Karyotype, reproductive organs, and pattern of gametogenesis in *Zorotypus hubbardi* Caudell (Insecta: Zoraptera, Zorotypidae), with discussion on relationships of the order

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Abstract: For the first time, the karyotype is described in a representative of the order Zoraptera. *Zorotypus hubbardi* Caudell (Zorotypidae) have holokinetic chromosomes and male karotype of $2n = 38$ (36 + neo-XY). Males possess two follicles in each testis and females have six panoistic ovarioles in each ovary. Oogenesis and, more closely, spermatogenesis, including meiosis and sperm formation, have been studied. Based on the presence of panoistic ovaries and holokinetic chromosomes, Crampton's hypothesis that Zoraptera represent a group of Polyneoptera nearest to the origin of Paraneoptera is considered the most plausible.

Résumé : On trouvera ici la description inédite du caryotype d'un zoraptère. *Zorotypus hubbardi* Caudell (Zorotypidae) a un caryotype de $2n = 38$ (36 + néo-XY) chez les mâles et des chromosomes holocinétiques. Les mâles comptent deux follicules dans chaque testicule et les femelles, six ovarioles panoïstiques dans chaque ovaire. L'ovogenèse, et plus particulièrement, la spermatogenèse avec méiose et formation des spermatozoïdes, ont été étudiées. La présence d'ovaires panoïstiques et de chromosomes holocinétiques permet de croire que l'hypothèse de Crampton, qui veut que les zoraptères représentent le groupe de polynéoptères qui se rapproche le plus du point d'origine des paranéoptères, est la plus plausible.

[Traduit par la Rédaction]

Introduction

Zoraptera are minute (less than 3 mm long) insects of cryptic habits living under bark, in humus, near termite nests, etc. Zorapterans are gregarious, but no evidence of true sociality has been found. Facultatively, they may reproduce by thelytokous parthenogenesis (Choe 1992). Thirty-two living and two fossil species are known, most of them inhabiting tropical or subtropical environments on nearly all continents. All zorapterans are allocated to a single genus, *Zorotypus* Silvestri, 1913, constituting the family Zorotypidae; seven additional monobasic genera were synonymized by Engel and Grimaldi (2000).

The morphology of zorapterans is characterized by numerous reductions, leading to major controversies over their relationships (most often this order was affiliated either to one of the polyneopteran groups or to Psocoptera; see Discussion). The results of molecular and total-evidence cladistic analyses are likewise conflicting (Wheeler 1998; Carpenter and Wheeler 1999; Wheeler et al. 2001). Cytogenetic data (karyotype, chromosome structure, meiosis) are useful in determining the taxonomy and phylogeny of some insect groups (Blackman 1980; Ueshima and Ashlock 1980; Kuznetsova 1986; Petitpierre 1997; Maryańska-Nadachowska et al. 2001). Zoraptera represent the only pterygote order for which no data on chromosome systems have been reported.

Remarkably little is known about the internal reproductive organs of zorapterans. *Zorotypus hubbardi* Caudell has been shown to display panoistic ovaries in females (Gurney 1938). The ovary pattern is reputed to provide a good basis for phylogenetic consideration (Štys and Bilinski 1990); however, with rare exceptions (Büning 1994, 1996), it has never been discussed when evaluating the phylogenetic relationship of Zoraptera.

Here karyotype and pattern of gametogenesis, mainly of male *Z. hubbardi*, are described for the first time. Data on the internal male and female reproductive systems are also presented, these being more comprehensive and differing in part from those reported for this species by Gurney (1938).

Material and methods

Collections of *Z. hubbardi* were made in August 1998 and January 2000 in U.S.A. (sawdust pile from MacLeansboro, Hamilton County, southern Illinois) by Dr. Roman Rakitov. Live specimens were preserved in 3:1 ethanol : glacial acetic acid. Both collections represented a clear female-biased sex ratio, with no more than 1 male per 10 females.

In our study of anatomy, the insect abdomen was opened under a stereomicroscope, and testis and ovary structure was examined. Then, the gonads were removed from the abdo-

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men, placed in a drop of 45% acetic acid on a slide, covered by a cover slip, and examined using phase-contrast microscopy.

For chromosomal study, extracted gonads were gently squashed on a microscope slide. The cover slips were removed by a dry-ice method (Conger and Fairchild 1953) and slides were air-dried. Slides were stained with the Feulgen–Giemsa procedure as described by Grozeva and Nokkala (1996). C-band staining for revealing constitutive heterochromatin was performed according to Sumner's (1972) protocol. Fluorochrome chromomycin A_3 (CMA_3) staining for revealing GC base pairs of DNA was carried out according to Schweizer (1976). Chromosome spreads were analyzed using a BX 50 light microscope with a OM-4 camera; CMA_3-labelled slides were studied using the fluorescent microscope Dialux 22 (Leitz, Wetzlar, Germany).

Results

Morphology of the male and female internal reproductive systems

The internal reproductive system of females includes paired ovaries. The number of ovarioles varies between ovaries of the same female from four (two cases) to eight (one case), but ovaries containing six ovarioles each clearly prevail (Fig. 1A). The ovarioles are elongated tubes, and their basal attachments to the lateral oviducts are independent of each other, resulting in a "comb-shaped" structure. The lateral oviducts are slender and rather long, their distal ends, to which the ovarioles are attached, being much enlarged and probably representing a common pedicel of the ovarioles. The lateral oviducts are united proximally to form a common median oviduct. An oval spermatheca joins the posterior end of the oviduct by a fairly long spermathecal duct. Each ovary is attached distally to the inner body wall by a thin terminal filament of 15–20 cells arranged linearly. The ovariole proper is differentiated into germarium and vitellarium, the latter each with 4–5 oocyte chambers in single file, each from apex to base in a more advanced stage of development. Each oocyte is surrounded by a layer of follicular cells, and there are no nurse cells in the ovarioles (Figs. 1B, 3A).

