td>
<td>group A-2</td>
<td>Costa Rica, Dominican Republic, Panama, Trinidad, Venezuela, French Guiana</td>
</tr>
<tr>
<td><em>Z. hubbardii</em></td>
<td>uniform hexagonal pattern</td>
<td>two-layered</td>
<td>8 μm</td>
<td>absent</td>
<td>absent</td>
<td>3 mm × 2 μm</td>
<td>large (400 × 150–400 μm)</td>
<td>500 μm × 20–50 μm</td>
<td>group B</td>
<td>Southcentral-southeastern United States</td>
</tr>
<tr>
<td><em>Z. impolitus</em></td>
<td>hexagonal pattern</td>
<td>regionally different in sculpture</td>
<td>8 μm</td>
<td>absent</td>
<td>absent</td>
<td>3 mm × 3 μm</td>
<td>large (1 mm × 70–150 μm)</td>
<td>1.4 mm × 20–60 μm</td>
<td>group C</td>
<td>Malay Peninsula</td>
</tr>
<tr>
<td><em>Z. brasiliensis</em></td>
<td>uniform hexagonal pattern</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>?</td>
<td>Brazil</td>
</tr>
<tr>
<td><em>Z. neotropicus</em></td>
<td>uniform hexagonal pattern</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>?</td>
<td>Brazil</td>
<td></td>
</tr>
</tbody>
</table>

Groundplan features of egg structure are excluded.

* a Mashimo et al., 2011, 2013; Dallai et al., 2011, 2012a.
  b Mashimo et al., 2013.
  c Choe, 1989.
  d Caudell, 1920.
  e Gurney, 1938; Choe, 1989.
  f Gurney, 1938; Dallai et al., 2012b.
  g Mashimo et al., 2013; Dallai et al., 2014a.
  h Silvestri, 1946.
  i Silvestri, 1946.
  j Posteriorly bifurcated sclerite on the ventral side in male genitalia.
genes (Misof et al., 2014), and should be considered as preliminary. Analyses of multiple morphological character systems, additional embryological data and transcriptome analyses with extended taxon sampling (1KITE) will likely lead toward a solution to the Zoraptera problem in the foreseeable future.

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