SYSTEMATICS OF PHASMIDA

by

ERICH HUNTER TILGNER

(Under the Direction of Dr. Joseph V. McHugh)

ABSTRACT

The results of a cladistic analysis of Phasmida are presented. The study is based on an examination of the morphology of the exoskeleton of the adult female form and egg capsule. Thirty-two species of Phasmida were included along with representatives of Isoptera, Grylloblattodea, Embiidina, and Orthoptera: Ensifera and Caelifera. Hypotheses of Phasmida and Euphasmida monophyly were not falsified. Contrary to preconceived expectations, *Orthomeria pandora* (Westwood) and *Dajaca monilicornis* Redtenbacher formed a clade at the base of the Euphasmida tree, one node below *Agathemera maculafulgens* Camousseight which was presumed to be the most basal Euphasmida taxon. Areolatae, Anareolatae, Pseudophasmatidae, Bacillidae, Phasmatidae, Heteronemiidae, Pseudophasmatinae, Bacillinae, Cladomorphinae, Phasmatinae, Necrosciinae, Lonchodinae, *sensu* Bradley & Galil were not monophyletic, but Heteronemiinae were monophyletic.

A review of the Phasmida fossil record is provided. No fossils of *Timema* Scudder are known. Euphasmida fossils include: *Agathemera reclusa* Scudder, *Electrobaculum gracilis* Sharov, *Eophasma oregonense* Sellick, *Eophasma minor* Sellick, *Eophasmina manchesteri* Sellick, *Pseudoperla gracilipes* Pictet, *Pseudoperla lineata* Pictet and various unclassified species from Grube Messel, Baltic amber, and Dominican Republic amber. The oldest documented Euphasmida fossils are 44-49 million years old; molecular clock dating underestimates the origin of the sister group *Timema* by at least 24 million years.

The phasmid *Lamponius nebulosus* new species is described. It is endemic to the cloud forest habitat of the Luquillo Experimental Forest (altitude above 762m). Females look like sticks overgrown with lichens or moss. Host plants include *Miconia* sp. (Melastomataceae) and *Guzmania* sp. (Bromeliaceae) and possibly *Tabebuia rigida* Urban (Bignoniaceae) and *Calycogonium squamulosum* Cogniaux (Melastomataceae).

The argument of Klass et al. that Mantophasmatodea is a new order of Insecta is inconclusive. Instead of a performing a cladistic analysis, these authors rely on subjective opinions of character polarity and possible autapomorphic characters to support their hypothesis.

INDEX WORDS: Fossil Insects, Leaf Insect, Mantophasmatodea, Phasmatodea, Phasmatoptera, Phasmid, Phasmoptera, Stick Insect

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TABLE OF CONTENTS

Page

CHAPTER 1

INTRODUCTION

Phasmida are terrestrial, nocturnal, phytophagous insects found in nearly all temperate and tropical ecosystems (Günther 1953; Bedford 1978). Scientists have described over 3,000 species (Bragg, 1995), yet this figure is uncertain since some taxon names are synonyms, and many new species have not been formally described.

They are variable in appearance, ranging from relatively plain forms, to some that are wonderful mimics of sticks and/or leaves. They display varying degrees of brachyptery, and can be winged or wingless. The tarsi have three articles in *Timema* Scudder and five in other Phasmida. Cerci are composed of one article, except for adult males of *Timema* which have a lobe on the right cercus (Tilgner *et al.* 1999).

Sexual dimorphism is usually extreme: the males are smaller and more gracile than the females. Reproduction is typically sexual, but parthenogenesis occurs frequently (Bedford 1978). The egg capsule is distinctively shaped, possessing a lid called the operculum and a micropylar plate (Sellick, 1997). Eggs are large and oftentimes highly sculptured resembling plant seeds. They are laid singly, and are either dropped, flicked, buried, glued to a surface, or riveted to a leaf (Carlberg 1983). Some species that drop the eggs rely on ants to disperse them in a process analogous to myrmecocory (Windsor *et al.*, 1996). The entire life cycle from egg to adult can take from several months to several years depending on the species (Brock 1999).

The order consists of two extant monophyletic groups: the genus *Timema* and the remaining species of Phasmida termed the Euphasmida (see Chapter 2). These two clades diverged at least 50 million years ago (Tilgner 2001) and are phenotypically distinct (Tilgner *et al.* 1999).

Timema are small, wingless Phasmida that lack elongation of the body segments. They have three tarsal articles, unlike other Phasmida which have five (Tilgner *et al.* 1999). Using her large paddle-shaped cerci, the female coats each egg with defecated soil before oviposition. This forms a coating that may protect the egg from fire (Sandoval 2000).

Timema are found only in the mountains of the western United States and are primarily associated with the Chaparral biome. They live on their host plants and are highly cryptic due to their coloration and habits while resting. When disturbed they release a spray from the prothoracic exocrine glands, but it has not been demonstrated to have a defensive function. They are unusual in that they feed on a variety of plants including both Gymnosperms and Angiosperms (Crespi & Sandoval 2000). Sandoval *et al.* (1998) and Crespi and Sandoval (2000) have examined the internal phylogeny of *Timema* using mitochondrial DNA sequence data from cytochrome oxidase I.

Euphasmida are commonly referred to as stick and leaf insects. Exquisitely camouflaged, many species look like twigs and may have the appearance of being covered by lichens, mold, bird feces, or moss. The leaf-like forms usually bear a striking resemblance to foliage, exhibiting leaf veins, mildew spots and even apparent insect feeding damage (e.g., see Figs in Brock 1999; Seiler *et al.* 2000; Seow-Choen 1997).

Their primary means of avoiding predators is crypsis. If discovered, they either play dead (catalepsy), or they try to scare the predator with a startle display which can include wing flashing, leg kicking, or spastic motion (Bedford 1978; Seiler *et al.* 2000). Some species also release an irritating "tear gas-like" spray when disturbed (Eisner *et al.* 1997). In addition, many Euphasmida can purposely lose some of their legs to help free them from a predator's grasp, or free them from the exuvia while molting. If this occurs during the immature stages, they can regenerate the lost limbs during successive molts (Brock 1999; Camousseight & Zapata 1981; Schindler 1979).

Compared to other insects, Euphasmida are large, and a few are gigantic. Several species measure over 200 mm in length (Brock 1999), and the worlds' largest extant insect is the Euphasmida *Phobaeticus serratipes*, with one documented female measuring 555 mm (Seow-Choen 1995).

Since Euphasmida are wonderful looking insects, and are relatively easy to rear in captivity, they are popular as pets and for displays at zoological gardens (Seiler *et al.* 2000).

This dissertation presents the results of the author's investigations into the systematics of this fascinating order of Insecta.

An earlier version of this chapter has appeared on the Tree of Life web page: http://tolweb.org/tree/eukaryotes/animals/arthropoda/hexapoda/phasmida/phasmida.html and another version will appear in "Encyclopaedia of Insects" (in press).

Chapter 2 presents a cladistic analysis of the Phasmida based on an extensive study of the morphology of thirty-two species of Phasmida, along with representatives of Isoptera, Grylloblattodea, Embiidina, and Orthoptera: Ensifera and Caelifera. This is the first phylogenetic study of the order ever conducted. It has been submitted to the Journal of Orthoptera Research for publication.

Chapter 3 interprets the fossil record of the order. This work has been published in the journal Insect Systematics and Evolution.

Chapter 4 provides a formal scientific description of a Phasmida species new to science. This report has been published in the Journal of Orthoptera Research.

Chapter 5 is an unpublished note submitted to the Journal of Orthoptera Research regarding the report of the discovery of a new order of Insecta Mantophasmatodea. The author is convinced that these insects are aberrant Orthoptera, a conclusion reached in 1998 after studying the external anatomy of a specimen housed in the British Museum of Natural History.

Chapter 6 presents the main conclusions of the dissertation research.

CHAPTER 2

A CLADISTIC STUDY OF PHASMIDA (INSECTA: NEOPTERA)[1]

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[1] Tilgner, E.H. Submitted to the Journal of Orthoptera Research, 5/15/02.

Abstract

This article describes the results of a cladistic analysis of Phasmida based on an examination of the morphology of the exoskeleton of the adult female form and egg capsule. Thirty-two species of Phasmida were included along with representatives of Isoptera, Grylloblattodea, Embiidina, and Orthoptera: Ensifera and Caelifera. Hypotheses of Phasmida and Euphasmida monophyly were not falsified. Contrary to preconceived expectations, *Orthomeria pandora* (Westwood) and *Dajaca monilicornis* Redtenbacher formed a clade at the base of the Euphasmida tree, one node below *Agathemera maculafulgens* Camousseight which was presumed to be the most basal Euphasmida taxon. Areolatae, Anareolatae, Pseudophasmatidae, Bacillidae, Phasmatidae, Heteronemiidae, Pseudophasmatinae, Bacillinae, Cladomorphinae, Phasmatinae, Necrosciinae, Lonchodinae, *sensu* Bradley & Galil were not monophyletic, but Heteronemiinae were monophyletic.

Key words

Cheleutoptera, Lower Neoptera, Phasmatodea, Phasmatoptera, stick-insect, leaf-insect, phylogeny, morphology

Introduction

Phasmida, commonly known as stick and leaf insects, comprise over 3000 species of nocturnal, exopterygote insects found in most temperate and nearly all tropical ecosystems (Günther 1953; Bragg 1995). Variable in appearance, they range from relatively plain forms to some that are wonderful mimics of sticks and leaves (e.g., see Figs in Seiler *et al.* 2000; Brock 1999; Seow-Choen 1997). In addition to crypsis, they are exceptional for their large size (Seow-Choen 1995), and for the ability of many species to regenerate limbs lost during nymphal stages (Carlberg 1994; Camousseight & Zapata 1981; Schindler 1979).

Phasmida are one of the principal groups of lower neopterous insects, and knowledge of their phylogeny is critical for helping to understand relationships of basal Insecta taxa. Surprisingly, only four published studies have examined the issue of Phasmida phylogeny (Bradler 1999; 2000; Kristensen 1975; Tilgner *et al.* 1999), and these works did not invoke formal methods of the phylogenetic analysis. Given this consideration, the author wanted to test the claims made in these works by conducting a cladistic analysis to evaluate the hypotheses (Fig. 1) that:

1) Phasmida are monophyletic
2) The genus *Timema* is the sister group to remaining Phasmida species termed "Euphasmida"
3) The genus *Agathemera* is the sister group to all other Euphasmida

In light of the results, remarks are made about the classification of Phasmida, their defensive behavior, feeding habits, and possible systematic position among lower Neoptera.

History of Phasmida Classification

Phasmida were first classified as Coleoptera by Linnaeus (1758) and later as Gryllidae and Mantidae by Drury (1773). Latrielle (1825) was the first to recognize them as a family and they were subsequently referred to as Phasmidae, a family of Orthoptera, in important works such as Gray (1835), Westwood (1859), Kirby (1904), Brunner von Wattenwyl & Redtenbacher (1908).

Brunner von Wattenwyl & Redtenbacher (1908) provided the first authoritative classification of the Phasmida that has served as a foundation for all subsequent works. They divided the Phasmida into two groups based on whether or not they possessed the area apicalis, a sclerotized triangular groove located on the ventral apices of the tibiae. Although a major scientific advance at the time, their monograph is plagued by errors and has been extensively criticized, e.g., Dohrn (1910), Hebard (1919), Karny (1923), and Brock (1999).

Günther (1953) proposed a revised classification of the order based on the Brunner von Wattenwyl & Redtenbacher (1908) system. His system was among the first to recognize Phasmida as a distinct order using the name Phasmatodea proposed by Jacobson & Bianchi (1902). Later, Bradley & Galil (1977) made changes to the nomenclature provided by Günther (1953) and classified the known genera.

Phasmida have been referred to by many different ordinal names, including Cheleutoptera, Phasmatodea, Phasmatoptera, Phasmoptera, among others. The author prefers Phasmida, however, because it is euphonious, brief, and easily linked to the vernacular name phasmid. First proposed by Leach (1815) it is from the name for *Phasma* the type genus, and is derived from the Greek "phasma" meaning an apparition.

Biology of the Phasmida

Phasmida are the only insect order whose members feed solely on the tissues of leaves (including flower petals and grasses) of terrestrial plants. Nearly all species eat angiosperms (e.g., see Tay & Seow-Choen 1996), but a few have secondarily evolved to feed on gymnosperms, or ferns (Crespi & Sandoval 2000). Both nymphs and adults feed on the same types of plants.

Like grasshoppers, Euphasmida (note: the feeding behavior of *Timema* is not known) feed by straddling the leaf margin and biting. Chewing continues in a downward semicircular motion until the leaf margin is reached. The insect then raises itself to resume chewing at the original starting point, and the downward movement is repeated.

Timema, winged forms, and the stick and leaf like species tend to spend the day hiding on the host plant, moving to feed at night (Brock 1999). Some wingless, lubberly, or warty/spiny species (e.g., some Heteropteryginae) descend from the food plant before daylight and hide about the surrounding substrate or in crevices (Bragg 1998).

Facultative and obligate parthenogenesis sometimes occurs, but reproduction is usually sexual. Copulation involves transfer of a spermatophore into the genital tract of the female by eversion of balloon-like male copulatory organ. Coupling between sexes before and after mating is usually brief, but in few species the male rides on the back of the female (e.g., *Anisomorpha buprestoides* Stoll) and remains coupled by attaching a sclerotized hook-like structure, termed the vomer, into a pregenital pouch located on the female abdomen.

The eggs are laid singly, and can be placed by the female in various ways, including simply dropping them, actively flicking them away, carefully burying them in the soil, gluing them to a substrate, or even riveting them into the phylloplane of a leaf (Carlberg 1983). In a few spectacular cases the eggs are transported by ants in a process analogous to myrmecocory exhibited by plants with ant-dispersed seeds (Windsor *et al.* 1996). The egg capsule bears a cap called the operculum, and is adorned on one side by a micropylar plate (Sellick 1998). Eggs are highly sculptured and often bear a striking resemblance to seeds but this is probably not mimesis, rather convergence for similar function of protecting a developing embryo exposed to the environment. The shape of the egg is often species specific, and several workers (notably Sellick) use its structure to distinguish morphologically similar species that would otherwise not be recognizable.

In general, Phasmida are cryptic but a few are aposematically colored. Cryptic species may merely have coloration that camouflages them, or more interestingly, may exhibit modifications of the exoskeleton such as dorso-ventral flattening of the body, lobes, spines, and warts, etc. that make them look like twigs, bark, or leaves, etc.

Although the order is well defined by several subtle morphological features (see Tilgner *et al.* 1999) a wide range of body forms occurs and it is very hard to make generalizations about the appearance of a typical Phasmida. Size can vary greatly from the relatively small *Timema* (about 2.5 mm) to giant species like *Pharnacia serratipes* that can measure over 500 mm in length (Seow-Choen 1995). Males and females are always dimorphic for size and may look dissimilar. Also, size, coloration, development of integumental processes, wings, etc., can vary dramatically between individuals of a species, but the details of body morphology, e.g., mouthparts, thoracic structure, male or female genitalia, etc. are usually homogenous. It should be noted that variation of easily observable external features has caused great confusion among both past and current taxonomists who have relied on these features when carrying out taxonomic work (Brock 1999).