The internal reproductive system of males includes paired testes each with two small, drop-shaped follicles (testis tubes) with a short stalk, or vas efferens (Fig. 2). Sister stalks are drawn proximally closer to each other and empty into a large, round, flat seminal vesicle, representing a strongly expanded part of the long tubular vas deferens. Bundles of spermatozoa occur within the vesicles and a pair of large accessory glands opens where the lateral vasa deferentia enter the common ejaculatory duct. The latter lacks a bulbus ejaculatorius.

Patterns of oogenesis and spermatogenesis

In ovarioles, oocytes at consecutive stages of meiotic prophase up to the diffuse stage were found. The apex of an ovariole with approximately 10 filament cells and an oocyte in pachytene is shown in Fig. 3A. During the diffuse stage, the oocyte increases in size many times. Mature females have a few developing oocytes in each ovariole and one large chorionated egg, which is discharged from the ovariole and is easily seen through the abdominal wall. One egg seems to mature at a time. The mature egg is full of yolk, the particles of which obscure the small nucleus, so no meiotic figures could be observed.

Within the testicular follicles, male gonial cells proliferate synchronously within large cysts. Cysts contain eight primary spermatocytes, indicating that there are three spermatogonial divisions after enclosure of the definitive spermatogonium (Fig. 3B). Cells in pachytene, and particularly in the diffuse stage, are most frequently found; thus, these two stages must be longest during meiotic prophase (Figs. 3C–3F). In early pachytene, the bivalents show a bouquet orientation, whereas in late pachytene they shorten and become more condensed. No heteropycnotic bodies were found during pachytene in primary spermatocytes.

In pachytene, there is one peculiar bivalent composed of two distinct parts (arrowheads in Fig. 3E). The thicker part consists of two homologous chromosomes, whereas the thinner part seems to be represented by a single chromosome. Such bivalent structure probably indicates the presence of a neo-XY system of sex determination. The diffuse stage, following just after pachytene, is easily recognizable by the presence of a comparatively large nucleus, fully dispersed autosomal chromatin, and the presence of 1–3 heteropycnotic bodies (Fig. 3D). After C-banding, a single large nucleolus becomes evident in the C-banded diffuse stage (Fig. 3F), whereas no C-positive blocks have been detected at any meiotic stages examined. Two brightly fluorescent dots observed in CMA_3-labelled interphase spermatocytes probably represent nucleolar organizing regions in a pair of the autosomes (Fig. 3G).

Chromosomes in diakinesis, metaphase I (MI), and anaphase I (AI) were observed on rare occasions allowing for the determination of the karyotype pattern of the species (Figs. 4A–4D). This issue is discussed below.

An early spermatid shown in Fig. 4E represents a nucleus–Nebenkern complex that includes a nucleus with chromatin and the spherical unstained Nebenkern (NK), which is known to consist of the coalesced mitochondria of the spermatid (Roosen-Runge 1977).

Sperm derived from the first definitive spermatogonium of each cyst keep together and form a sperm bundle. Each cyst contains 32 spermatids (Fig. 4F).

Karyotype

Diakinetic and MI spermatocytes show a total of 19 bivalents, whereas AI spermatocytes show 38 chromosomes, suggesting the presence of 18 autosomal pairs and that of XY sex chromosomes in the karyotype (Figs. 4A–4D). The bivalents, except for the three largest ones, demonstrate an even gradation in size. All bivalents are chiasmatic. Every examined chromosome plate included three ring bivalents, probably with two chiasmata each, and 16 dumbbell-shaped bivalents with one chiasma each. The sex-chromosome bivalent could not be distinguished from autosomal bivalents in MI and AI, at which stages bivalents are condensed and homomorphic. All chromosomes undergo reduction in AI (Fig. 4D). Chromosomes lack primary constrictions at any meiotic stages examined.

Fig. 1. Photomicrographs of squash preparations of the ovarioles of *Zorotypus hubbardi* Caudell. (A) Ovary with six ovarioles (each marked with a dot), pedicels (small arrowhead), and lateral oviduct (large arrowhead). (B) Two ovarioles with terminal oocyte chambers of different size. Scale bars = 10 μm.

