

The usefulness of chromosomes of parasitic wasps of the subfamily Eupelminae (Hymenoptera: Chalcidoidea: Eupelmidae) for subfamily systematics

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Abstract. Karyotypes of 16 Eupelminae species were analyzed. The haploid chromosome number was found to be much more diverse than the $n = 5$ previously recorded for three studied species. All species investigated of *Eupelmus* (*Eupelmus*), *Eupelmus* (*Macroneura*), *Merostenus excavatus* and three species of *Anastatus* have a karyotype of $n = 5$; $n = 7$ in *Eupelmus* (*Episolidelia*) *linearis*, $n = 8$ in *Arachnophaga picardi*, and $n = 10$ in *Anastatus ruficaudus*. The modal chromosome number is $n = 5$ and this is considered to be the plesiomorphic character state for the subfamily; karyotypes with higher chromosome numbers are hypothesized to be derived independently from the ancestral state by centric fission. This cytogenetical data provides important information on the subfamily systematics, especially the subgeneric classification of *Eupelmus* and generic placement of *Eupelmus rostratus*.

INTRODUCTION

Eupelmidae are a relatively small family of parasitic Hymenoptera of the superfamily Chalcidoidea which includes more than 900 recognized species, varying in life history and morphology (Gibson, 2006). The family is divided into three subfamilies (Calosotinae, Neanastatinae, and Eupelminae), Eupelminae being the most diverse with almost 80 species in Europe, but many of them are rarely collected.

Recent studies illustrated the usefulness of karyotypic features in the taxonomy of other chalcid wasp families. Different aspects of the karyotype were shown to be useful in separating closely related species of *Encarsia* (Aphelinidae) (Baldanza et al., 1999; Baldanza & Giorgini, 2000; Giorgini & Baldanza, 2004); Gokhman (2004a) discussed the patterns of evolution and the main characteristics of the karyotype in parasitic Hymenoptera and argued (Gokhman, 2006a, b) that karyotype is of taxonomic significance in this group. Cytogenetical data on members of Eupelmidae are scarce. Only recently, Gokhman & Quicke (1995) and Gokhman (2002) published the first data on three species, two *Eupelmus* and one *Anastatus*. All these species have a karyotype of $n = 5$. The aim of this study is to provide new information on the subject and to assess the usefulness of the karyotype for the systematics of the group.

MATERIAL AND METHODS

Adult females used in this study were collected by the author in Romania (except *Eupelmus vuilleti*) using a sweep net or reared from their hosts; for *E. vuilleti*, specimens of a laboratory strain of African origin were used (Table 1). All species were identified by the author and voucher specimens are deposited in the author's collection at “Al. I. Cuza” University of Iași, Romania.

Chromosome preparations were obtained from the ovaries of adult females as suggested by Gokhman & Quicke (1995), using the technique of Imai et al. (1988). This method combines col-

chicine and hypotonic treatments in a single step and the fixing, dissociation and spreading of cells are all done consecutively on a microscope slide; this results in good chromosome spreading and minimizes the loss of valuable cellular material. Chromosomes were stained with 6% Giemsa in a phosphate buffer solution (M/15, pH 6.8). Karyotypes were assembled by arranging the chromosomes in order of decreasing length; the classification, based on the centromere position, followed Levan et al. (1964) and Imai et al. (1977). The fundamental number (NF) was calculated as the number of chromosome arms in a diploid cell. The relative chromosome length (RCL) was not calculated and comparisons using absolute chromosome length in individual preparations were not possible (not informative due to different degrees of condensation). This is because there were only a few preparations for each species. The species used in this study are small, several of them rarely collected, thus few cells were available for cytogenetic investigation; a specimen usually provides 1–2 suitable metaphasic plates, at most.