Enemies of Phasmida are many and include both vertebrate and invertebrate predators and parasites from which the insects must hide and defend themselves. One aspect of defense that has been studied is a tear gas-like defense spray produced and discharged by a pair of prothoracic exocrine glands. These sprays are effective at deterring both vertebrate and invertebrate predators (Eisner *et al.* 1997). Although their function seems obvious, these glands are enigmatic because many members of the order, including *Timema*, produce sprays that are apparently innocuous, at least to human observers, and many species do not seem to produce a spray at all although they possess the glands that should make them. Other defenses employed by Phasmida include leg kicking or pinching, wing flashing startle displays, death feigning (i.e. catalepsy), purposeful limb loss (i.e. autotomy), and apparent scorpion/or ant mimicking appearance and behavior (Brock 1999).

Fossil record of Phasmida

Phasmida were believed to be an ancient group of insects whose ancestry could be traced back to the Paleozoic. However, a review of the known fossil record revealed that none of the Paleozoic or Mesozoic fossils were Phasmida (Tilgner 2001). The oldest Euphasmida fossils are from the Eocene found in Grübe Messel (Germany) and Clarno Nut Beds (USA). Oligocene and Miocene fossils are known from Florissant shale, Baltic and Dominican Republic amber. No fossils of *Timema* Scudder are known.

Phylogenetic position among Neoptera

The exact phylogenetic relationship of Phasmida to other Insecta has always been controversial. It is generally conceded that they are orthopteroid-type insects and represent a distinct clade that is part of the lower Neoptera assemblage *sensu* Kristensen (1975). For a historical review of previous views of their phylogenetic affinities, and for reasons why they are untenable, the reader is referred to Kristensen's extensive summaries. Wheeler *et al.* (2001) and Flook & Rowell (1998) determined, largely based on molecular sequence data, that Orthoptera and Phasmida were sister groups and this can be taken as the current consensus view. Maekawa *et al.* (1999), Tilgner (2001) and Klass *et al.* (2002) have suggested other alternatives.

Materials and Methods

Observations and illustrations of specimens were performed on a Leica MZ8 stereomicroscope fitted with a Wild 308700 drawing tube. Dissections were performed in 75% ethanol. Exoskeletal preparations were made using weak solutions of potassium hydroxide. It was found that heating the KOH solution before adding the partially dissected specimen yielded the best results. To observe and manipulate small structures, a piece of cotton was put in ethanol and the part to be observed was placed on it. This was superior to the traditional method of using sand, or glass "micro beads." Dissected specimens were individually stored in labeled, 50ml Poly- Propylene screw cap tubes (SARSTEDT- PP RPK GWB) filled with 75% ethanol.

Figure Abbreviations

Head

af, antennifer; ap, tentorial apodemes; br, brustia; cp, clypeus; d, dentes; e, compound eye; es, epistomal sulcus; f, swollen lateral region of frons; g, galea; gal, galealobulous; il, incisor lobe; lb, swollen lacinial base; lm, labrum; lo, lobe; lt, lacinial teeth; ml, molar lobe; ms, midcranial sulcus; mxp, maxillary palp; poc, postoccipital carina; poc, postoccipital carina; ptp, posterior tentorial pit; sc, scape; sd, salivary ducts; slt, subapical lacinial tooth; ta, anterior tentorial arm; tb, tubercles; tp, posterior tentorial arm; vs, ventral sclerite

General

a, apodemes; c, carina

Cervix

cv, cervical sclerite; p, cervical plate; g, gula

Thorax

aa, area apicalis; adg, aperture of defense gland; an, anapleurite; bs, basisternum; cp, coxopleurite; cx, coxa; dt, dorsal tubercle; p, anterior projection of apodeme; pn, paranotum; pt, planta; sa, sternal apophyses; sm, spinasterna, spine; sn, spina; sp, spiracle; t, tergite; tn, trochantin; un, ungue; ungitractor, plate; w, wing

Abdomen

c, cercus; cr, crop; gap, gonapophyses; g, gonangulum; gpl, gonoplac; mes, mesenteron; n, proventricular neck; op, operculum; ov, oesophageal valve; pp, paraproct; pv, proventriculus;

Egg

c, capitulum; m, micropyle; mp, micropylar plate; op, operculum;

On all figures stipples indicate membrane; simple broken lines, underlying structures; and parallel lines, cut structures. Pictures are not to scale.

This study includes data from the morphology of the adult female form and egg capsule. Features observed that exhibited continuous variation and unique structures, i.e. autapomorphies, were excluded along with ones of pteralia and wing venation. Too many taxa lacked wings and this would have resulted in the optimization of wing characters for wingless taxa, an approach the author did not feel comfortable with because too much data was missing and optimization could be misleading. Missing characters were indicated with a "?", while inapplicable ones were scored with "-" (Fig. 50). All characters were analyzed as unordered and unweighted. Since more than 20 taxa were studied, exact searches for the most parsimonious trees were not feasible and heuristic search methods were employed instead. The data matrix was constructed with WinClada (Nixon 1999). NONA (Goloboff 1994) was used to perform heuristic searches for the most parsimonious trees, for successive weighting, for calculating ensemble consistency indices, retention indices, and Bremer support values. The characters cited as supporting monophyletic groups are those that are independent of optimization.

Mastotermes darwiniensis Froggatt were chosen to root the trees because they are not believed to be part of the ingroup whose relationships are under investigation (Wheeler *et al.* 2001).

The author felt that either an Orthoptera (Wheeler *et al.* 2001; Flook & Rowell 1998) or Embiidina (Tilgner 2001) sister group relationship to Phasmida was the best supported hypotheses available (see Maekawa *et al.* 1999; Engel & Grimaldi 2000; Klass *et al.* 2002, Kristensen 1975, for alternative views). Representatives of basal and derived Ensifera and Caelifera clades (*sensu* Flook & Rowell 1998) were, therefore, included in the study, along with a species of Grylloblattodea and the basal Embiidina taxon *Clothoda nobilis* Gerstaecker.

Listing of taxa studied

Isoptera: Mastotermitidae: *Mastotermes darwiniensis* Froggatt; Grylloblattodea: Grylloblattidae: *Galloisiana nipponensis* (Caudell); Orthoptera
Gryllidae: *Gryllus* sp.; Tettigoniidae: *Pterophylla camellifolia* (Fabricius); Proscopiidae: *Cephalocoema* sp.; Acrididae: *Melanoplus differentialis* (Thomas); Tridactylidae: *Tridactylus apicalis* Say; Embiidina: Clothodidae: *Clothoda nobilis* Gerstaecker

Phasmida *sensu* Bradley & Galil (1977): Suborder: Areolatae: Bacillidae: Bacillinae: *Antongilia muricata* Redtenbacher, *Bacillus rossius* (Rossi); Heteropteryginae: *Heteropteryx dilatata* (Parkinson); Pseudophasmatidae: Aschiphasmatinae: *Orthomeria pandora* (Westwood); Korinninae: none; Pseudophasmatinae: *Agathemera maculafulgens* Camousseight, *Anisomorpha buprestoides* (Stoll), *Dajaca monilicornis* Redtenbacher, *Pseudophasma rufipes* (Redtenbacher); Pygirhynchinae: none; Phylliidae: *Phyllium bioculatum* Gray; Timematidae: *Timema cristinae* Vickery. Suborder: Anareolatae: Heteronemiidae: Heteronemiinae: Diapheromera femorata (Say), Libethra regularis, Brunner, *Oreophoetes peruana* (Saussure); Lonchodinae: *Carausius morosus* Sinéty, *Lonchodes brevipes* (Gray), *Phenacephorus auriculatus* (Brunner); Necrosciinae: *Necroscia westwoodi* Kirby, *Orxines macklottii* (de Haan), *Oxyartes spinosissimus* Carl, *Sipyloidea sipylus* (Westwood); Pachymorphinae: *Gratidia hispidulus* (Wood-Mason); Palophinae: *Palophus tiaratus* Stal; Phasmatidae: Cladomorphinae: *Aplopus ligia* Westwood, *Phibalosoma phyllinum* (Gray); Eurycanthinae: *Eurycantha calcarata* Lucas; Phasmatinae: *Acrophylla wuelfingi* (Redtenbacher), *Acanthoxyla prasina* (Westwood), *Baculum insueta* (Brunner), *Phasma gigas* (L); Platycraninae: *Megacrania tsudai* Shiraki; Tropidoderinae: none; Xeroderinae: *Nisyrus spinulosus* Stål; *Incertae sedis*: *Extatosoma tiaratum* (Macleay).

Both captive bred and wild caught species were included in the study. Identifications were verified with the literature, and in many cases by experts such as P. Bragg, P. Brock, A. Camousseight, E. Ross, amongst others who kindly assisted the author with this matter. It is believed that this sample spans the range of morphological variation exhibited by the Phasmida since representatives of aberrant and typical genera were included. Voucher specimens were deposited in the Georgia Museum of Natural History, Athens, Georgia, USA.

Morphological data matrix

0. Labrum: not emarginated (0) (Fig. 2); emarginated (1) (Fig. 3). An emarginated labrum is clearly correlated with feeding on leaf margins being exhibited by Caelifera and Phasmida that have this habit.

1. Molar lobe: cusp (0) (Fig. 4); toothed lobe (1) (Fig. 5). The cusp-like molar lobe is a striking modification of the orthopteroid mandible exhibited by the Euphasmida. Presumably, it aids the insects to cut the phylloplane, while the grinding function provided by a toothed lobe is lost. This unique morphology explains the observation of Gangwere (1960) that unlike grasshoppers, there is no distinct pause for mastication of the leaf material while Euphasmida are feeding.

2. Left mandible incisor lobe: crenate (0) (Fig. 6); entire (1) (Fig. 7).

3. Left mandible: incisor lobe does not form a ventral cusp (0); incisor lobe forms a ventral cusp (1).

4. Left mandible, inner face: dentes absent (0) (Fig. 6); dentes present (1) (Fig. 7).

5. Left mandible: inner face with one dente (0); two dentes (1) (Fig. 7).

6. Maxillary palpi: oriented down (0); neutral/curled up (1) (Fig. 8).

7. Apical lacinial teeth: one (0); two (1) (Fig. 9); three (2) (Figs 10, 11).

8. Subapical lacinial tooth: absent (0) (Fig. 10); present (1) (Fig. 9). The subapical tooth of *Melanoplus* was larger than the other Orthoptera taxa, but is considered homologous.

9. Galealobulous: absent (0) (Fig. 9); present (1) (Figs 10, 11). Bradler (2000) stated the galealobulus is absent from *Agathemera*, but it is present in *A. maculafulgens* (Fig. 1).

10. Galealobulous: vestigial (0) (Fig. 12); lobe-like (1) (Figs 10, 11).

11. Lacinial base: not swollen (0) (Figs 9, 10, 11, 12); swollen (1) (Fig. 13).

12. Lobe over lacinia base: absent (0) (Figs 9, 10, 13); present (1) (Figs 11, 12).

13. Paired salivary ducts: merge anterior to junction with hypopharynx (0); join hypopharynx separately (1) (Fig. 14).

14. Lateral margins of glossae: not grooved (0); grooved (1). This was noted for *Timema* by Tilgner *et al.* (1999) and the author was surprised to find this state present in *Cephalocoema*, *Melanoplus*, and *Clothoda* where it has never been observed.

15. Antennifer: vestigial (0) (Fig. 15); well-defined (1) (Fig. 16). The reduction of the antennifer is a synapomorphy homoplastically exhibited by Orthoptera and Euphasmida.

16. Corpotentorium: without a medial carina (0) (Fig. 17); with a medial carina (1) (Figs 18, 19).

17. Corpotentorium, posterior face: without a depression (0); with a depression (1).

18. Apophyses of tentorium: absent (0) (Fig. 18); present (1) (Figs 17, 19).

19. Apophyses of tentorium: on dorsal arms (0) (Fig. 19); on corpotentorium (1) (Fig. 17).

20. Tubercles of posterior tentorial arms: absent (0); present (1) (Figs 17, 18, 19).

21. Lateral apodemes of tentorium: absent (0) (Fig. 20); present (1) (Fig. 21). Surprisingly all morphologists who have previously studied the grasshopper head capsule did not report these apodemes.

22. Occipital carina: absent (0) (Fig. 20); present (1) (Fig. 21). This feature is usually referred to as the occipital sulcus, but it formed a carina in all of the taxa that possessed it, so the author renamed it.

23. Postocciput internal medial carina: not enlarged (0); enlarged (1). In some taxa a carina on the inside of the head capsule on the postocciput was greatly enlarged to form a plate-like apodeme.

24. Cervix: membranous (0); one medial plate (1) (Fig. 22); two lateral plates (2) (Fig. 23); gula (3) (Fig. 24). Contrary to what has been stated by Kristensen (1975) Embiidina do not have a true gula (Ross 2000). The postocciput of Embiidina is "pinched together" ventrally forming "gula-like" closure to the head capsule. In Euphasmida, the gula is similar to that of gula bearing Coleoptera in that it is a well formed plate that spans the cervix and connects the postocciput ventrally.

25. Prothoracic exocrine gland: absent (0); present (1) (Figs 25, 26, 27). The only objective way to determine the presence or absence of this gland is to perform a dissection of the prothorax since many species of Phasmida do not have an obvious external aperture to the gland.

26. Prothoracic ana- & coxapleurite: not divided (0) (Figs 25, 26); divided (1) (Fig. 27). In the divided state, the two-sclerotized plates are separated by a thin piece of membrane.

27. Pro-anapleurite: connected to basisternum (0) (Fig. 25); connected to cvII (1) (Fig. 27); not connected to any sclerite (2) (Fig. 26). State 1 is a rather striking modification exhibited by *Dajaca* and *Orthomeria* and represents a radical structural reconfiguration of the sclerotization of this region, which is otherwise uniform among Euphasmida.

28. Paranota: absent (0) (Fig. 26); present (1) (Figs 25, 27).

29. Spiracle II: engulfed by pronotum (0) (Figs 25, 27) ; not engulfed (1) (Fig. 26). It was found that certain taxa that possessed the paranotum do not have spiracle II engulfed so characters 28 and 29 were deemed independent.

30. Pro-basiternum: not connected to notum (0) (Figs 25, 26, 27); connected to notum (1). Certain taxa had a thin band of sclerite connecting the basisternum to the notum.

31. Prothoracic sternal apophyses: elongated (0); vestigial (1) (Fig. 28). The vestigial prothoracic sternal apophyses of *Tridactylus* are homoplasious with those of Euphasmida and probably a synapomorphy with the Rhiphterygidae.