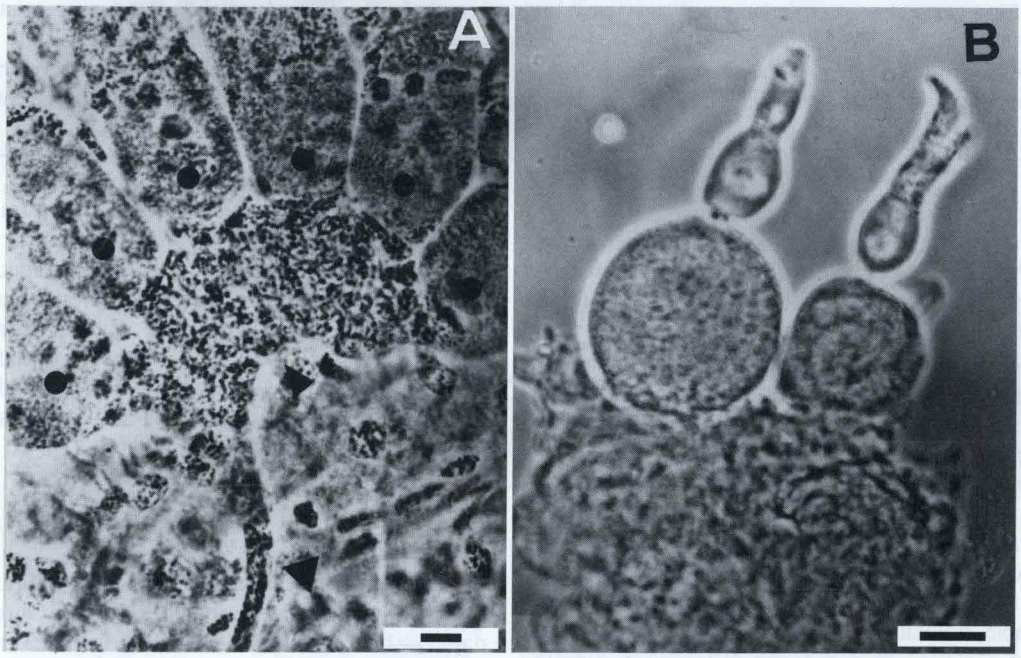
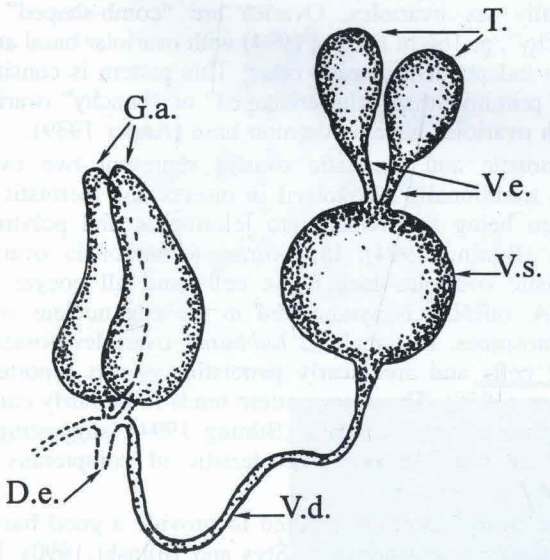


Fig. 2. Schematic representation of the male reproductive system of *Z. hubbardi* Caudell (dorsal view, left testis omitted). T, testis with two follicles; V.e., vas efferens; V.s., vesicula seminalis; V.d., vas deferens; G.a., glandula accessorius; D.e., ductus ejaculatorius.



Discussion

Chromosome number and sex-chromosome system

Male *Z. hubbardi* display a chromosome complement of $2n = 38$, a relatively high value when Insecta is considered as a whole. In most insect species, diploid chromosome numbers vary between 12 and 40, although higher and lower numbers are not uncommon (White 1973). In Psocoptera, diploid chromosome numbers vary between 14 and 30 with a marked mode at 18, the number of autosomes in this modal karyotype being 16 (Golub 1999). Representatives of polyneopteran orders affiliated with Zoraptera often have higher chromosome numbers: Embiida, $2n = 20–24$ (modal autosome number 20); Grylloblattida, $2n = 30$ (28); Dermaptera, $2n = 8–60$ (22); Isoptera, $2n = 22–52$ (modal autosome number not yet determined); and Blattida, $2n = 16–80$ (with modal autosome number 36 as in *Z. hubbardi*) (White 1973; our estimates).

Male *Z. hubbardi* appear to have a XY sex-determining system. On the basis that X and Y chromosomes form a chiasmatic bivalent in meiosis, we believe that this system is of the neo-XY type and could result from one X-autosome fusion in the initial karyotype of $2n = 40$ (38 + X0). This is consistent with the fact that in insects the only chiasmatic sex-chromosome system known is neo-XY (Blackman 1995). The autosomally derived Y chromosome of *Z. hubbardi* is still homologous with the autosomal part of the neo-X that is evidenced by their synapsis in meiosis. This suggests a relatively recent origin for this sex-determining system in this species. Evolutionary reversions from X0 to neo-XY are known to have occurred independently in many insect groups (White 1973; Blackman 1995). They occur in single species or, rarely, in closely related species or, exceptionally, in tribes (Kuznetsova 1986) within groups having the X0 system.

Holokinetic chromosomes

No primary constrictions were detectable in spermatocyte I metaphases and anaphases. This suggests that chromosomes of *Z. hubbardi* are holokinetic. It has traditionally been suggested that localized or individualized centromeres are absent in holokinetic chromosomes, spindle microtubules

Fig. 3. Photomicrographs of different stages of female (A) and male (B–G) meiosis. (A) Ovariole with five to six filament cells (large arrowhead) and oocyte nucleus in pachytene (small arrowhead). (B) Cyst with eight primary spermatocyte nuclei in pachytene. Arrowheads point to three nuclei at the diffuse stage of another cyst. (C) Four primary spermatocytes in pachytene. (D) Primary spermatocyte nucleus in diffuse stage showing one large and two small heteropycnotic bodies (arrowhead). (E) Primary spermatocyte nucleus in pachytene with bivalent of neo-XY. The autosomally derived (synaptic) part is indicated by a large arrowhead and the original X-part (univalent) by a small arrowhead. (F) Primary spermatocyte nucleus in C-banded diffuse stage with nucleolus (large arrowhead) and heteropycnotic bodies (small arrowhead). (G) Fluorochrome CMA_3-labelled interphase nuclei each with two fluorescent dots. Scale bars = 10 μm.

being attached throughout the entire chromosome surface, whereas they are restricted to centromeres in monokinetic chromosomes. Wolf (1994) considered holokinetic chromosomes to possess relatively large centromeres covering a wide expanse of poleward chromosomal surface.