RESULTS

Eupelminae Walker

Eupelmus Dalman, 1820

Eupelmus (*Eupelmus*) *urozonus* Dalman. $n = 5$; $2n = 10$ (8M + 2ST); NF = 20. Chromosomes 1–4 are metacentric and gradually decrease in length; chromosome 5 is subtelocentric and much smaller than the first four (Fig. 1a). A similar karyotype was reported by Gokhman (2002). Five bivalents were observed during diakinesis, of which four are closed, ring-shaped, each with two chiasmata and one open with a single chiasma (Fig. 1b).

Eupelmus (*Eupelmus*) *alloysii* Russo. $2n = 10$ (8M + 2SM); NF = 20. The first four pairs of metacentric chromosomes gradually decrease in length; chromosome 5 is submetacentric, much smaller than chromosomes 1–4 and with the homologues differing in size, indicating a possible polymorphism in the population investigated (Fig. 1c).

Eupelmus (*Eupelmus*) *microzonus* Förster. $n = 5$; $2n = 10$ (8M + 2ST); NF = 20. The karyotype is similar to that

TABLE 1. Collection data for the 16 Eupelminae species used in the chromosome analysis.

Species	Number of specimens and collection data
<i>Eupelmus urozonus</i> Dalman, 1820	2 ♀ Romania, Iași county, Bârnova Forest, from <i>Diastrophus rubi</i> galls on <i>Rubus</i> sp. 1 ♀ Romania, Pașcani, Codrii Pașcanilor Forest, from <i>Biorhiza pallida</i> galls on <i>Quercus</i> sp. 1 ♀ Romania, Iași, Botanical Garden, from <i>Diplolepis rosae</i> galls on <i>Rosa</i> sp.
<i>Eupelmus aloysii</i> Russo, 1938	1 ♀ Romania, Iași, Botanical Garden, branches of <i>Pinus</i> sp. with <i>Pityogenes bistridentatus</i> , <i>Pityophthorus buyssoni</i>
<i>Eupelmus microzonus</i> Förster, 1860	4 ♀ Romania, Constanța county, “Agigea maritime dunes” Nature Reserve
<i>Eupelmus vuilleti</i> (Crawford, 1913)	Burkina Faso, Ouagadougou region Laboratory strain from “Institut de Recherche sur la Biologie de l’Insect”, Tours, France, maintained on <i>Callosobruchus maculatus</i>
<i>Eupelmus vindex</i> Erdős, 1955	1 ♀ Romania, Iași, Botanical Garden
<i>Eupelmus atropurpureus</i> Dalman, 1820	3 ♀ Romania, Iași county, “Valea lui David” Nature Reserve
<i>Eupelmus falcatus</i> (Nikol’skaya, 1952)	1 ♀ Romania, Iași county, “Valea lui David” Nature Reserve
<i>Eupelmus vesicularis</i> (Retzius, 1783)	6 ♀ Romania, Iași, Botanical Garden, from <i>Tetramesa brevicornis</i> galls on <i>Festuca</i> sp., <i>Tetramesa</i> sp. galls, <i>Tetramesa hyalipennis</i> galls on <i>Agropyron</i> sp., <i>Diplolepis rosae</i> galls on <i>Rosa</i> sp.
<i>Eupelmus linearis</i> Förster, 1860	1 ♀ Romania, Galați county, “Gârboavele Forest” Nature Reserve 2 ♀ Romania, Constanța county, “Agigea maritime dunes” Nature Reserve
<i>Eupelmus rostratus</i> Ruschka, 1921	1 ♀ Romania, Botoșani county, Leorda
<i>Merostenus excavatus</i> (Dalman, 1820)	1 ♀ Romania, Iași county, Bârnova Forest
<i>Anastatus lichtensteini</i> (Ruschka, 1921)	1 ♀ Romania, Constanța county, “Gura Dobrogei” Nature Reserve
<i>Anastatus catalonicus</i> Bolivar, 1935	1 ♀ Romania, Iași county, Bârnova forest
<i>Anastatus bifasciatus</i> (Geoffroy, 1785)	1 ♀ Romania, Vaslui county, Chircești
<i>Anastatus ruficaudus</i> Ferrière, 1954	2 ♀ Romania, Constanța county, “Alah Bair” Nature Reserve
<i>Arachnophaga picardi</i> (Bernard, 1936)	1 ♀ Romania, Constanța county, “Agigea maritime dunes” Nature Reserve

of *Eupelmus urozonus*. The short arm of chromosome 3 has an evident secondary constriction (Fig. 1d). Five bivalents were observed at diakinesis, of which three are closed, ring-shaped, with two chiasmata and two open with a single chiasma (Fig. 1e).