32. Prothoracic sternal apophyses: not interlocking with pleural apophyses (0); interlocking with pleural apophyses (1).

33. Mesothoracic sternal apophyses: interlocking with pleural apophyses (0); not interlocking (1).

34. Mesothoracic apophyses: without anterior projection (0); with anterior projection (1).

35. Metathoracic sternal apophyses: interlocking with pleural apophyses (0); not interlocking (1).

36. Metathoracic apophyses: without anterior projection (0); with anterior projection (1) (Fig. 29).

37. Metatergum & abdominal tergum I: not fused (0); fused (1). Clearly this is a homoplasious similarity between Caelifera and Euphasmida.

38. Metasternum & abdominal sternum I: not fused (0); fused (1).

39. Pro-spina: absent (0); present (1).

40. Pro-spina: conical (0); carina (1) (Fig. 28). The prospina of *Dajaca* and *Orthomeria* differed from that of other taxa in that it was a carina and not a conical apodeme. Given

the fact that the carina was on the spina sternum in an identical location to a true spina the homology between the spina and carina was made.

41. Meso-spina: absent (0); present (1) (Fig. 29).

42. Meso-spina: not elongated (0); elongated (1) (Fig. 29).

43. Tergal apodemes anterior to metanotum: absent (0); present (1) (Fig. 30 a1).

44. Tergal apodemes anterior to abdominal tergum I: absent (0); present (1) (Fig. 30 a2).

45. Tergal apodemes issuing from abdominal tergum II: absent (0); present (1) (Fig. 30 a3).

46. Tergal apodemes issuing from abdominal tergum III: absent (0); present (1) (Fig. 30 a4).

47. Coxae: close (0); widely separate (1).

48. Procoxa: without a dorsal tubercle (0); with a dorsal tubercle (1) (Fig. 31).

49. Hind tibia: without parallel rows of spines (0); with parallel rows of spines (1). This character was first discussed by Kristensen (1975) as a synapomorphy of Orthoptera, but it was observed in several Euphasmida taxa.

50. Apex of tibia: with ventro-medial spine (0) (Fig. 32); without ventro-medial spine (1).

51. Area apicalis: absent (0); present (1) (Figs 33, 34). It seems most parsimonious to assume that the area apicalis was homologous among all Phasmida contrary to the opinion of Kristensen (1975). This structure is a traditional taxonomic character for distinguishing the two suborders areolate and anareolate *sensu* Bradley & Galil (1977) (families Phylliidae and Phasmidae *sensu* Günther (1953)).

52. Area apicalis: membranous (0) (Fig. 33); plate-like (1) (Fig. 34). The author was startled to observe a nearly identical area apicalis in *Timema* and *Clothoda* (compare Fig. 20 Tilgner *et al.* 1999 to Fig. 34 of this study). Contrary to the view of Bradler (2000) the area apicalis of *Agathemera* is plate-like although it does bear membrane on its apex.

53. Mesothoracic leg with tarsal article I: one euplantula (0); two (1); three (2). It was noted that variation existed for the number of tarsal euplantulae and this character was made.

54. Mesothoracic leg with euplantula I: not longitudinally divided (0); divided longitudinally (1).

55. Mesothoracic leg with euplantula of ultimate tarsal article: absent (0); present (1).

56. Unguis: not pectinate (0); pectinate (1) (Fig 35). This has been a traditional taxonomic character of Aschiphasmatinae and unexpectedly it was observed on *Necrosia*. This has prompted the author to ask taxonomists to reconsider the phylogenetic position of *Pinnispinus harmani* Brock since it may not belong to the clade with *Dajaca*, *Orthomeria* and presumably other Aschiphasmatinae (see Discussion).

57. Mesothoracic leg, planta: lateral setae absent (0); lateral setae present (1) (Fig 35).

58. Paraprocts form a plate: no (0); yes (1) (Fig 36).

59. Gonangulum: absent (0); present (1) (Fig. 37).

60. Gonangulum: not projecting (0); projecting (1) (Fig. 37). Many Euphasmida have a gonangulum that forms a flap that overlaps the bases of the ovipositor blades.

61. Gonapophysis II: enclosed by gonoplac (0); with gonoplac dorsally fused (1) (Fig. 37).

62. Subgenital plate: not notched apically (0); notched apically (1) (Fig. 37).

63. Tube over oviductus communis: absent (0); present (1).

64. Numerous tubules on lateral margin pouch II: present (0) (see Heather 1965); absent (1).

65. Proventriculus: with tubular "neck" (0) (Fig. 38); without tubular "neck" (1) (Fig. 39).

66. Oesophageal valve: absent (0) (Fig. 38); present (1) (Fig. 39).

67. Operculum of egg capsule: absent (0); present (1) (Fig. 40). Embiidina eggs possess an operculum like Phasmida.

68. Capitulum: absent (0); present (1) (Fig. 40).

69. Mycropylar plate *sensu* Sellick (1998): absent (0); present (1) (Fig. 40).

Results

The analysis using equal weights yielded three most parsimonious cladograms, each with 217 steps, a CI of 35 and a RI of 72. Uncertainty centered on the relationship between *Acrophylla*, *Extatosoma*, *Phasma* and the clade *Acanthoxyla*+*Nisyrus* (Fig. 41). When successive weighting was applied, one most parsimonious cladogram was found with 7651 steps, a CI of 54 and a RI of 84. This cladogram was the same as one of the three most parsimonious cladograms and its topology was chosen as the preferred tree (Fig. 42).

Phasmida monophyly (hypothesis #1) was not falsified (Fig. 43). Autapomorphies include characters 25(1), prothoracic exocrine gland present and 69(1), micropylar plate present. Homoplasious synapomorphies include character 0(1), labrum emarginated and character 38(1), thoracic metasternum and abdominal sternum I fused. This clade had a Bremer support value of 3 (Fig. 44).

Hypothesis #2 that *Timema* is the sister group to the Euphasmida was not falsified (Fig. 43). Autapomorphies of Euphasmida include characters 1(0), molar lobe a cusp; 3(1), left mandible with a ventral cusp formed by lacinia dentes; 6(1), maxillary palpi oriented upwards or in a neutral position; 13(1), paired salivary ducts joining hypopharynx separately; 31(1), prothoracic sternal apophyses vestigial; 52(1), area apicalis plate-like; 58(1), paraprocts form a plate and character 66(1), oesophageal valve present.

Homoplasious synapomorphies include characters 15(0), antennifer vestigial; 36(1), metathoracic sternal apophyses with an anterior projection; 37(1), metatergum and abdominal tergum I fused and character 42(1), meso-spina elongated. This clade had a Bremer support value of 5 (Fig. 44).

Hypothesis #3 that *Agathemera* is the sister group to all other Euphasmida was falsified. *Dajaca* and *Orthomeria* are a clade that is sister group to all other Euphasmida. Synapomorphies for *Dajaca* + *Orthomeria* include characters 27(1), pro-anapleurite connected to cervical sclerite II and character 57(1), mesothoracic leg planta with lateral setae.

Homoplasious synapomorphies include characters 16(0), corpotentorium without a medial carina; 18(1), apophyses of tentorium present; 24(1), cervix with two lateral plates; 34(1), mesothoracic apophyses with an anterior projection; and character 56(1), unguis pectinate (Fig. 43). This clade had a Bremer support value of 5 (Fig. 44).

Synapomorphies for all other Euphasmida include characters 9(1), gealealobulous present and character 39(0), pro-spina absent. Homoplasious synapomorphies include characters 26(0), prothoracic anapleurite and coxopleurite not divided; 41(0), meso-spina absent and character 54(1), mesothoracic leg with euplantula I divided (Fig. 43). This clade had a Bremer support value of 4 (Fig. 44).

Agathemera is sister group to a clade containing all remaining Euphasmida species studied. The monophyly of the remaining Euphasmida is supported by autapomorphous character 68(1), capitulum of egg capsule present. Homoplasious synapomorphies include characters 17(1), corpotentorium with a depression; 24(3), cervix with a gula; 27(0), pro-anapleurite not connected to any sclerite (Fig. 43). This clade had a Bremer support value of 3 (Fig. 44).

Supporting characters for the relationships of the remaining Phasmida taxa are illustrated in Figs 45, 46, 47. With the exception of the unresolved node all clades had Bremer support values of 1.

Synapomorphies of Clothoda and Phasmida include characters 22(0), occipital carina absent; 51(1), area apicalis present; 67(1), operculum of egg capsule present. Homoplasious synapomorphies include characters 21(0), lateral apodemes of tentorium absent; 47(1), coxae widely separate (Fig. 43). This clade had a Bremer support value of 5 (Fig. 44).

Supporting characters for the relationships of the non-Phasmida ingroup taxa are provided in Fig. 48. For Bremer Support values see Fig. 49.

Comparison of results to previous taxonomy

Suborders Areolatae and Anareolatae recognized by Bradley & Galil (1977) were not monophyletic. The Pseudophasmatidae, Bacillidae, Phasmatidae, Heteronemiidae, were also not monophyletic. The monophyly of the Phylliidae and Timematidae were not tested. Subfamilies Pseudophasmatinae, Bacillinae, Cladomorphinae, Phasmatinae, Necrosciinae, Lonchodinae, were not monophyletic. The Heteronemiinae was monophyletic. The monophyly of the Heteropteryginae, Aschiphasmatinae, Korinninae, Pygirhynchinae, Pachymorphinae, Palophinae, Eurycanthinae, Tropidoderinae, Xeroderinae were not tested.

Discussion

Support for results

Ensemble Consistency Index value is comparable to that reported for other phylogenetic studies that included similar numbers of taxa and characters (Sanderson & Donoghue 1989). High Bremer support values for the basal Phasmida clades give the author confidence in this part of the phylogeny. Relationships between the remaining Euphasmida species, i.e. after the *Agathemera*/*Bacillus* split on the cladogram, are weakly supported, however, with low Bremer support values of 1 and should be treated with skepticism. Denser taxon sampling, inclusion of data from the male form, and possibly, other forms of data, e.g., molecular sequence data, might yield a more robust result. If the Phasmida have undergone an evolutionary radiation, however, the large amount of homoplasy observed might not be an artifact of this particular data set, and the addition of more, and/or different, data still may not get a strongly supported topology for the crown group.

The emarginated labrum and fused thorax/abdominal sternum I have never been proposed as autapomorphies of Phasmida, but the other characters supporting Phasmida monophyly were predicted in earlier studies (see summary Tilgner *et al.* 1999). The pyriform filament bearing processes on the mesenteron, listed as an autapomorphy in Tilgner *et al.* (1999), were observed in all ethanol preserved Phasmida examined but this character was excluded from consideration since only dried specimens of *Orthomeria* and *Dajaca* (and a few other species as well) were available and the trait could not be checked. It is probably safe to say that the pyriform processes are an autapomorphy of the order since none of the outgroup taxa were observed to possess them. Other potential apomorphies excluded from the study were the vomer, a male character that the author did not have sufficient data to score, and the "swollen lateral region of the frons" (Tilgner *et al.* 1999) since it could not be objectively determined as present or absent in certain cases (e.g., Gryllidae: *Gryllus*).

Hypothesis #2: *Timema* is the sister group to the Euphasmida

Of the characters listed by Tilgner *et al.* (1999) as possible synapomorphies of Euphasmida only the cusp-like molar lobe and vestigial prothoracic sternal apophyses were included in the analysis or were not rejected as synapomorphies. The testis morphology was not considered. The development of the proventricular teeth varied extensively among Phasmida forming a gradation between species so it was excluded from the analysis. *Orthomeria* and *Dajaca* had a divided prothoracic ana-coxopleurite so the fusion of these sclerites was not upheld as synapomorphy of Euphasmida.

Hypothesis #3: *Agathemera* is the sister group to all other Euphasmida

Falsification of Bradler's hypothesis was one of the most surprising results obtained in this study. *Agathemera* seem more "primitive" *A priori* than *Orthomeria* and *Dajaca* which actually are phylogenetically more basal than *Agathemera* on the Euphasmida tree. The "primitive nature" of *Agathemera* is due to their wingless, lubberly appearance and because they occur in high elevation habitats in Chile, a region known to harbor other kinds of relic species.

Unfortunately, the author was not able to include the one morphological character that supported Bradler's (2000) hypothesis in this study due to a lack of suitably preserved specimens. This character: ventral abdominal muscles spanning the length of each segment, is a supposed plesiomorphic trait possessed by *Timema* and *Agathemera* that distinguishes them from other Euphasmida that have a shortening of these muscles. Bradler was not certain of the condition of this character for *Orthomeria* and *Dajaca* since only dried specimens of Aschiphasmatinae were available for him to study, and the muscles were poorly preserved (pers. comm.). The author feels that a slight modification of Bradler's hypothesis for *Agathemera*, namely that *Agathemera* represent the most basal clade of non-Aschiphasmatinae Euphasmida is a workable hypothesis.

The monophyly of Aschiphasmatinae

Dajaca belongs in a taxon with *Orthomeria*. Males of *Aschiphasma annulipes* (the type species of the Aschiphasmatinae) and *Prebistus peleus* were examined and they possessed the synapomorphies of the aforementioned species. The author believes that a redefined Aschiphasmatinae may be a monophyletic group. A critical evaluation of the taxa included, partiularly *Pinnispinus harmani*, and a survey of species perhaps wrongly excluded, e.g., Korinninae, needs to be conducted before more conclusions that are definite can be drawn.

Aschiphasmatinae are only found in tropical Asia and range from the Indian subcontinent to Borneo (Günther 1953). They do not occur in the Australasian region. Aschiphasmatinae species generally have greatly reduced, or absent, forewings and large colorful hindwings. They do not have a particularly stick-like appearance and look somewhat like the neotropical genera *Prisopus*, *Pseudophasma*, and *Anisomorpha*. It is interesting to note that they also bear a superficial resemblance to *Pseudoperla* species found in Eocene age Baltic amber, from Europe, particularly in regards to the proportions of the head capsule which is very globular by Phasmida standards. The author could find no potential synapomorphies linking them to the Baltic amber species, however, and believes the similar appearance is due to the retention of a plesiomorphic body form.

Taxonomy

Given the low Bremer support values for the Euphasmida crown group and the relatively small percentage of taxa studied (less than 1% of all described species) the author feels uncomfortable proposing a new taxonomic scheme to reflect the phylogeny hypothesis presented here. This is in spite of the strong support for the basal part of the phylogeny. The author hopes this study will guide future taxonomists by serving as a basis for further studies to merge our knowledge of Phasmida phylogenetics with their classification.

Hypotheses about the evolution of defense spray

All Phasmida examined possess prothoracic exocrine glands. These glands are located in the anterior lateral corners of the prothorax, but in many species the apertures of the glands are not sclerotized and the glands appear to be absent unless a careful dissection is performed to reveal them.

Of the Phasmida that were considered in this study twelve produce a noticeable spray (*Timema*, *Agathemera*, *Pseudophasma*, *Oreophoetes*, *Anisomorpha*, *Heteropteryx*, *Phibalosoma*, *Extatosoma*, *Oxyartes*, *Orxines*, *Sipyloidea*, and *Megacrania*), but only *Anisomorpha*, *Oreophoetes* and *Megacrania* have had the chemical nature of the spray analyzed. In these three taxa the sprays components are different. *Oreophoetes* produces a spray composed of Quinoline, while *Anisomorpha* and *Megacrania* produce two different cyclopentanoid monoterpenes (Eisner *et al.* 1997). The sprays of these three taxa are toxic to both vertebrates and invertebrates and their primary role is presumed to be defense. This idea is partly supported by the observation that *Oreophoetes* and *Anisomorpha* are aposematically colored. The sprays of these last two taxa are known not to be directly derived from the foodplants (Eisner *et al.* 1997).