Holokinetic chromosomes are found in Odonata, Dermaptera, Lepidoptera, Trichoptera, and all orders of Paraneoptera (see Hughes-Schrader and Schrader 1961; White 1973; Kuznetsova 1979). Paraneoptera is the only major pterygote lineage with all representatives possessing holokinetic chromosomes, which indicates a common origin of this chromosome type for paraneopterans. Since it is clear that holokinetic chromosomes have arisen several times during pterygote evolution, they may differ in detail between members of the various groups. For example, holokinetic chromosomes of lepidopterans and trichopterans are now known to be different in kinetic structure from those in other taxa mentioned above (Wolf 1994).

Zoraptera could have acquired holokinetic chromosomes independently. Alternatively, such chromosomes may constitute a synapomorphy of Zoraptera and Paraneoptera or of Zoraptera and Dermaptera (direct relationship to Trichoptera + Lepidoptera or to Odonata seems hardly possible).

Spermatogonial meiosis and spermiogenesis

Modified and aberrant meiosis occurs in members of those insect groups having holokinetic chromosomes, such as Odonata (Oksala 1952); Heteroptera (Nokkala and Nokkala 1984); Homoptera, Coccoidea (Nur 1981); and Phthiraptera (Tombesi and Papeshi 1993). However, several taxa whose member species have holokinetic chromosomes show "normal" meiosis in all species examined. Examples are provided by Dermaptera (White 1976); Psocoptera (Golub 1999); and Homoptera, Auchenorrhyncha and Psylloidea (Halkka 1959; Kuznetsova et al. 1997). *Zorotypus hubbardi* shows the orthodox sequence of chromosome synapsis, chiasma formation, and segregation during male meiosis. Most bivalents have a single chiasma and only three bivalents have two chiasmata. Thus, males of this species exhibit Darlington's recombination index (RI = n + number of chiasmata per nucleus) of approximately 41. According to Halkka (1964), restriction of chiasma frequency to a maximum of two per bivalent is characteristic of holokinetic chromosomes.

After spermiogenesis, the spermatozoa of *Z. hubbardi* are arranged in bundles similar to those of most insects. In insects, the number of spermatozoa per bundle may vary between 16 (2^4) and 65536 (2^{16}) (Virkki 1973). Virkki (1973) concluded that members of higher orders are characterized by a lower number of spermatozoa per bundle than members of the more basal ones, and within an order or a lower taxon, the most advanced species have lower numbers than the more basal ones. In other words, the number of spermatozoa per bundle tends to be reduced during specialization. Data on *Z. hubbardi* support this conclusion because zorapterans are markedly specialized. The spermatozoa per bundle in male *Z. hubbardi* is 32 (2^5), suggesting that there are three mitoses in definitive spermatogonia followed by two meiotic divisions. Spermatocysts (as well as oocytes) are very large and few in number (approximately 15–20 in a male), so sperm production is probably low.

Reproductive system

The internal reproductive organs of male and female *Z. hubbardi* were described briefly by Gurney (1938). Our data confirm that males have two follicles in each testis and two accessory glands. Each follicle has a vas efferens. According to Gurney, the double vasa deferentia lead to a common seminal vesicle; however, we found that each vas deferens had a seminal vesicle. The ejaculatory bulb is absent. According to Gurney (1938), females have four to six ovarioles in each ovary, but according to our data there are typically six ovarioles. Ovaries are "comb-shaped" (not "bunchy", p. 166 in Büning 1994) with ovariolar basal attachments independent of each other. This pattern is considered more primitive than "cluster-shaped" or "bunchy" ovaries in which ovarioles have a common base (Ander 1939).

Panoistic and meroistic ovaries represent two ovarian types traditionally recognized in insects, the meroistic type in turn being subdivided into telotrophic and polytrophic types (Büning 1994). In contrast to meroistic ovarioles, panoistic ovarioles lack nurse cells and all oocyte RNA (rRNA, mRNA) is synthesized in the egg nucleus on the chromosomes. In female *Z. hubbardi*, ovarioles possess no nurse cells and are clearly panoistic, as was reported by Gurney (1938). The ovary pattern tends to be fairly constant at higher taxonomic levels (Büning 1994), suggesting that panoistic ovarioles are characteristic of zorapterans as a whole.

The ovary pattern is reputed to provide a good basis for phylogenetic consideration (Štys and Bilinski 1990). In insects, panoistic ovaries are probably plesiomorphic and are most probably inherited from a hexapod ancestor (Bilinski 1998). This ovariolar type is retained in females of basal taxa, such as Odonata of Palaeoptera, and in those of all polyneopteran orders except Dermaptera. Females of most paraneopteran and oligoneopteran orders have meroistic ovaries, polytrophic or telotrophic, the latter representing the more advanced state (Büning 1994).