Eupelmus (Eupelmus) vuilleti (Crawford). $2n = 10$ (8M + 2A); NF = 18. Chromosomes 1–4 are large and metacentric, their lengths form a gradually decreasing series; chromosome 5 is much smaller than the first 4 chromosomes and acrocentric (Fig. 1f).

Eupelmus (Eupelmus) vindex Erdős. $n = 5$; $2n = 10$ (8M + 2A); NF = 18. The karyotype (Fig. 1g) is similar to that of *Eupelmus vuilleti*. Five bivalents were observed at diakinesis, of which four are closed, with two chiasmata and one open with a single chiasma (Fig. 1h).

Eupelmus (Eupelmus) atropurpureus Dalman. $2n = 10$ (8M + 2A); NF = 18. The karyotype (Fig. 1i) is similar to that of *Eupelmus vuilleti* and *E. vindex*.

Eupelmus (Macroneura) falcatus (Nikol’skaya). $n = 5$; $2n = 10$ (8M + 2A), NF = 18. The karyotype (Fig. 1j) is similar to that of *Eupelmus vuilleti*, *E. vindex* and *E. atropurpureus*. Five bivalents were observed at diakinesis, of which four are closed and one open. One of the closed bivalents has an interstitial chiasma in addition to the two terminal chiasmata (Fig. 1k).

Eupelmus (Macroneura) vesicularis (Retzius). $2n = 10$ (8M + 2A); NF = 18. The karyotype (Fig. 1l) is similar to that of the four previously mentioned species. This data supports that of Gokhman & Quicke (1995), who mention $2n = 10$ for this species, but without further details.

Eupelmus (Episolidelia) linearis Förster. $2n = 14$ (2M + 4SM + 4ST + 4A); NF = 24. The karyotype is formed by chromosomes that gradually decrease in size, only those of the first pair being slightly larger. Chromosomes 1 and 5 are submetacentric, chromosome 2 – metacentric, chromosomes 3 and 4 – subtelocentric, 6 and 7 apparently acrocentric (Fig. 1m).

Eupelmus rostratus Rushka. $2n = 10$ (10M), NF = 20. The karyotype is symmetrical, with five pairs of metacentric chromosomes that gradually decrease in length (Fig. 2a).

Merostenus Walker, 1837

Merostenus excavatus (Dalman). $n = 5$; $2n = 10$ (10M), NF = 20. The karyotype (Fig. 2b) is similar to that of *Eupelmus rostratus*. Five bivalents were observed at diakinesis, of which two are closed, with two terminal chiasmata and three open with a single chiasma (Fig. 2c).

Anastatus Motschulsky, 1859

Anastatus lichtensteini (Ruschka). $2n = 10$ (10M), NF = 20. The karyotype is symmetrical, with five pairs of metacentric chromosomes that gradually decrease in length (Fig. 2d).

Anastatus catalonicus Bolivar. $2n = 10$ (10M), NF = 20. The karyotype has five pairs of metacentric chromosomes that gradually decrease in length (Fig. 2e) and is very similar to that of *A. lichtensteini*. Similar data were presented for this species by Gokhman & Quicke (1995).