Of the other spray releasing species considered in this study only *Agathemera* can be said to have a toxic spray and their vernacular name, "chinchemoya," translates to "powerful udder" in deference to the potent milky white fluid released by these insects upon irritation (Camousseight 1995).

While a defensive function of the sprays seems to represent an adaptation for defense, two observations question this assumption. Namely: why do the majority of species produce sprays that are seemingly non-toxic, at least to human observers (many even have a pleasant smell)? Why do species that apparently do not produce sprays at all have the exocrine glands needed to create and discharge them?

It seems that the species whose defensive compounds are most offensive to human observers are the ones that have received attention and that these sprays are exceptional and homoplastically evolved by the different taxa that possess them. This is supported by the fact that they have different chemical compositions and are possessed by species that do not come out together on the cladogram. The author also speculates that all Phasmida release a spray and if careful testing were done they would be detected. Perhaps the primary purpose of the Phasmida spray is not defense at all for the majority of species that possess them, and instead they serve as pheromones that are only secondarily co-opted for defense in a few relatively rare cases. Perhaps sexual selection or genetic drift drives variation in the composition of the spray and by chance, certain pheromone blends provide a defensive function. More research on the components of the sprays would be very interesting, particularly if the components of the spray of *Timema* were identified and compared to those of other species.

Evolution of feeding habits

It is interesting to note that the Euphasmida and several *Timema* feed almost exclusively on the leaves of Angiosperm plants. Crespi and Sandoval (2000) hypothesized that angiosperms were the foodplant of ancestral *Timema* and that gymnosperm feeding is secondarily derived. The results of this study imply a similar evolutionary pattern for the Euphasmida with the one fern feeding species, *Oreophoetes*, being well nestled within the Euphasmida that as far as the author is aware feed exclusively on Angiosperms.

Labandeira *et al.* (1993) hypothesized that Angiosperms did not increase family level evolutionary diversification of Insecta, and they cite Phasmida as a data point supporting their hypothesis. Tilgner (2001), however, demonstrated that pre-Cenozoic Phasmida fossils are not known to exist and since all extant species appear to feed primarily on Angiosperms, it suggests that the diversification of the Phasmida, particularly the Euphasmida, was due to an ability to exploit the resources provided by these plants. It is the author's opinion that Phasmida are a modern clade of Insecta that probably first appeared in the Cretaceous and subsequently diversified during the latter part of that period or during the Cenozoic era.

Sister group relationship

While the purpose of this study was not to draw conclusions about the relationships of the various orders of lower Neoptera considered, it is intriguing that based on the morphological evidence Embiidina is resolved as the sister group to Phasmida. In addition to the characters mentioned previously in the results, another character that may support a Phasmida + Embiidina sister group relationship is the micropylar plate. Based on the photographs provided by Ross (2000) *Haploembia solieri* (Rambur) clearly has a micropylar plate structure similar to *Timema* located in an identical position near the operculum of the egg capsule (compare Fig. 17, Ross 2000 to Fig. 3, Sellick 1998). The eggs of *Clothoda* examined appeared to lack the micropylar plate, so it had to be scored as absent for this taxon, but its absence could easily be a derived trait not characteristic of other Embiidina. Overall, the author believes that several of the proposed synapomorphies linking Phasmida and Embiidina are quite convincing and that they demand further evaluation of the question of Phasmida's sister group.

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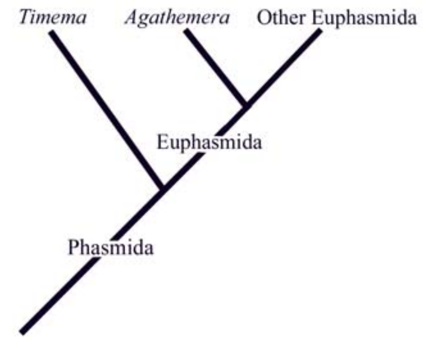
Figure legends

Fig. 1. Cladogram representing the hypotheses of Kristensen (1975), Bradler (1999, 2000), Tilgner *et al.* (1999).

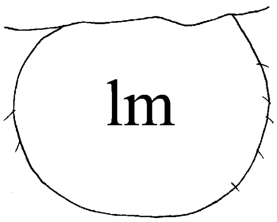
Figs 2-3. Labra, frontal views. 2. *Pterophylla*. 3. *Phyllium*.

Figs 4-5. Mandibles, right, ventrolateral views. 4. *Anisomorpha*. 5. *Melanoplus*.

Figs 6-7. Mandibles, left, ventrolateral views. 6. *Agathemera*. 7. *Phyllium*.


Timema
Agathemera
Other Euphasmida
Euphasmida
Phasmida

1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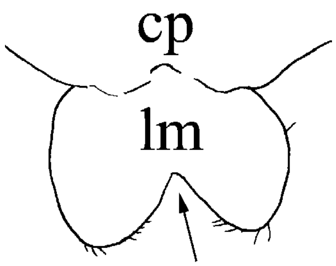
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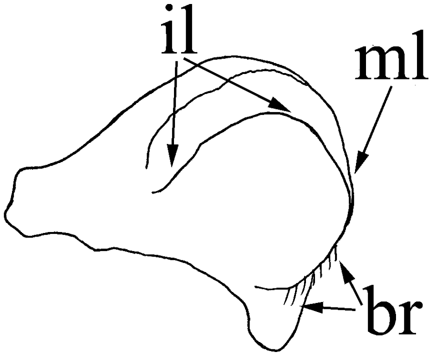
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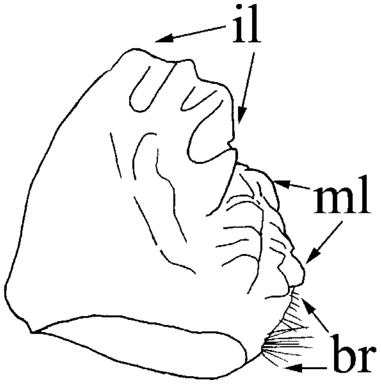
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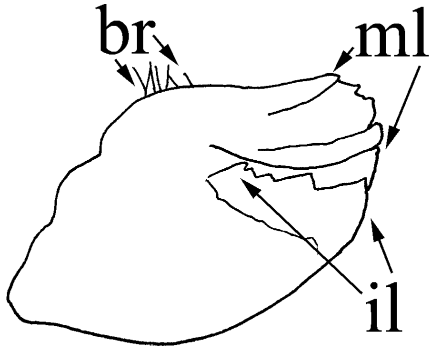
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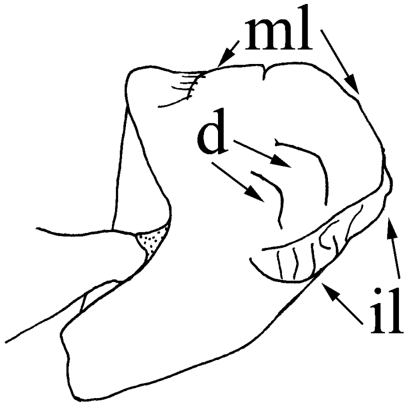
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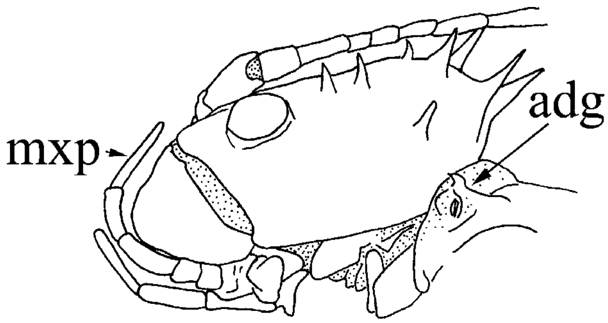
7

Fig. 8. Head, *Heteropteryx*, lateral view.

Figs 9-13. Maxillae, left, dorsal views. 9. *Pterophylla*. 10. *Agathemera*. 11. *Anisomorpha*. 12. *Oreophoetes*. 13. *Melanoplus*.



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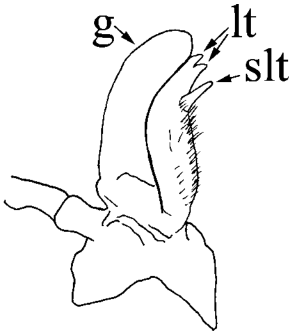

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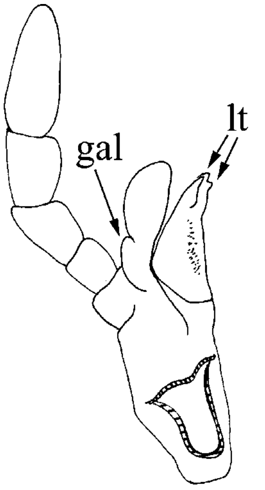

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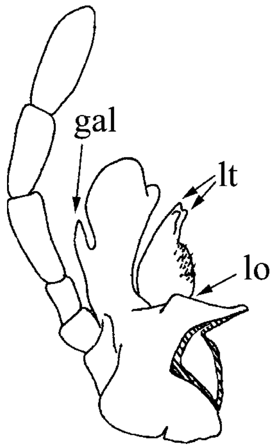

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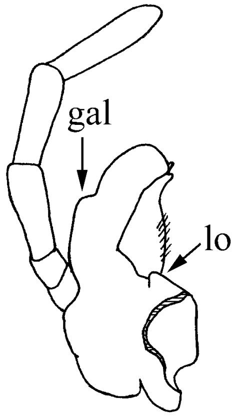

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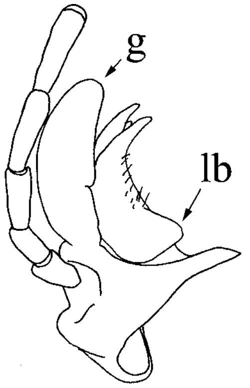

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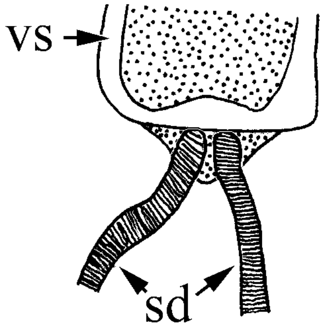
Fig. 14. Hypopharynx union with salivary ducts, ventral view, *Anisomorpha*.

Figs. 15-16. Anterolateral views of head capsules. 15. *Anisomorpha*. 16. *Clothoda*.



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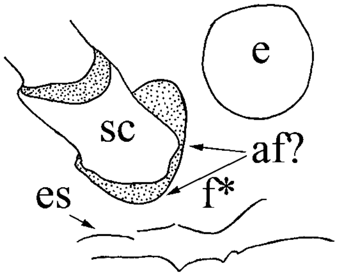

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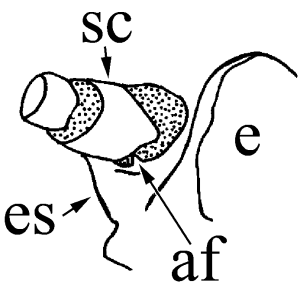
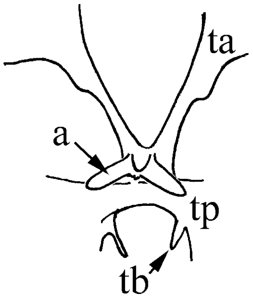


16

Figs 17-19. Tentoria, frontal views. 17. *Megacrania*. 18. *Anisomorpha*. 19. *Orxines*.

Figs 20-21. Head capsules, posteriorlateral views. 20. *Anisomorpha*. 21. *Melanoplus*.



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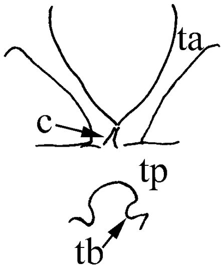

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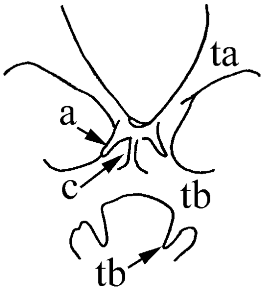

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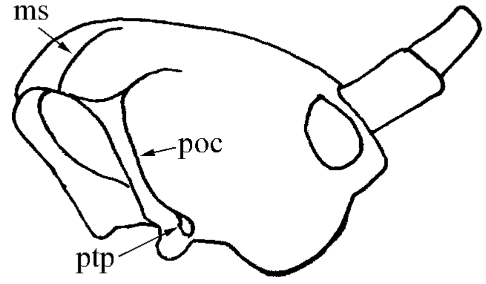

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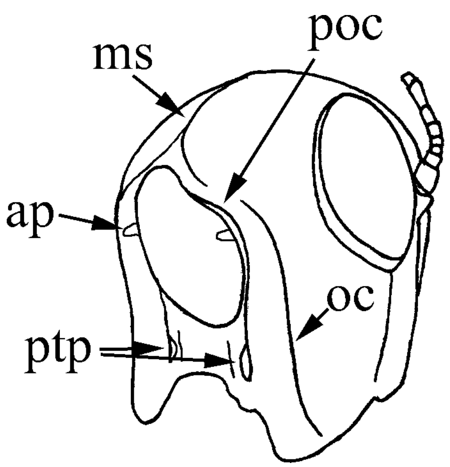

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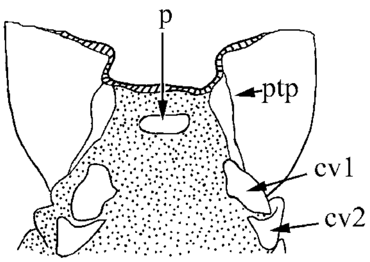
Figs 22-24. Head capsules and cervices, ventral views. 22. *Paraphasma*. 23. *Nisyrus*. 24. *Megacrania*.

Figs 25-27. Pro-thoraces, lateral views. 25. *Anisomorpha*. 26. *Agathemera*. 27. *Orthomeria*.