In females of several taxa (e.g., Thysanoptera), ovaries are believed to be secondarily panoistic (Pritsch and Büning

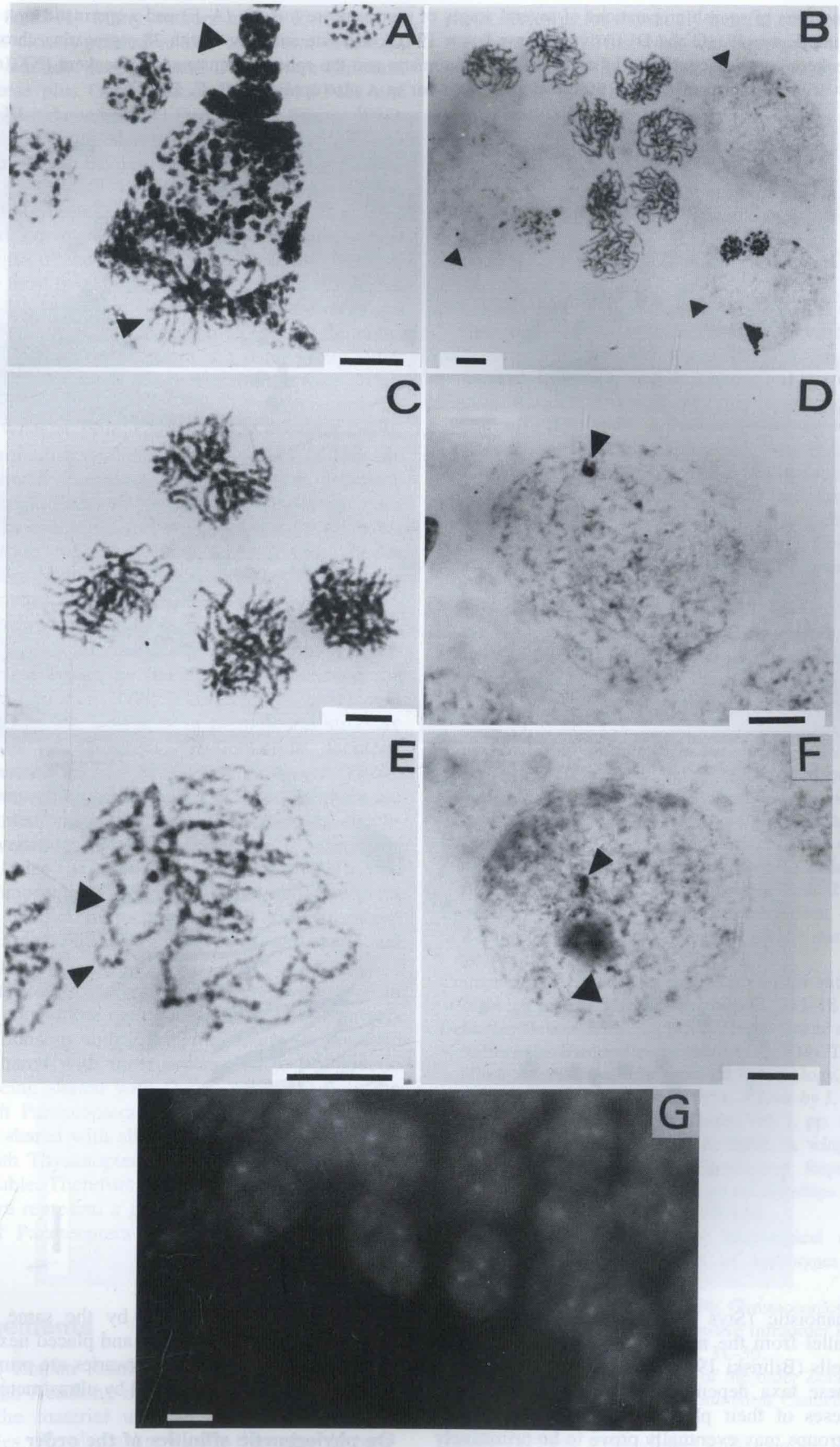
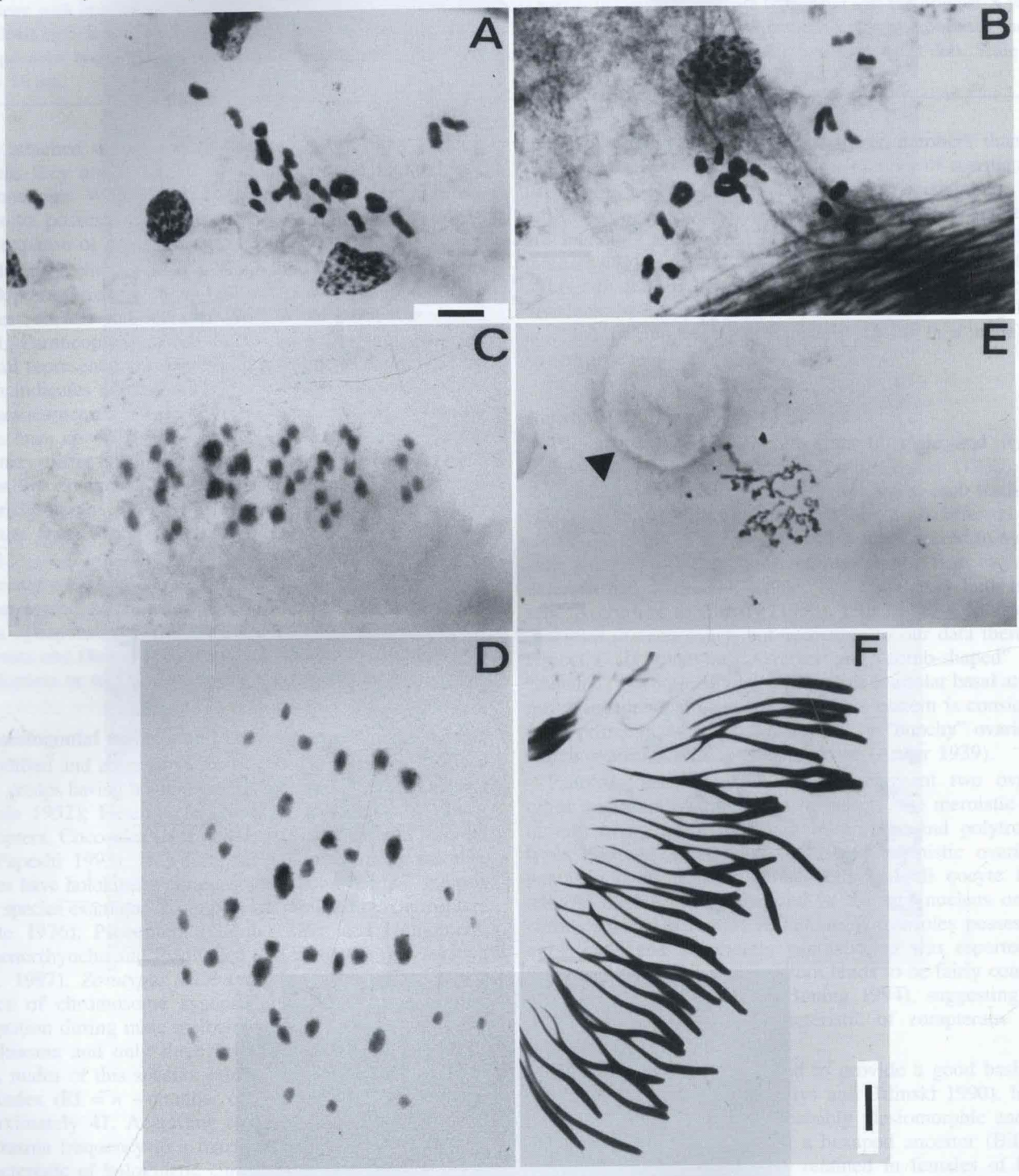