Anastatus bifasciatus (Geoffroy). $n = 5$. Only meiotic chromosomes were observed during diplotene. The mei-



Fig. 1. Mitotic (a, c, d, f, g, i, j, l, m) and meiotic (b, e, h, k) karyograms. a, b – *Eupelmus urozonus*; c – *E. aloysii*; d, e – *E. microzonus*; f – *E. vuilleti*; g, h – *E. vindex*; i – *E. atropurpureus*; j, k – *E. falcatus*; l – *E. vesicularis*; m – *E. linearis*. Scale bar 10 μ m.

otic karyotype reveals five closed bivalents, each with two terminal chiasmata (Fig. 2f).

Anastatus ruficaudus Ferrière. $n = 10$; $2n = 20$. Chromosomes were counted during mitotic metaphase, but it was impossible to assess the morphology of the chromosomes. Meiotic chromosomes observed at diakinesis showed ten pairs of open bivalents, each with a single terminal chiasma. All chromosomes appear to have two arms, with a short or very short small arm (Fig. 2g).

Arachnophaga Brues, 1907

Arachnophaga (Parasolindenia) picardi (Bernard). $2n = 16$ (2M + 12ST + 2A), NF = 30. Chromosome 1 is a large, metacentric element; chromosomes 2 to 7 –

medium to small size submetacentrics; chromosome 8 is small and acrocentric (Fig. 2h).

DISCUSSIONS AND CONCLUSIONS

The haploid chromosome number in Eupelmidae was found to be more diverse than the $n = 5$ previously reported, which was based on *Eupelmus urozonus* (Gokhman 2002), *E. vesicularis* and *Anastatus catalonicus* (Gokhman & Quicke 1995). The 16 species of Eupelminae examined have a chromosome complement with $n = 5$ predominating, but also karyotypes of $n = 7$, $n = 8$, and $n = 10$.

Information on the ancestral karyotype of Eupelmidae is provided by outgroup comparison with the related Pteromalidae (Gibson, 1989). Members of this family