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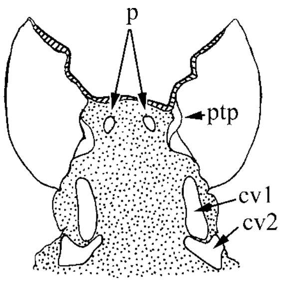

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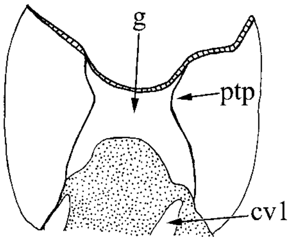

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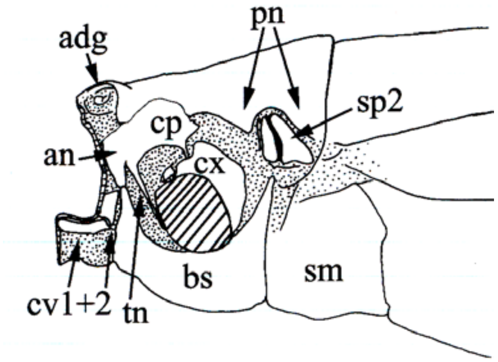

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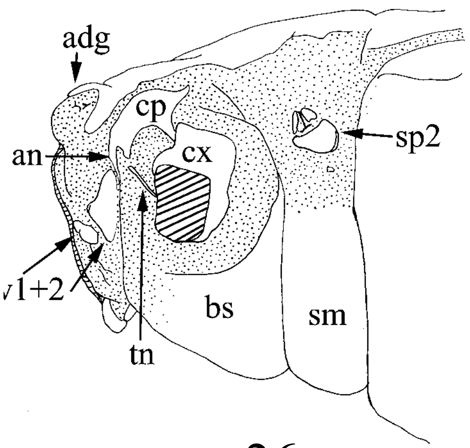

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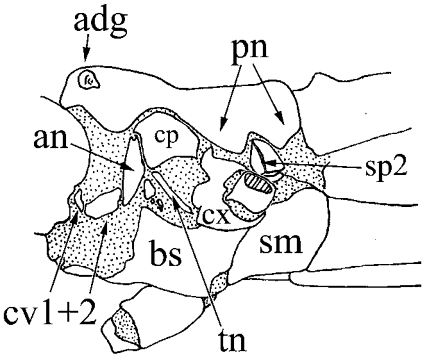

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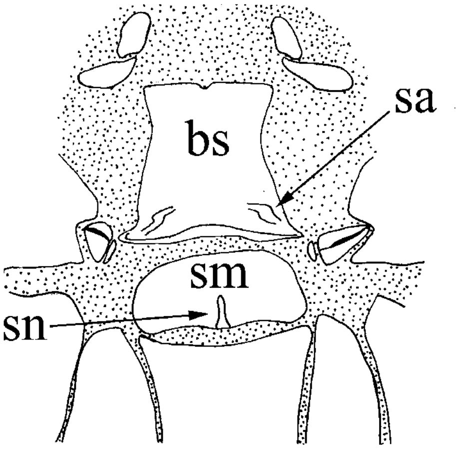
Fig. 28. Prosternum, dorsal view, internal, *Orthomeria*.

Fig. 29. Meso- and metasternum, dorsal view, internal, *Orthomeria*.

Fig. 30. Meso-, metathorax and abdominal segments, dorsal view, cleared specimen, *Megacrania*.



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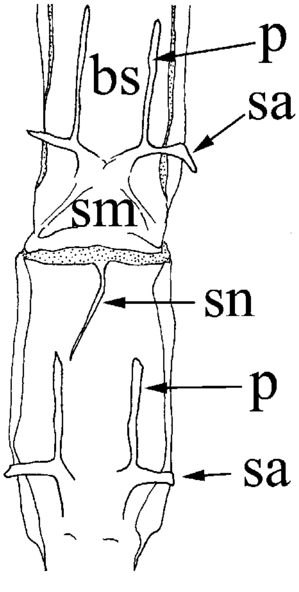

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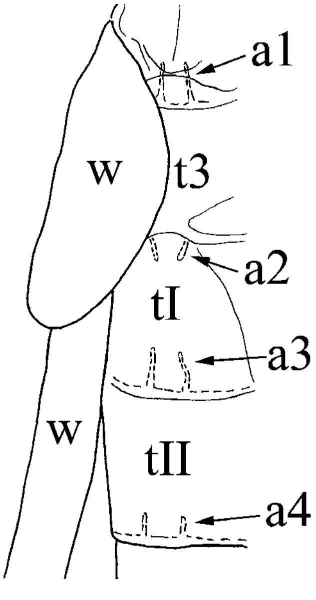
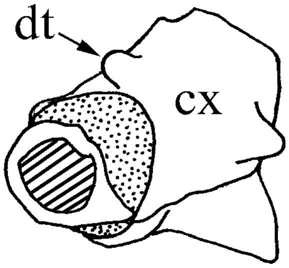


30

Fig. 31. Procoxa, left, lateral view, *Nisyrus*.

Figs 32-34. Mesothoracic legs, apices, right, ventral views. 32. *Acanthoxyla*. 33. *Agathemera*. 34. *Clothoda*.

Fig. 35. Pretarsus, mesothoracic leg, lateral view, *Orthomeria*.



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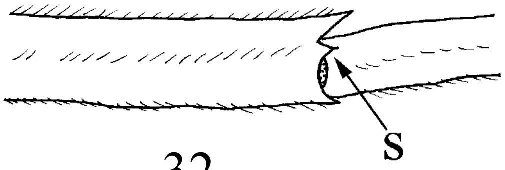

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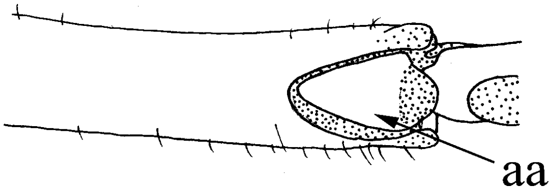

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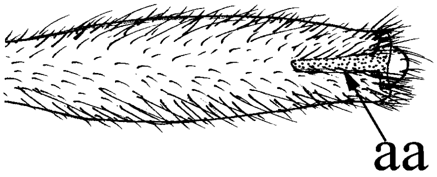

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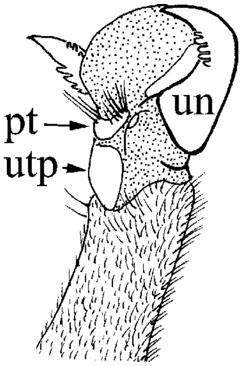
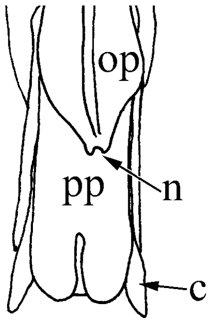


35

Figs 36-37. Terminalia, *Palophus*. 36. Ventral view. 37. Lateral view.

Figs 38-39. Crops and midguts, sagittal sections, lateral views. 38. *Pterophylla*. 39. *Acanthoxyla*.

Fig. 40. Egg capsule, frontal view, *Aplopus*.



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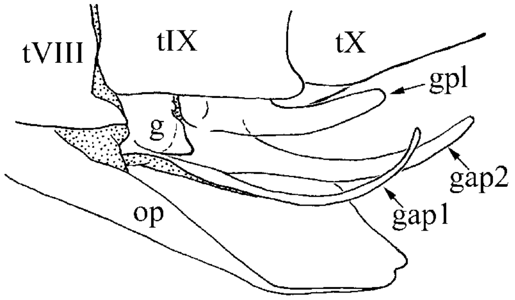

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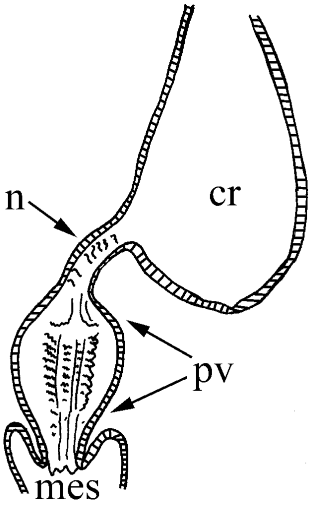

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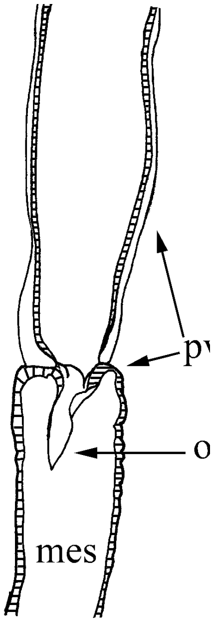

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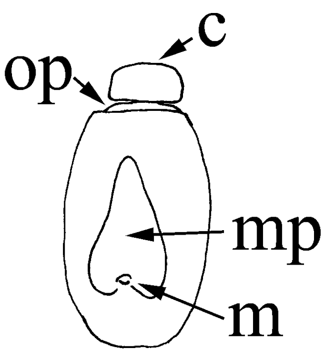

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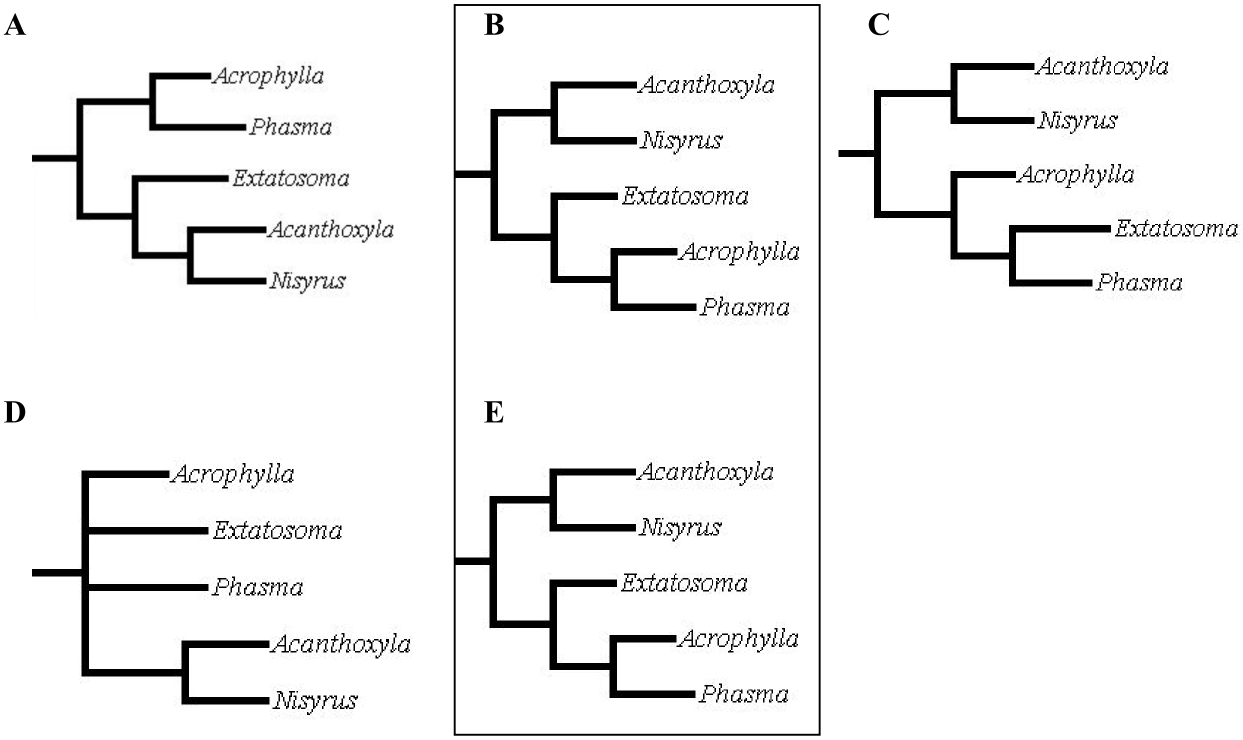
Fig. 41. A, B, C alternative topologies for the ambiguous region of the three most parsimonious cladograms. D strict consensus. E successive weighting.


A
Acrophylla
Phasma
Extatosoma
Acanthoxyla
Nisyrus
B
Acanthoxyla
Nisyrus
Extatosoma
Acrophylla
Phasma
C
Acanthoxyla
Nisyrus
Acrophylla
Extatosoma
Phasma
D
Acrophylla
Extatosoma
Phasma
Acanthoxyla
Nisyrus
E
Acanthoxyla
Nisyrus
Extatosoma
Acrophylla
Phasma

41

Fig. 42. Tree depicting preferred hypothesis of relationships. This tree was one of three most parsimonious trees. It was chosen since it matched the tree obtained from the successive weighting procedure.