Fig. 4. Photomicrographs of squash preparations of several stages of spermatocyte meiosis (A–E) and a spermatid cyst (F). (A and B) Metaphase I, $n = 19$. (C and D) Early anaphase I, $n = 19$ (C), and late anaphase I with 38 segregating chromosomes (D). (E) Nucleus–Nebenkern complex consisting of a nucleus with chromatin and the spherical, unstained Nebenkern (NK, arrowhead). (F) Spermatid cyst with 32 spermatids. Scale bars = 10 μm; scale bar in A also applies to B, C, D, and E.



1989) or neopanoistic (Štys and Bilinski 1990), having evolved in parallel from the meroistic–polytrophic ones by loss of nurse cells (Bilinski 1998). However, inferring neopanoism for these taxa depends chiefly on acceptance of current hypotheses of their phylogenetic relationship, and some of these groups may eventually prove to be primitively panoistic. So Zoraptera, first shown by Büning (1994) to be neopanoists and to have descended from polytrophic ancestors, were later considered by the same author (Büning 1996) to be primary panoists and placed next to Blattida and Isoptera. Whether zorapteran ovaries are primitively panoistic or neopanoistic may be solved by ultrastructural investigation.

On phylogenetic affinities of the order

The systematic position of Zoraptera within Neoptera is not clear. There are three groups of hypotheses based on

morphological data. (1) Many authors assign Zoraptera to Polyneoptera as closely related to one or several orders of this group: (*a*) Blattida, Isoptera, and Dermaptera (Silvestri 1913), or to these plus Grylloblattida (Kukalová-Peck and Peck 1993); (*b*) Blattida and extinct Protoblattida (Karny 1922); (*c*) Blattida, Isoptera, and Mantida (Delamare-Debouteville 1952; Boudreaux 1979; Büning 1996; Wheeler et al. 2001); (*d*) Isoptera (e.g., Caudell 1918); and (*e*) Embiida (Minet and Bourgoin 1986; Engel and Grimaldi 2000). (2) Some authors considered Zoraptera either to be in the Polyneoptera close to the origin of the Paraneoptera (Crampton 1920) or to constitute the most basal lineage of Paraneoptera (Crampton 1921; Hennig 1969; Kristensen 1975); sometimes the order was even included in Psocoptera (Crampton 1938). (3) Recently, a few authors claimed that Zoraptera is a sister group either to Oligoneoptera (placing it in an ancestral, otherwise extinct subgroup of Paraneoptera; Rasnitsyn 1998) or to Paraneoptera + Oligoneoptera (Wheeler 1998; Carpenter and Wheeler 1999).

Analysis of homologous nucleotide sequences of 18S and 28S rDNA showed Zoraptera to be a sister group to Trichoptera + Lepidoptera (Wheeler et al. 2001), but once combined with morphological evidence gave more plausible results: sister group to Dermaptera (Carpenter and Wheeler 1999; discussed in Engel and Grimaldi 2000) or to Blattida + Mantida + Isoptera (Wheeler et al. 2001).