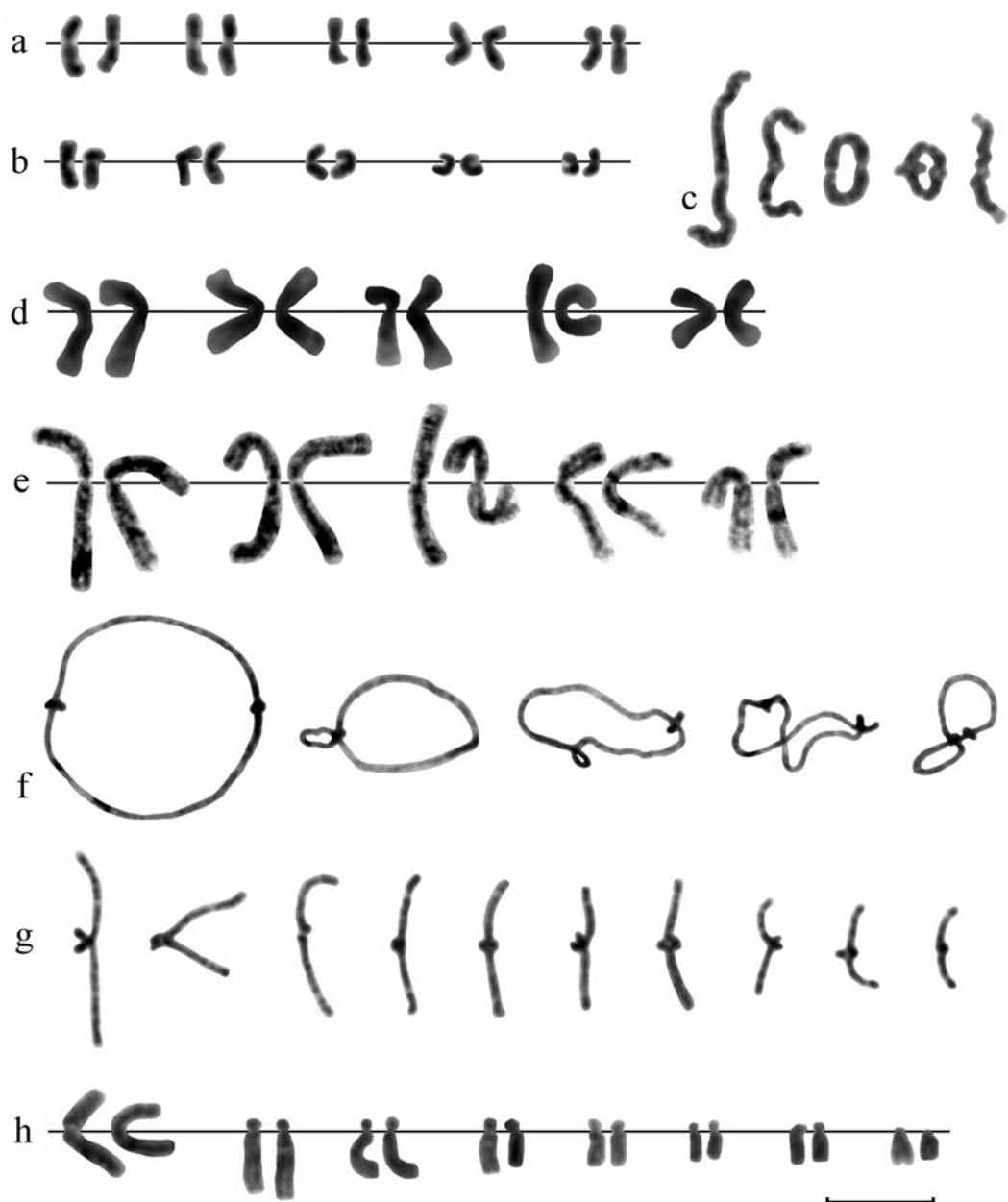


Fig. 2. Mitotic (a, b, d, e, h) and meiotic (c, f, g) karyograms. a – *Eupelmus rostratus*; b, c – *Merostenus excavatus*; d – *Anastatus lichtensteini*; e – *A. catalonicus*; f – *A. bifasciatus*; g – *A. ruficaudus*; h – *Arachnophaga picardi*. Scale bar 10 μ m (but for Fig. 2f – 20 μ m).

have $n = 5$ –7 with a modal value of 5, and $n = 5$ is supposed to be the plesiomorphic character state (Gokhman, 2004a). Unfortunately there are no data on the chromosomes of *Phenaceupelmus* Gibson (known only from Chile). This genus is considered basal to all other Eupelminae due to its reduced sexual dimorphism and notauli structure (Gibson, 1995). Other fairly basal genera are *Eupelmus* and *Merostenus* (Fig. 3), ten and one species respectively from these genera were analyzed cytogenetically in this study; all these species except one have a karyotype of $n = 5$. Outgroup comparison and predominance of $n = 5$ (this being also the modal value for the whole subfamily) in the relatively basal genera *Eupelmus* and *Merostenus* strongly support the hypothesis that $n = 5$ is the plesiomorphic character state for Eupelminae. The

main trends in karyotype evolution in the subfamily are better visualized by mapping the karyological data on the cladogram presented by Gibson (1995). A karyotype with the ancestral $n = 5$ is preserved in most species, including three species of *Anastatus* (the most derived species used in this study). Rare karyotypes with chromosome numbers higher than $n = 5$, found in three species from three not directly related genera ($n = 7$ in *Eupelmus linearis*, $n = 8$ in *Arachnophaga picardi*, $n = 10$ in *Anastatus ruficaudus*), suggest that karyotype evolution by centric fission occurred in parallel in different evolutionary lines (Fig. 3). The existence of karyotypes with both low ($n = 5$) and high ($n = 7, 8, 10$) chromosome numbers in Eupelminidae is an unusual feature for Chalcidoidea. The existing data summarized by Gokhman (2006b) shows