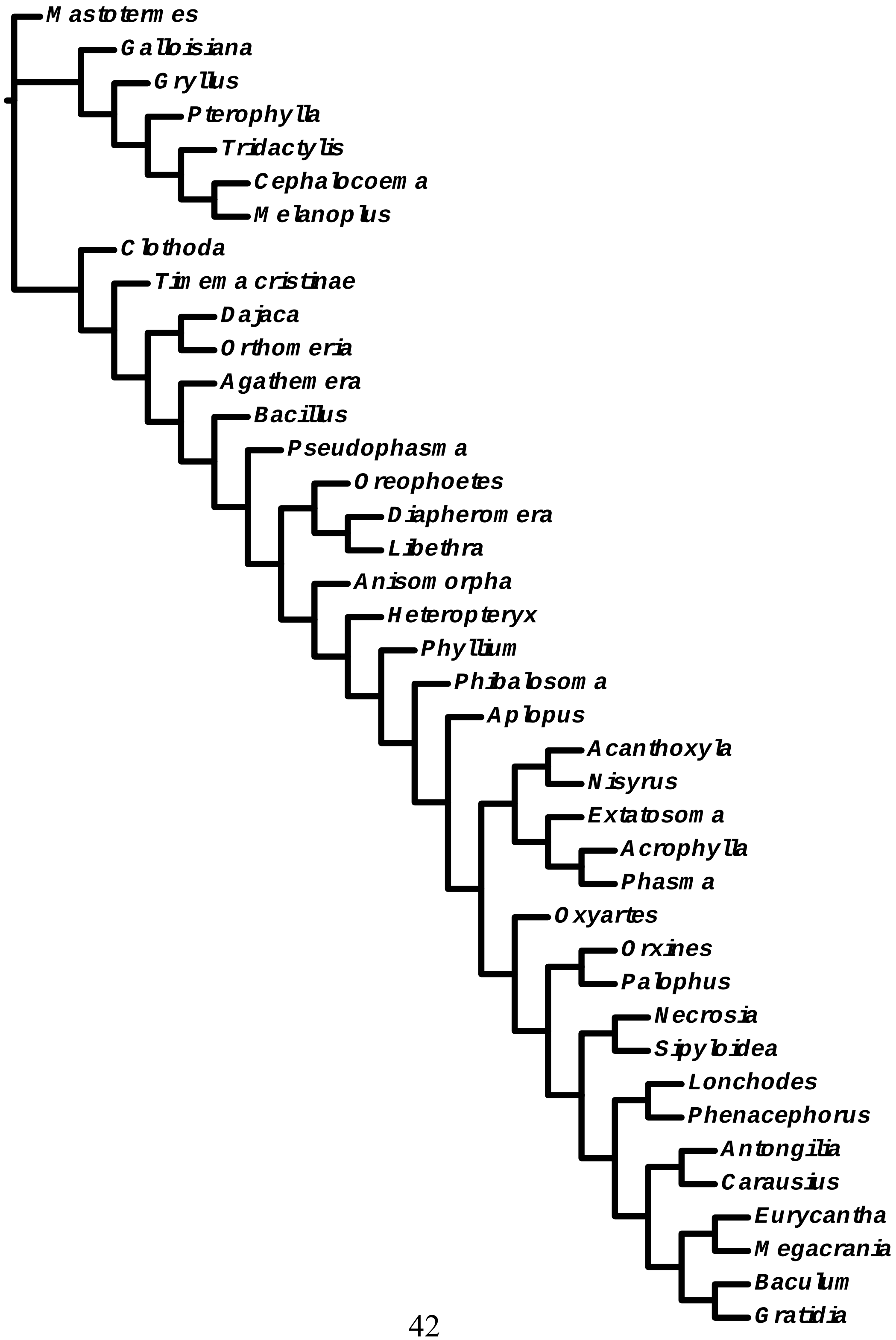
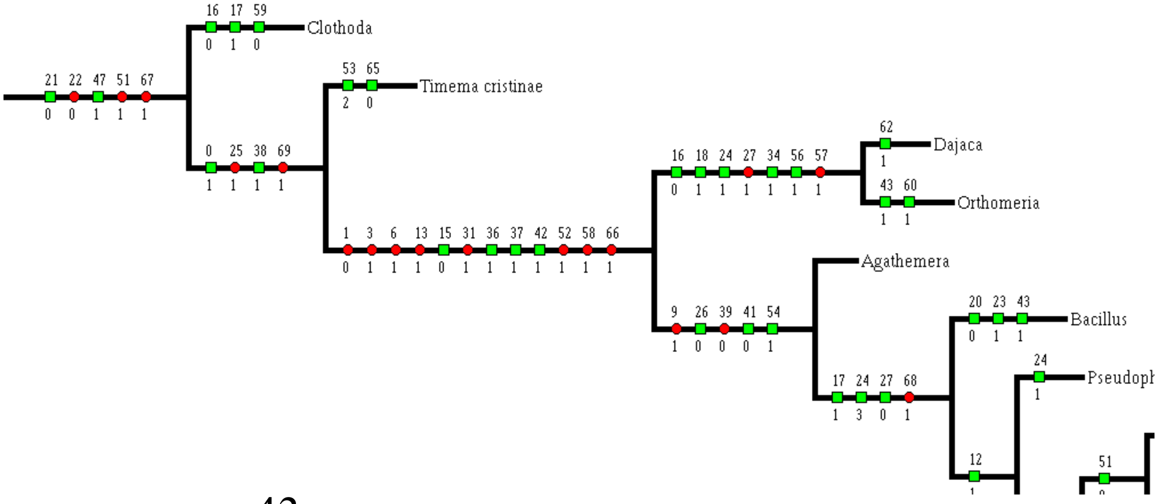
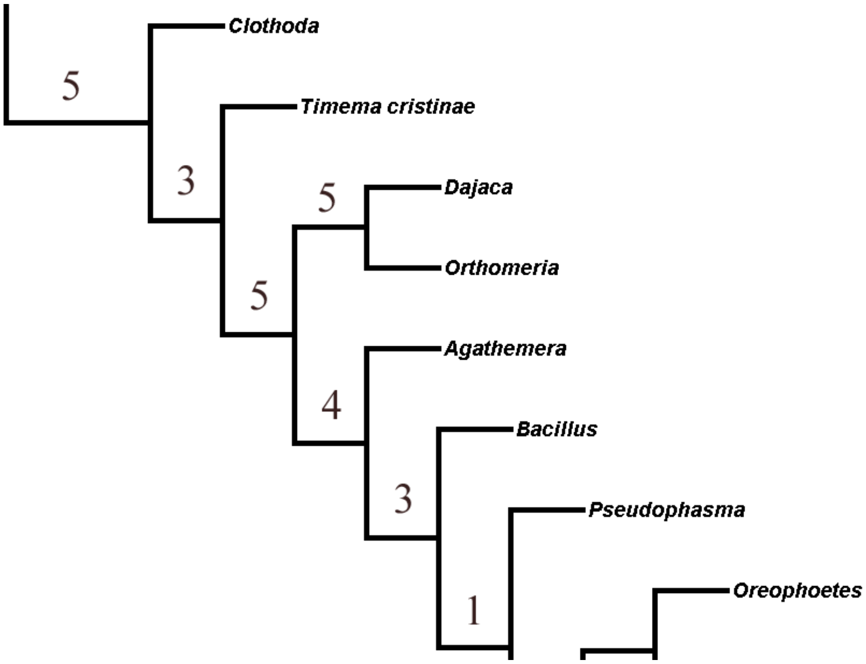

Mastotermes
Galloisiana
Gryllus
Pterophylla
Tridactylis
Cephalocoema
Melanoplus
Clothoda
Timema cristinae
Dajaca
Orthomeria
Agathemera
Bacillus
Pseudophasma
Oreophoetes
Diapheromera
Libethra
Anisomorpha
Heteropteryx
Phyllium
Phibalosoma
Aplopus
Acanthoxyla
Nisyrus
Extatosoma
Acrophylla
Phasma
Oxyartes
Orxines
Palophus
Necrosia
Sipyloidea
Lonchodes
Phenacephorus
Antongilia
Carausius
Eurycantha
Megacrania
Baculum
Gratidia

Fig. 43. Character support at the base of the Phasmida tree. Green rectangles are homoplasious characters while red circles are non-homoplasious characters.


16 17 59
0 1 0
Clothoda
21 22 47 51 67
0 0 1 1 1
53 65
2 0
Timema cristinae
0 25 38 69
1 1 1 1
1 3 6 13 15 31 36 37 42 52 58 66
0 1 1 1 0 1 1 1 1 1 1 1
16 18 24 27 34 56 57
0 1 1 1 1 1 1
62
1
Dajaca
43 60
1 1
Orthomeria
Agathemera
9 26 39 41 54
1 0 0 0 1
20 23 43
0 1 1
Bacillus
24
1
Pseudoph
17 24 27 68
1 3 0 1
12
51

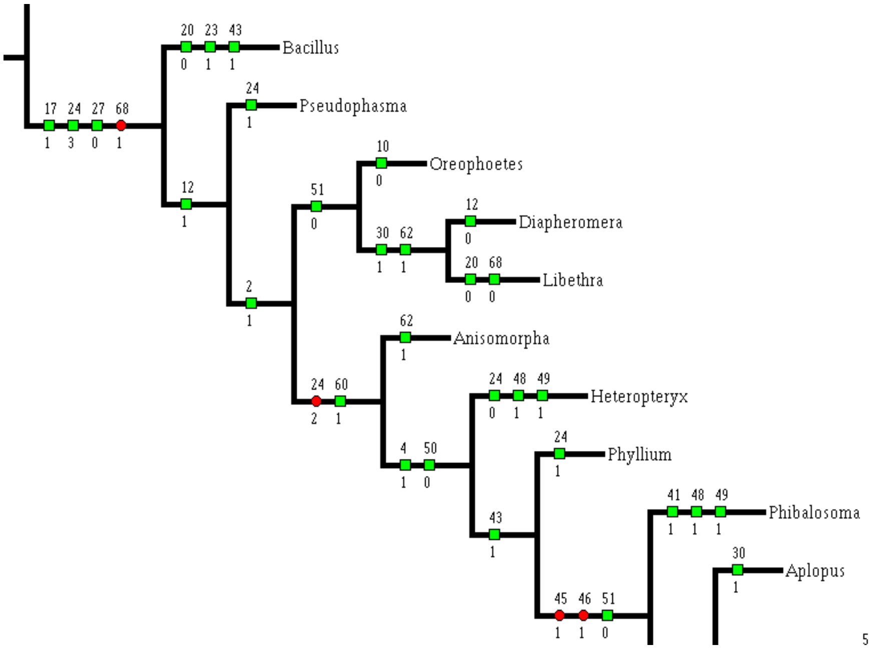
43

Fig. 44. Bremer Support values at the base of the Phasmida tree.

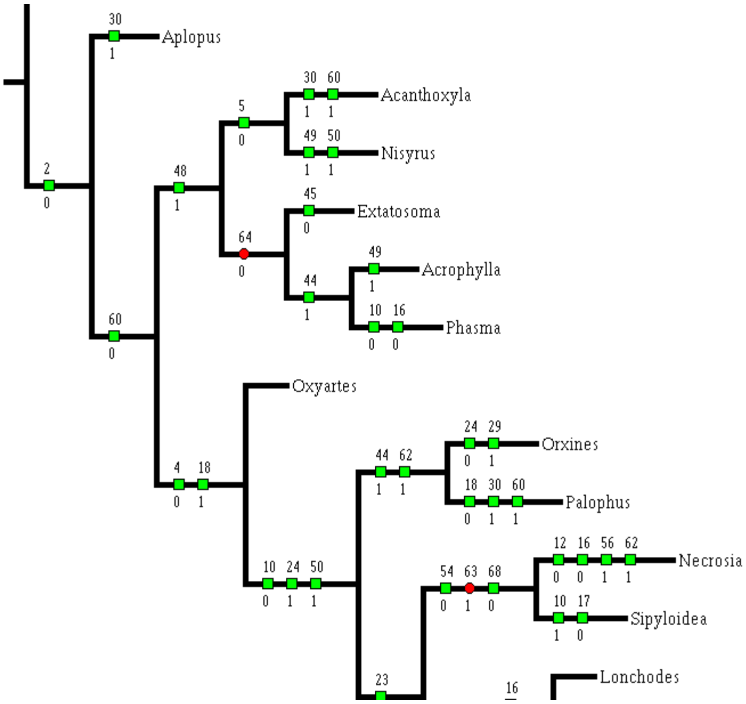

Clothoda
Timema cristinae
Dajaca
Orthomeria
Agathemera
Bacillus
Pseudophasma
Oreophoetes
5
3
5
5
4
3
1

44

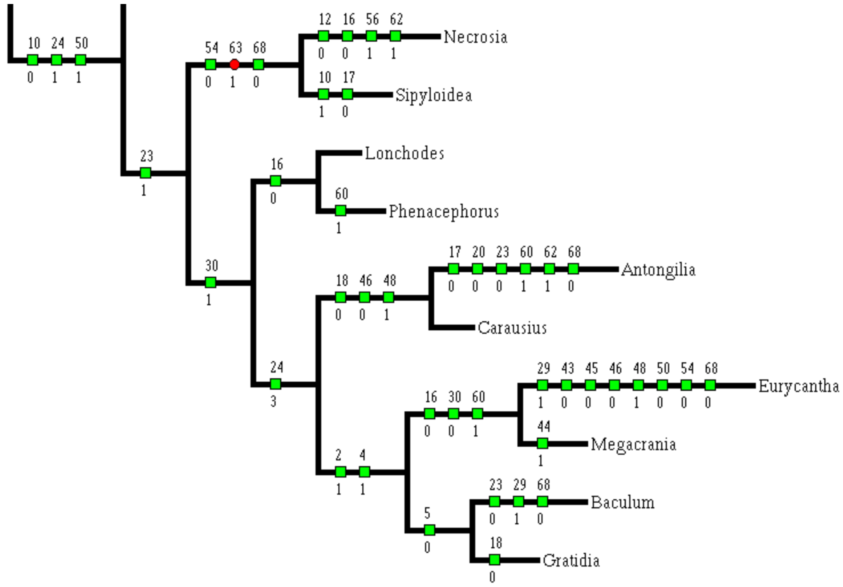
Figs 45, 46, 47. Character support within Euphasmida.


Bacillus
Pseudophasma
Oreophoetes
Diapheromera
Libethra
Anisomorpha
Heteropteryx
Phyllium
Phibalosoma
Aplopus
5

45

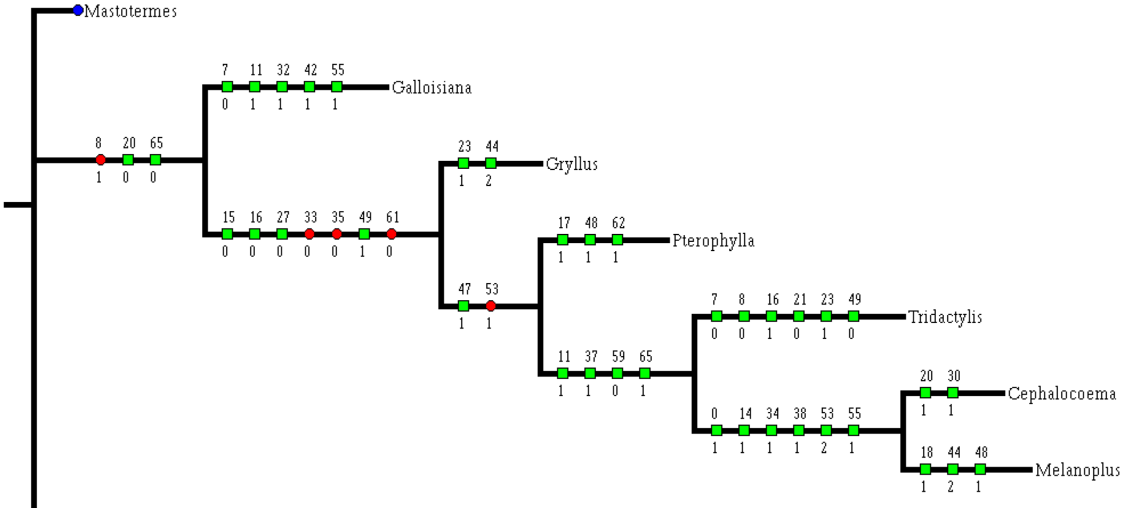

Aplopus
Acanthoxyla
Nisyrus
Extatosoma
Acrophylla
Phasma
Oxyartes
Orxines
Palophus
Necrosia
Sipyloidea
Lonchodes
16

46


10 24 50
0 1 1
23
1
54 63 68
0 1 0
12 16 56 62
0 0 1 1
Necrosia
10 17
1 0
Sipyloidea
30
1
16
0
Lonchodes
60
1
Phenacephorus
24
3
18 46 48
0 0 1
17 20 23 60 62 68
0 0 0 1 1 0
Antongilia
Carausius
2 4
1 1
16 30 60
0 0 1
29 43 45 46 48 50 54 68
1 0 0 0 1 0 0 0
Eurycantha
44
1
Megacrania
5
0
23 29 68
0 1 0
Baculum
18
0
Gratidia

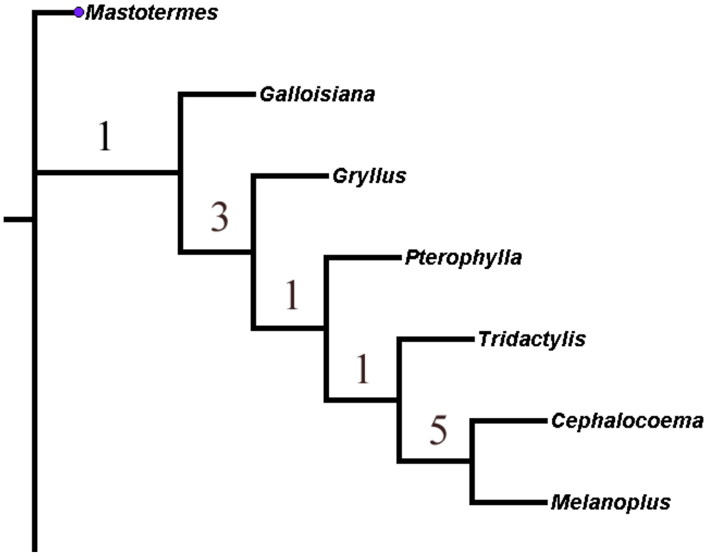
47

Fig. 48. Character support for the non-Phasmida ingroup taxa, i.e. clade of Orthoptera.