Of the three hypotheses based on morphological evidence, the earliest one, of a polyneopteran relationship of Zoraptera, seems at present to be the most viable (Kristensen 1995; Engel and Grimaldi 2000; Wheeler et al. 2001). It is further supported by the similarity in pteropleural structure of representatives of Zoraptera, Grylloblattida, Isoptera, Embiida, and Dermaptera as first noted by Crampton (1920). Putative synapomorphies of Zoraptera and Paraneoptera are few and represented mainly by reductions (including simplified forewing venation with "areola postica"), which are possible homoplasies (Kukalová-Peck and Peck 1993). Two putative synapomorphies of Zoraptera and Oligoneoptera (discrimen and medial mesocoxal articulation; Rasnitsyn 1998) were discarded by Shcherbakov (1999) and Engel and Grimaldi (2000).

Data on the chromosome type and on the ovary type in *Z. hubbardi* are compatible both with the polyneopteran version of the relationship with Zoraptera (owing to panoistic ovaries being shared with most orders and to holokinetic chromosomes being shared with Dermaptera) and with the relationship with Paraneoptera (owing to holokinetic chromosomes being shared with all orders and panoistic ovaries being shared with Thysanoptera), the former looking somewhat more probable. Therefore, Crampton's (1920) hypothesis that Zoraptera represent a group of Polyneoptera nearest to the origin of Paraneoptera should be tested by further study.

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References

Ander, K. 1939. Vergleichend-anatomische und phylogenetische Studien über die Ensifera (Saltatoria). Opusc. Entomol. Suppl. **2**: 1–306.

Bilinski, S.M. 1998. Introductory remarks. Folia Histochem. Cytobiol. **36**: 143–145.

Blackman, R.L. 1980. Chromosome numbers in the Aphididae and their taxonomic significance. Syst. Entomol. **5**: 7–25.

Blackman, R.L. 1995. Sex determination in insects. *In* Insect reproduction. *Edited by* S.R. Leather and J. Hardie. CRC Press, Boca Raton, Fla.. pp. 57–94.

Boudreaux, H.B. 1979. Arthropod phylogeny with special reference to insects. Wiley-Interscience, New York.

Büning, J. 1994. The insect ovary. Ultrastructure, previtellogenic growth and evolution. Chapman and Hall, London.

Büning, J. 1996. Germ cell cluster variety creates diversity of ovary types in insects. Verh. Dtsch. Zool. Ges. **89**: 123–137.

Carpenter, J.M., and Wheeler, W.C. 1999. Numerical cladistics, simultaneous analysis and hexapod phylogeny. Boletin de la Sociedad Entomologica Aragonesa, **26**: 333–346. [*Edited by* A. Melic, J.J. De Haro, M. Mendez, and I. Ribera.]

Caudell, A.N. 1918. *Zorotypus hubbardi,* a new species of the order Zoraptera from the United States. Can. Entomol. **50**: 375–381.

Choe, J.C. 1992. Zoraptera of Panama with a review of the morphology, systematics and biology of the order. *In* Insects of Panama and Mesoamerica. Selected studies. *Edited by* D. Quintero and A. Aiello. Oxford University Press, Oxford. pp. 249–256.

Conger, A.D., and Fairchild, L.M. 1953. A quick-freeze method for making smear slides permanent. Stain Technol. **28**: 289–293.

Crampton, G.C. 1920. Some anatomical details of the remarkable winged zorapteron, *Zorotypus hubbardi* Caudell, with notes on its relationships. Proc. Entomol. Soc. Wash. **22**: 98–106.

Crampton, G.C. 1921. A further comparison of the wings of Zoraptera, psocids, and aphids, from the standpoint of phylogeny. Can. Entomol. **53**: 110–117.

Crampton, G.C. 1938. The interrelationships and lines of descent of living insects. Psyche (Camb.), **45**: 165–181.

Delamare-Debouteville, C. 1952. L'homogeneité de la morphologie sternale des Blattopteroides (Martynov 1938). Transactions of the Ninth International Congress of Entomology, Amsterdam, the Netherlands, 17–24 August 1951. *Edited by* J. de Wilde. Excelsior, The Hague, the Netherlands. Vol. 1. pp. 147–150.

Engel, M.S., and Grimaldi, D.A. 2000. A winged *Zorotypus* in Miocene amber from the Dominican Republic (Zoraptera: Zorotypidae), with discussion on relationships of and within the order. Acta Geol. Hisp. **35**: 149–164.

Golub, N.V. 1999. Review of karyological data on psocids (Psocoptera) and description of karyotypes of 13 species. Entomol. Rev. **79**: 412–424.

Grozeva, S., and Nokkala, S. 1996. Chromosomes and their behavior in two families of the primitive infraorder Dipsocoromorpha (Heteroptera). Hereditas, **125**: 31–36.

Gurney, A.B. 1938. A synopsis of the order Zoraptera, with notes on the biology of *Zorotypus hubbardi* Caudell. Proc. Entomol. Soc. Wash. **40**: 57–87.

Halkka, O. 1959. Chromosome studies on the Hemiptera Homoptera Auchenorrhyncha. Ann. Acad. Sci. Fenn. Ser. A IV Biol. **43**: 1–71.

Halkka, O. 1964. Recombination in six homopterous families. Evolution, **18**: 81–88.

Hennig, W. 1969. Die Stammesgeschichte der Insekten. Kramer, Frankfurt am Main, Germany.

Hughes-Schrader, S., and Schrader, F. 1961. The kinetochore of the Hemiptera. Chromosoma (Berl.), **12**: 327–350.