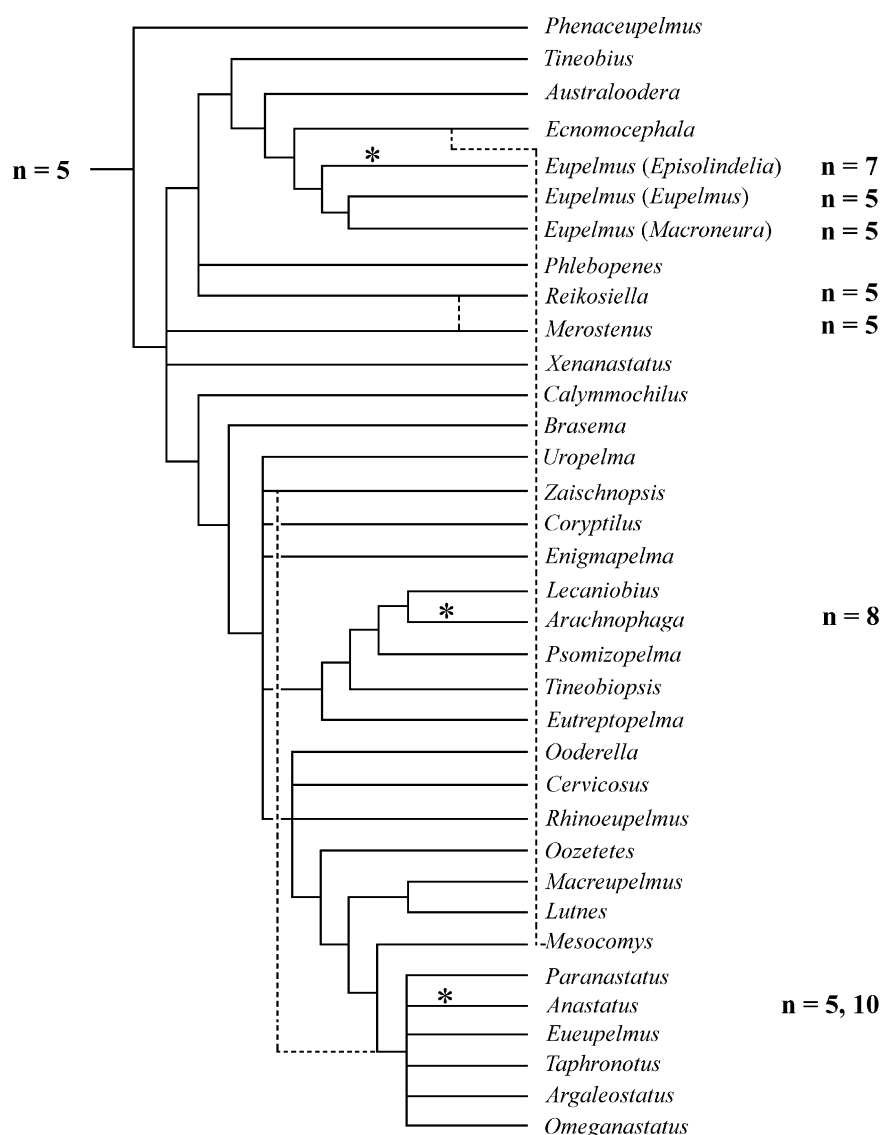


Fig. 3. Karyotype evolution in Eupelminae. Haploid chromosome numbers were mapped on the phylogenetic tree of the subfamily (simplified after Gibson, 1995; dashed lines indicate some of the alternative relationships); parallel karyotype evolution (homoplasy) by centric fission is indicated by stars.