Mastotermes
7 11 32 42 55
0 1 1 1 1
Galloisiana
8 20 65
1 0 0
23 44
1 2
Gryllus
15 16 27 33 35 49 61
0 0 0 0 0 1 0
17 48 62
1 1 1
Pterophylla
47 53
1 1
7 8 16 21 23 49
0 0 1 0 1 0
Tridactylis
11 37 59 65
1 1 0 1
20 30
1 1
Cephalocoema
0 14 34 38 53 55
1 1 1 1 2 1
18 44 48
1 2 1
Melanoplus

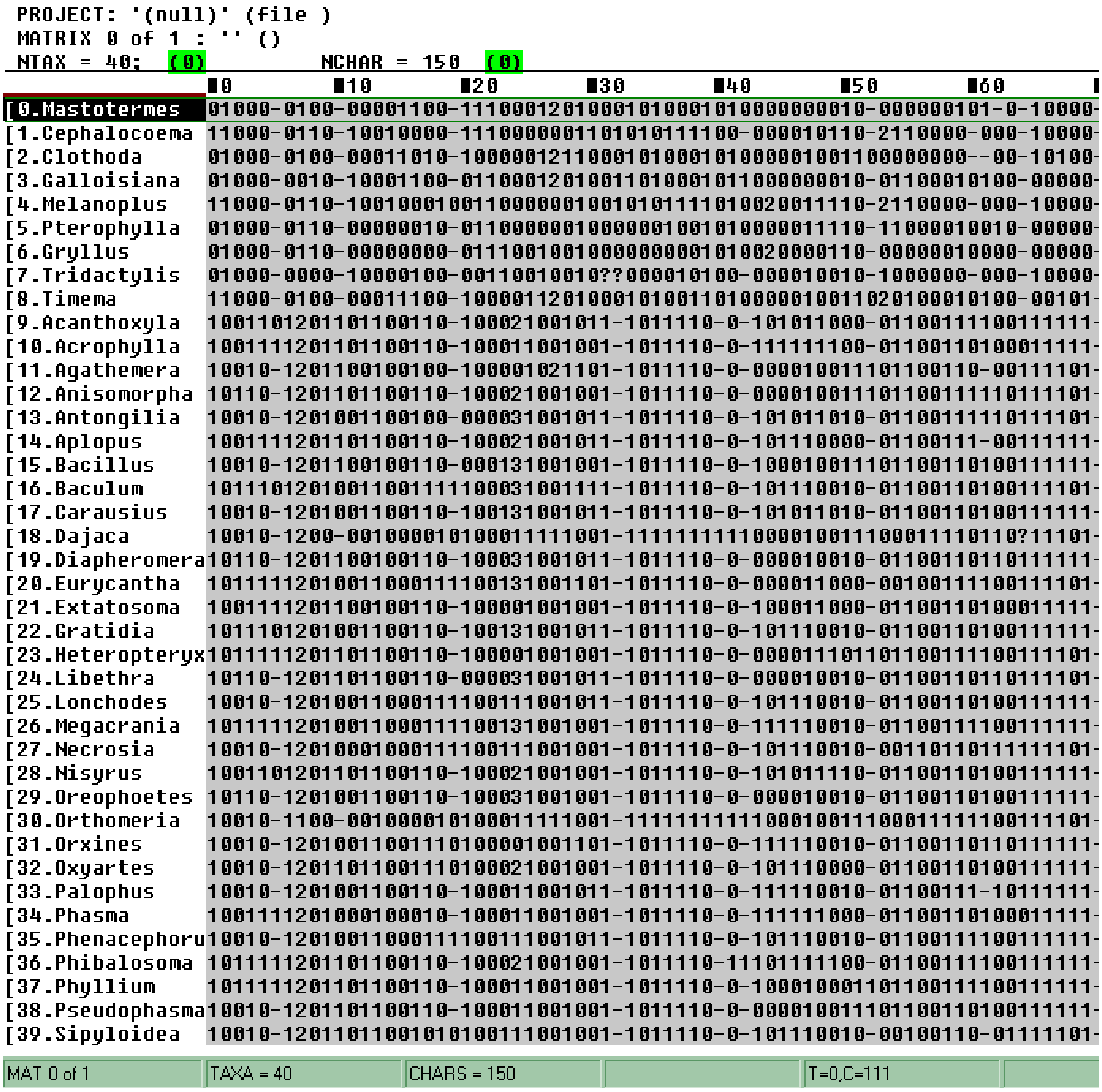
48

Fig. 49. Bremer support values for the non-Phasmida ingroup taxa, i.e. clade of Orthoptera.


Mastotermes
Galloisiana
Gryllus
Pterophylla
Tridactylis
Cephalocoema
Melanoplus
1
3
1
1
5

49

Fig. 50. Data matrix.



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50

CHAPTER 3

THE FOSSIL RECORD OF PHASMIDA (INSECTA NEOPTERA)[1]

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[1] Tilgner, E. 2001. Insect Syst. Evol. 31:473-480.

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Abstract

A review of the Phasmida fossil record is provided. No fossils of *Timema* Scudder are known. Euphasmida fossils include: *Agathemera reclusa* Scudder, *Electrobaculum gracilis* Sharov, *Eophasma oregonense* Sellick, *Eophasma minor* Sellick, *Eophasmina manchesteri* Sellick, *Pseudoperla gracilipes* Pictet, *Pseudoperla lineata* Pictet and various unclassified species from Grube Messel, Baltic amber, and Dominican Republic amber. The oldest documented Euphasmida fossils are 44-49 million years old; molecular clock dating underestimates the origin of the sister group *Timema* by at least 24 million years.

Introduction

Order Phasmida consists of over 3000 extant species[1] of cryptic phytophagous insects from temperate and tropical areas of all biogeographic regions (Bragg 1995; Günther 1953). Although originally treated as a subgroup of Orthoptera (e.g., Kirby 1904; Brunner von Wattenwyl & Redtenbacher 1908), they are now recognized as a distinct order with several classification schemes proposed (Günther 1953; Beier 1957, 1968; Bradley & Galil 1977; Kevan 1982).

Evidence exists that extant Phasmida are monophyletic (Tilgner *et al.* 1999). *Timema* Scudder is recognized as the sister group to the remainder of Phasmida, termed Euphasmida by Bradler (1999). The author believes that the clade *Timema* + Euphasmida should be recognized at the ordinal level maintaining the standard use of the ordinal name Phasmida. A problem occurs, however, when trying to determine which fossil species belong to Phasmida. Paleoentomologists have not based their taxon Phasmida on apomorphic characters (e.g., Martynov 1928; Sharov 1971) and thus it may not be a monophyletic group. The objective of this study, therefore, is to determine which fossil species belong to a monophyletic Phasmida, and to discuss the implications of this view for estimating the age of the order in geological time.

Materials & Methods

Literature on fossil species assigned to the order Phasmida was reviewed. Observations and illustrations of the fossil specimens were performed on a Leica MZ8 stereomicroscope fitted with a Wild 308700 drawing tube. List of figure abbreviations: aa- area apicalis, adg- aperture of defense gland, b- air bubble, cv- cervical sclerite, f*- swollen lateral region of frons, lm- labrum, m- mandible, ps- pleurostoma, sc- scape, sos- subocular sulcus, sp- spiracle, t1, t2, t3- thoracic terga, tI- abdominal tergum.

Results

Ragge (1955b) doubted the hypothesis of Martynov (1928) that *Aerophasma* Martynov, *Aeroplana* Tillyard, *Chresmoda* Germar, and *Necrophasma* Martynov were Euphasmida. He stated that "These genera have little in common with modern stick-insects, and the relationship should not be looked upon as more than a possibility." Based on Martynov (1928), *Aerophasma* possesses an unbranched cubitus in the forewing like Euphasmida (see Ragge 1955b). Sharov (1971), however, illustrates a two-branched cubitus for *Aerophasma*, and Gorokhov (1994) interprets it as having three branches.

An unbranched radial sector is exhibited by *Necrophasma*, *Aeroplana* (Martynov 1928), and Euphasmida (Ragge 1955b). However, it is also found in Embiidina (Ross 1991), the possible sister group of Phasmida (see Discussion), some Mantodea (Ragge 1955a), and certain Protorthoptera, e.g., Lemmatophoridae (see Carpenter 1992). It may be a plesiomorphic character state of Phasmida *sensu* Martynov (1928).

Phasmida *sensu* Sharov (1971) consists of two superfamilies, the Xiphopteridea and the Chresmodidea. The latter includes the extinct family Chresmodidae, extant Euphasmida, and *Timema*. Sharov (1971) proposed that extant Phasmida evolved from Chresmodidae. The characters he presented in support of this hypothesis are discussed below to evaluate the monophyly of Chresmodidae and *Timema* + Euphasmida.

Sharov (1971) hypothesized that the cubitus of Euphasmida has an anterior branch CuA1 fused to vein Mp2 *sensu* Ragge (1955b), and, therefore, Mp2 *sensu* Ragge (1955b) is actually a composite vein MP+CuA1 *sensu* Sharov (1971). He cited the figure of a male of *Pharnacia serratipes* (Gray) to support his hypothesis. The tracheae of the hindwing of a male of *P. serratipes* were observed by the author, and Mp2 was clearly a branch of Mp1, while Cu was unbranched. This observation supports Ragge's (1955b) claim that Cu is simple in the hindwing of Euphasmida, and it falsifies Sharov's (1971) hypothesis that CuA1 exists. An unbranched Mp+CuA1 is not a synapomorphy of Chresmodidae and Euphasmida.

A three-branched medial vein of the forewing is listed by Sharov (1971) as a character of Chresmodidae and Euphasmida. To support this claim Sharov (1971 Fig. 46 A) provided an illustration of the forewing of *Acrophylla titan* (Macleay)[3], with a three branched media. Ragge (1955b), however, stated that the media of the forewing of Euphasmida is "simply bifurcate as a rule" casting doubt on the validity of this character. A male of *A. titan* had a bifurcate media in the forewing examined by the author. A female of *A. titan* was not available, but a female of a similar species, *A. wülfingi* (Redtenbacher), had a three-branched media. It should be noted that the male of *A. titan* and the female of *A. wülfingi* differed from Sharov's (1971) figure 46 A of *A. titan* in that

MP+CuA and CuP *sensu* Sharov (1971) were not fused. A three branched medial vein of the forewing has been recorded from the possible sister group of Phasmida, Orthoptera (see Discussion) (Ensifera: Prophalangopsidae, Caelifera: Locustopsidae), and from some Mantodea (Ragge 1955a), casting doubt on the hypothesis that it represents a synapomorphy of Chresmodidae and Euphasmida.

In the hindwing, pectinate branching of the second anal vein is listed by Sharov (1971) as a similarity between *Prochresmoda* Sharov (Chresmodidae) and Euphasmida wings. This condition, however, has been recorded from several other Insecta including: Protoblattodea, Blattodea (Sharov 1971; see Ragge 1955a for a different interpretation of Blattodea 2A), certain Mantodea (Ragge 1955a), nymphs of Orthoptera (Sharov 1971; Ragge 1955a), Hagiphasmatidae (Ren 1997, see below), the extinct orders Titanoptera (Sharov 1971) and Protorthoptera (e.g., Liomopteridae Carpenter 1992). The apomorphic status of this character for Chresmodidea seems doubtful based on the broad distribution in outgroup taxa.

Finally, Sharov (1971) noted that males of *Prochresmoda* Sharov and a few Euphasmida have metathoracic femora and tibiae curved, with the ventral side of the femora bearing spines. This character is found in males of some species in the Euphasmida subfamily Eurycanthinae *sensu* Günther (1953). This character is probably a homoplasious similarity, i.e. an autapomorphy of Prochresmodidae and some species of Eurycanthinae. The possibility that it represents a synapomorphy of Chresmodidae and Euphasmida cannot be ruled out, however, since a phylogenetic hypothesis for Euphasmida does not exist to allow determination of character polarity.

Kukalová-Peck (1991) provided a discussion of synapomorphies of Phasmida *sensu* Sharov (1971). The first character mentioned is a short stemmed Cu vein of the forewing. While true of *Aeroplana*, it is not true of Euphasmida that have an unbranched cubitus (Ragge 1955b), or Chresmodidae *sensu* Sharov (1971). The characters CuA braced with MP by a temporary fusion, and CuP fused with AA1 are not applicable to Euphasmida, since the cubitus is unbranched. The anal fan is the final character listed as a synapomorphy of Phasmida by Kukalová-Peck (1991), but this character is probably a plesiomorphy (see Kristensen 1975).

Carpenter (1992) also provided character support for Sharov's (1971) taxon Phasmida, including forewings long and narrow. This character is false for all Euphasmida, because they have abbreviated forewings that are not particularly narrow. Ostensibly, this character would be plesiomorphic for Phasmida anyway, being exhibited by Orthoptera (e.g., Caelifera), Embiidina and numerous extinct and extant lower Neoptera. Forewing with straight and parallel longitudinal veins, is possibly a synapomorphy of Chresmodidae and Euphasmida, but it is not characteristic of all Euphasmida, e.g., *Phyllium* Illiger (see Ragge 1955b), and the character polarity is unknown.

A toughened remigium is characteristic of Euphasmida hindwings, but based on Sharov (1971), Gorokhov (1994), and Carpenter (1992), it is not clear if it is also possessed by Xiphopteridea and Chresmodidae *sensu* Sharov (1971).

Cerci having only one article are possessed by Chresmodidae *sensu* Sharov, Euphasmida (condition of male *Timema* probably derived, see Tilgner *et al.* 1999), and Hagiphasmatidae Ren, but are also characteristic of most Orthoptera.

In summary, the monophyly of Chresmodidea *sensu* Sharov, including taxa considered by Ansorge (1996) and Martins-Neto (1989), seems doubtful based on the currently available evidence. Both Kristensen (1975) and Gorokhov (1994) reached similar conclusions, but did not explain their reasoning. Gorokhov (1994) excluded *Timema* + Euphasmida from his taxonomic scheme, using a new ordinal name Phasmoptera, possibly inadvertently, for the resulting taxon.