Karny, H.H. 1922. Zorapteren aus Süd-Sumatra. Treubia, **3**: 14–37.

Kristensen, N.P. 1975. The phylogeny of hexapod "orders": a critical review of recent accounts. Z. Zool. Syst. Evolutionsforsch. **13**: 1–44.

Kristensen, N.P. 1995. Forty years' insect phylogenetic systematics. Zool. Beitr. N.F. **36**: 83–124.

Kukalová-Peck, J., and Peck, S.B. 1993. Zoraptera wing structures: evidence for new genera and relationship with the blattoid orders (Insecta: Blattoneoptera). Syst. Entomol. **18**: 333–350.

Kuznetsova, V.G. 1979. Chromosomes of holokinetic type and their distribution among insects and other invertebrate animals. *In* Karyosystematics of invertebrate animals. [In Russian.] *Edited by* O.A. Scarlato. Zoological Institute, Russian Academy of Sciences, Leningrad. pp. 5–19.

Kuznetsova, V.G. 1986. Phylogenetical analysis of the chromosome variability and karyosystematics of the leaf-hoppers of the family Dictyopharidae (Homoptera, Auchenorrhyncha). Entomol. Rev. **65**: 88–106.

Kuznetsova, V.G., Nokkala, S., and Maryańska-Nadachowska, A. 1997. Karyotypes, sex chromosome systems, and male meiosis in Finnish psyllids (Homoptera: Psylloidea). Folia Biol. (Krakow), **45**: 143–152.

Maryańska-Nadachowska, A., Taylor, G., and Kuznetsova, V.G. 2001. Meiotic karyotypes and structure of testes in males of 17 species of Psyllidae: Spondyliaspidinae (Hemiptera: Psylloidea) from Australia. Aust. J. Entomol. **40**: 349–356.

Minet, J., and Bourgoin, T. 1986. Phylogenie et classification des Hexapodes (Arthropoda). Cah. Liaison, O.P.I.E. **20**: 23–28.

Nokkala, S., and Nokkala, C. 1984. Achiasmatic male meiosis in the heteropteran genus *Nabis* (Nabidae, Hemiptera). Hereditas, **101**: 31–35.

Nur, U. 1981. Evolution of unusual chromosome systems in scale insects (Coccoidea: Homoptera). Symp. R. Entomol. Soc. Lond. No. 10. pp. 97–117.

Oksala, T. 1952. Chiasma formation and chiasma interference in the Odonata. Hereditas, **38**: 449–480.

Petitpierre, E. 1997. The value of cytogenetics for the taxonomy and evolution of leaf beetles (Coleoptera, Chrysomelidae). Misc. Zool. **20**: 9–18.

Pritsch, M., and Büning, J. 1989. Germ cell cluster in the panoistic ovary of Thysanoptera (Insecta). Zoomorphology (Berl.), **108**: 309–313.

Rasnitsyn, A.P. 1998. On the taxonomic position of the insect order Zorotypida = Zoraptera. Zool. Anz. **237**: 185–194.

Roosen-Runge, E.C. 1977. The process of spermatogenesis in animals. Cambridge University Press, Cambridge.

Schweizer, D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma (Berl.), **58**: 307–324.

Shcherbakov, D.E. 1999. Controversies over the insect origin revisited. *In* Proceedings of the First International Palaeoentomological Conference, Moscow, 1998. AM/PFICM98/1.99. Bratislava, Slovakia. pp. 141–148.

Silvestri, F. 1913. Descrizione di un nuovo ordine di insetti. Bollettino del Laboratorio di Zoologica Generale e Agraria della R. Scuola Superiore d'Agricoltura in Portici. Portici, Premiato Stab. Tip. Vesuviano di Ernesto Della Torre, **7**: 193–209.

Štys, P., and Bilinski, S. 1990. Ovariole types and the phylogeny of hexapods. Biol. Rev. Camb. Philos. Soc. **65**: 401–429.

Sumner, A.T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304–306.

Tombesi, M.L., and Papeshi, A.G. 1993. Meiosis in *Haematopinus suis* and *Menacanthus stramineus* (Phthiraptera, Insecta). Hereditas, **119**: 31–38.

Ueshima, N., and Ashlock, P.D. 1980. Cytotaxonomy of the Lygaeidae (Hemiptera—Heteroptera). Univ. Kans. Sci. Bull. **51**: 717–801.

Virkki, N. 1973. Evolution of sperm cell number per bundle in insects. An. Esc. Nac. Cienc. Biol. Mex. **20**: 23–34.

Wheeler, W.C. 1998. Sampling, groundplans, total evidence and the systematics of arthropods. *In* Arthropod relationships. *Edited by* R.A. Fortey and R.H. Thomas. Systematic Association Spec. Vol. Ser. 55. Chapman and Hall, London. pp. 87–96.

Wheeler, W.C., Whiting, M., Wheeler, Q.D., and Carpenter, J.M. 2001. The phylogeny of the extant hexapod orders. Cladistics, **17**: 113–169.

White, M.J.D. 1973. Animal cytology and evolution. Cambridge University Press, Cambridge.

White, M.J.D. 1976. Animal cytogenetics 3: Insecta 2: Blattodea, Mantodea, Isoptera, Grylloblattodea, Phasmatodea, Dermaptera and Embioptera. Gebrüder Bornträger, Berlin and Stuttgart. pp. 49–59.

Wolf, K.W. 1994. The unique structure of lepidopteran spindles. Int. Rev. Cytol. **152**: 1–48.