that chromosome number vary little within a family: some less advanced families (Mymaridae and Eurytomidae) but also Encyrtidae have karyotypes of $n = 8-12$, whereas most other families have karyotypes with lower chromosome numbers ($n = 2-7$). A situation similar to that in Eupelmidae is recorded for Eulophidae, with most species having $n = 5-6$, but *Aprostocetus elongatus* (Förster) has $n = 7$ and a species of *Elachertus* $n = 8$ (Gokhman, 2004b); in Aphelinidae within a single genus (*Encarsia*) the haploid chromosome number varies from 3 to 10 (Baldanza et al., 1999). In Aphelinidae it is not evident whether high or low chromosome number is ancestral (Gokhman, 2004a) but in Eulophidae there is a clear case of increase in chromosome numbers (Gokhman, 2004b). This rare event of karyotype evolution by centric fission is also recorded for a few other parasitic Hymenoptera: one species of Braconidae, one of Ichneumonidae, and one of Chalcididae (Gokhman, 2004a).

Another important finding is that the karyotype is a useful additional character for resolving the systematics of the family Eupelmidae at the generic level. Each of the four genera studied has a characteristic karyotype, with only a few species not conforming. *Eupelmus* is characterized by a karyotype of $n = 5$; chromosome pairs 1-4 large and metacentric, chromosome 5 much smaller, acrocentric, subtelocentric or submetacentric. In the only species of the subgenus *Episolidelia* (*E. linearis*) studied the karyotype is different from that of the other two subgenera of *Eupelmus* in having $n = 7$ (Fig. 1). This agrees particularly well with the present concept of the genus *Eupelmus*. Gibson (1990) suggested that recognizing *Macroneura* as a separate genus would render *Eupelmus* paraphyletic; later Gibson (1995) treated *Macroneura* as a subgenus of *Eupelmus* and showed that *E. (Macroneura)* is close to species of *E. (Eupelmus)*. That the karyotypes of the two *E. (Macroneura)* species investigated (*E. vesicularis* and *E. falcatus*) are very similar to that of

some *E. (Eupelmus)* species suggests these subgenera are closely related and gives support for treating *Macroneura* as a subgenus of *Eupelmus*. *Episolidelia* Girault is considered a synonym of *Eupelmus* by Girault (1915), but Gibson (1995), considered *Episolidelia* to be a subgenus of *Eupelmus*. Cytogenetical data justify this separation: the karyotype of *E. (Episolidelia) linearis* is different in chromosome morphology and number from that of all other *Eupelmus* species investigated. The chromosomes of *E. vuilleti* (type species of *Bruchocida* Crawford) indicate that this species is only a modified *Eupelmus*, and this supports the synonymy of *Bruchocida* with *Eupelmus* by Bouček (1988).

E. rostratus has a karyotype dissimilar to that of other *Eupelmus* species and chromosome morphology very similar to that of *Merostenus excavatus* (Fig. 2a, b). Some characters of *E. rostratus* (head and metasoma structure) suggest that this species belongs to *Reikosiella* Yoshimoto and this species, together with other West Palearctic *Eupelmus* species, will be formally transferred to *Reikosiella* in a forecoming paper (L. Fusu, in prep.). Gibson (1995) suggests that *Merostenus* could render *Reikosiella* paraphyletic, and cytogenetic data are consistent with this opinion.

Of the four *Anastatus* species investigated, three have a karyotype of $n = 5$ (Fig. 2d, e, f), only in *Anastatus ruficaudus* is $n = 10$ and this is the highest chromosome number record for the family Eupelmidae. Most chromosomes in this karyotype seem to have a small short arm and the chromosome number is double that found in other *Anastatus* species (Fig. 2g). This indicate that it is probably derived from an $n = 5$ karyotype with metacentric chromosomes (similar to that found in other species of the genus) by multiple centric fissions. The karyotype of *Anastatus* species with $n = 5$ is apparently similar to that of *Merostenus* and *E. rostratus*. This could be explained by homoplasmy or retention of the ancestral karyotype, but cytogenetic data for more species is needed for a better resolution of the phylogenetic relationships.

The only species of *Arachnophaga* (*Parasolidenia*) analyzed (*A. picardi*) has a very peculiar asymmetrical karyotype of $n = 8$. The investigation of more species is needed to see if this is characteristic of the subgenus.

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