Ren (1997) provided descriptions of Hagiphasmatidae (late Jurassic [Ren 1997], upper Cretaceous [Barrett 2000]); vaguely Euphasmida-like insects that possess wing venation similar to Aerophasmatidae *sensu* Gorokhov (1994) and to Phasmomimidae (Ensifera: Haglidae) *sensu* Sharov (1971). He stated that these insects "undoubtedly" belong to Phasmida (Ren 1997). The evidence he provided for this ordinal placement consisted of plesiomorphic characters, e.g., pronotum lacking lateral lobes, absence of stridulatory organs, gressorial legs, five segmented tarsi. The wing venation is unlike that of extant Euphasmida (Ren 1997; see Ragge 1955b). The elongated operculum of female Hagiphasmatidae is superficially similar to that exhibited by females of certain Euphasmida, and is not known from other lower Neoptera. This character may be a synapomorphy of Hagiphasmatidae and Euphasmida. Ren (1997) claims that females of Hagiphasmatidae possess a preopercular organ, a fold of membrane on the hind margin of sternum VII that receives the male vomer during coupling of the sexes. This structure is only known from Euphasmida, and it may be a synapomorphy of Hagiphasmatidae and Euphasmida. If the preopercular organ of Hagiphasmatidae and Euphasmida are homologous, however, the males of Hagiphasmatidae should possess the vomer an autapomorphy of Phasmida (Tilgner *et al.* 1999). The one male specimen figured by Ren

(1997) apparently has an intact abdomen, but lacks the vomer. Additional detailed observations of male abdominal apex and the female preopercular organ of Hagiphasmatidae are needed to clarify the potential phylogenetic relationship of this family to Phasmida.

Based on a fossil forewing (Upper Cretaceous, Greenland), Birket-Smith (1981) proposed a new species *Coniphasma rosenkrantzi*. Birket-Smith concluded that it belonged to Euphasmida: Pseudophasmatidae. Several characters were mentioned supporting this placement, but their phylogenetic significance is unknown. Presence of a costa, forked radial sector, branched cubitus, and two anal veins demonstrate that this wing is quite different from that of extant Phasmida studied by Ragge (1955b). The author agrees with Carpenter (1992) and Ren (1997) that the ordinal assignment is doubtful.

Pike (1994) reported a Cretaceous Phasmida fossil in Grassy Lake amber from Alberta, Canada. Voucher specimens, identified as Phasmida by Pike (TMP 96.9.631, TMP 96.9.988, TMP 96.9.1116, and TMP 96.9.835), were borrowed from Royal Tyrell Museum and determined by the author to belong to Hymenoptera and Hemiptera.

Arillo *et al.* (1997) provided a report of an insect from Baltic amber that could not be classified in an extant order. They suggested that it was Euphasmida based on its filiform antennae, five tarsal articles, arolia, and possibly one-segmented cerci. These taxonomic characters help to allow recognition of Euphasmida among extant Insecta, but they are not unique to this group. The insect described by Arillo *et al.* does not appear to possess any of the phylogenetically informative characteristics of either Euphasmida or

Phasmida (see Tilgner *et al.* 1999). The large kidney-shaped eyes and elongated coxae are traits unknown for the order. This insect cannot be considered a Phasmida.

Rasnitsyn and Ross (2000) listed "Phasmatodea" as present in a piece of Burmese amber in the Natural History Museum, London (specimen NHM In.19117-22?), based on three eggs. Given the revised interpretation of Burmese amber as Cretaceous (Zherikhin and Ross 2000), this could be the oldest credible record of Phasmida. Unfortunately, no description or figure of the egg capsules was provided so the claim is unverified.

Eocene Phasmida

A compression fossil from Grube Messel (Darmstadt, Germany) appears to represent the oldest known Euphasmida fossil dating to 49 million years ago (Lutz 1990). Based on the figure provided by Lutz (1990), this species seems to possess an elongated mesothorax, and its habitus is strikingly Euphasmida-like. The tegmina are abbreviated, and their length is comparable to that of certain Euphasmida that have relatively long forewings, e.g., *Prisopus* Latreille. The presence of a poculum-like structure at the apex of the abdomen indicates the specimen is male. A detailed description of the fossil, accompanied by a critical evaluation of its phylogenetic position, will be provided by S. Bradler (pers. comm.).

Represented by egg capsules only, *Eophasma oregonense* Sellick, *Eophasma minor* Sellick*, Eophasmina manchesteri* Sellick are among the oldest Phasmida fossils, dating to 44 million years ago (Sellick 1994). The eggs were found in the Clarno Nut Beds (Oregon, USA) amongst debris of fossilized plant material (Sellick 1994). These species possess the micropylar plate identical to that of Euphasmida. Sellick (1994) classifies them as Pseudophasmatidae *sensu* Bradley & Galil (1977), although doubt exists, since they are similar to *Tectarchus* (Phasmatidae), and the monophyly of Pseudophasmatidae is uncertain.

Pictet (in Germar & Berendt 1856) described two species presumably in Baltic amber: *Pseudoperla gracilipes* Pictet and *Pseudoperla lineata* Pictet (Note: Spahr 1992 considered *P. lineata* a junior synonym of *P. gracilipes*). In a separate publication, Berendt (1845-1856) figured *P. gracilipes*, *P. lineata* and an unidentified Euphasmida nymph. The descriptions and figures are inadequate to determine if *P. gracilipes* and *P. lineata* are Phasmida. Attempts to locate the original specimens were unsuccessful. One Baltic amber fossil containing a *Pseudoperla lineata*-like inclusion was purchased and studied by the author (Figs 1, 2). Assuming the fossil is correctly identified, *P. lineata* possesses prothoracic defense glands and swollen lateral region of frons (Fig. 2), two characters hypothesized to be autapomorphies of Phasmida (Tilgner et. al. 1999). The first abdominal tergite is fused to the metathorax, a characteristic of Euphasmida. It should be noted that Weitschat & Wichard (1998) provide illustrations of several Baltic amber Phasmida nymphs that look like *P. lineata*, or a closely related species. Also included in Weitschat & Wichard (1998) is an illustration of a Euphasmida nymph similar to that figured in Berendt (1845-1856).

Oligocene Phasmida

Electrobaculum gracilis Sharov was found in amber from Yantarnyi near Kaliningrad, Russia. The description and illustration provided by Sharov (1971) are inadequate to verify that it is a Phasmida. It is shown to possess the area apicalis, however, and this structure is unique to certain extant Phasmida, implying that the species is correctly classified. Morphological discrepancies between this taxon and extant Phasmida, e.g., the Orthoptera-like head capsule, may be inaccuracies in rendering the illustration, or they may represent actual anatomical differences. This specimen needs to be reexamined, for it may clarify the character polarity of the area apicalis, the defining character for the two Phasmida suborders Areolatae and Anareolatae (e.g., Günther 1953; Beier 1957, 1968; Bradley & Galil 1977). If the head capsule is truly Orthoptera-like, the area apicalis may have a wider distribution in lower Neoptera than previously realized. This would provide support for the hypothesis of Bradler (1999) that it represents a plesiomorphy of Phasmida. Unfortunately, A. Rasnitsyn of the Paleontological Institute of Moscow, Russia has informed the author that the type specimen cannot be located.

Agathemera reclusa Scudder was described from Florissant shale. Poorly preserved, its anatomy is suggestive of that of an "*Agathemera*-like" taxon (Scudder 1890). Ordinal placement is justified by the elongated mesothorax, a unique characteristic of Euphasmida. The type specimen is apparently lost (Meyer pers. comm.).

Miocene Phasmida

Grimaldi (1996) and Poinar (1994) figure a nymph and egg capsule (Poinar 1999) in Dominican amber. An additional nymph was studied by the author (Fig. 3). They undoubtedly are Euphasmida (e.g., egg with micropylar plate, mesothorax elongated, etc.) and may represent extant species.

Discussion

Fossils of Phasmida are extremely rare, and their sudden appearance in Cenozoic fossil record is likely an artifact of better sampling provided by the exceptional fossil deposits in which these specimens were found. A question arises: what is the real age of Phasmida in geological time?

Sandoval *et al.* (1998) hypothesized that *Timema* Scudder originated 20 million years ago based on molecular clock analysis. If Euphasmida existed 44-49 million years ago and *Timema* is the sister group, molecular clock dating underestimates the age of *Timema* by at least 24 million years.

Sister group dating also suggests that the 44-49 million-year old age for Phasmida as evinced by the fossil record is an underestimate. Based on data from morphology and molecular phylogenetic analysis, the sister group of Phasmida is either Embiidina[2] (see Kristensen 1975), or Orthoptera (see Kristensen 1975, Wheeler 1997, Flook & Rowell 1998). The oldest well-documented fossils of Embiidina are from Cretaceous Burmese amber (Ross & York 2000, Zherikhin & Ross 2000), but one questionable fossil of Permian age has been figured by Kukalová-Peck (1991). Orthoptera have a fossil record extending to the Upper Carboniferous (Carpenter 1992).

Biogeography can potentially provide indirect evidence for the age of Phasmida, but since no cladistic hypothesis for Euphasmida phylogeny exists, it is premature to draw any conclusions.

Feeding habits of extant Phasmida may provide a clue to the geological age of Phasmida. Most Phasmida (particularly Euphasmida, but also some *Timema*) feed on the leaves of angiosperms. If the phylogenetic and morphological diversification of Phasmida were largely due to the ability to exploit the resources provided by these plants, their fossil record would be expected to extend into the Lower Cretaceous.

Anomalous habitat preferences of *Timema* may also suggest a Cretaceous age for the order. Grimaldi (1999) hypothesized that xeric and Mediterranean type environments might have served as Cretaceous refugia for pollinating Insecta raising the possibility that

Timema were present in the Cretaceous, since nearly all species are found in these habitats (see Vickery 1993).

Fossil evidence for Cretaceous Phasmida is likely to come from amber (see Results), or by analogy with the Eocene fossils described by Sellick (1994), from egg capsules preserved in deposits of flowers, fruits, and seeds (see Herendeen *et al.* 1999). Further research is needed to test this hypothesis and provide a more accurate estimate of the age of Phasmida in geological time.

Footnotes

1. A few recently extinct species such as *Dryococelus australis* Montrouzier are also included.
2. In addition to the dorsal flexor paraglossa muscle (see Kristensen 1975) Embiidina and *Timema* + Euphasmida are the only lower Neoptera that possess an egg capsule with an operculum. The operculum may be a synapomorphy of Embiidina + Phasmida.
3. *Ctenomorpha titan* (Macleay) is now classified as *Acrophylla titan* (Macleay).

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Figure Captions

Fig. 1. *Pseudoperla lineata*, dorsal habitus.

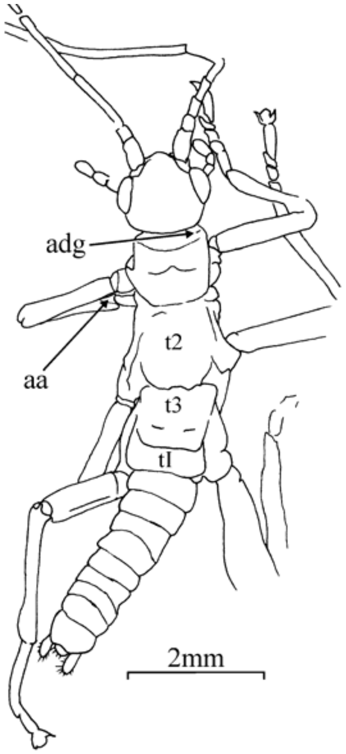

Fig. 1

Fossil Phasmida

Fig. 2. *Pseudoperla lineata*, head capsule and prothorax, lateral view. Note the swollen lateral region of frons and prothoracic defense glands, two features characteristic of Phasmida.

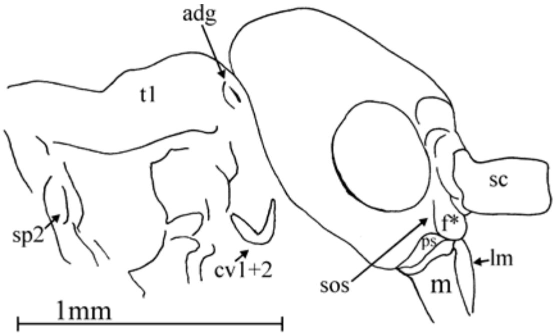

Fig. 2
Fossil Phasmida

Fig. 3. A Euphasmida nymph from Dominican amber (AMNH #DR-10-1826), lateral view. Note the elongated mesothorax characteristic of Euphasmida.

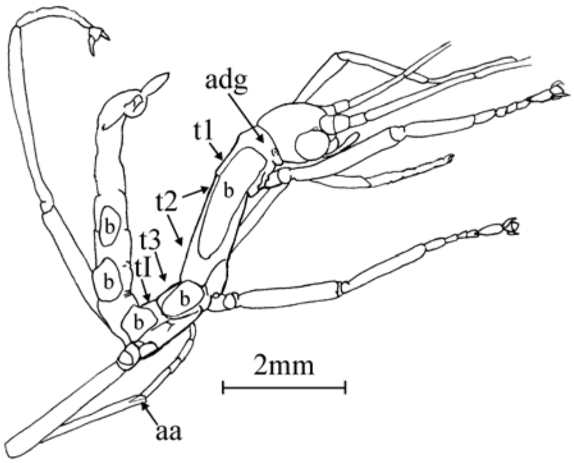

Fig. 3

Fossil Phasmida

CHAPTER 4

A NEW SPECIES OF LAMPONIUS (PHASMIDA: PHASMATIDAE)[1]

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[1] Tilgner, E.H., G.R. Camilo and C. F. Moxey 2000. Journal of Orthoptera Research. 9: 37-39. Reprinted here with permission of publisher.

Abstract

The phasmid *Lamponius nebulosus* is described and information is provided about host plants, defensive behavior, and distribution.

Key words

Caribbean National Forest, Cheleutoptera, cloud forest, dwarf forest, el Yunque, endemic, Luquillo, Phasmatodea, Phasmatoptera, stick insect

Introduction

Lamponius nebulosus was originally recognized as a new species by Moxey (1972), and given a manuscript name *Lamponius clytaemnestra* in his unpublished dissertation on the systematics of Antillean Phasmida. Van Den Bussche *et al.* (1988) referred to *L. nebulosus* as “species X” in their phenetic study of the Phasmida found in eastern Puerto Rico. They stated that “taxonomic work is being pursued” to formally name the insect, but no name has subsequently appeared for this species. To facilitate the study of this phasmid by ecologists working in the Caribbean National Forest, the opportunity is taken to publish the name and description of *L. nebulosus*.

Lamponius nebulosus new species

Diagnosis

This species can be distinguished from other *Lamponius* by the following characters: Female epiproct conspicuous, apex of operculum acute (Fig. 1); male pronotum bearing pair of strong spines, tubercles on anterior third of mesonotum strong (Fig. 4).

Description of Holotype: Body scabrous, adorned with tubercles and spines. Coloration a complex pattern of light and dark brown patches.

Head capsule rounded, bearing a pair of interocular tubercles; posteriorly four additional pairs of spines are present, penultimate pair strongest. Disk of head with a dark brown Y-shaped pattern interrupted by pale-colored midcranial sulcus. Genae with a pair of tubercles and dark brown postocular stripes.

Thorax stout, wingless. Meso- and metanotum with distinct longitudinal carina medially. Defense glands present. Pronotum bearing spines, with one strong pair posteriorly. Disk of pronotum divided by strong transverse depression and weaker longitudinal one and marked by two poorly defined dark brown longitudinal bands.

Pro- and mesothoracic coxae bear spines. Fore femora bowed basally, inner faces red. Femora marked by alternating light and dark brown bands. Dorsal and ventral carinae strongly dentate; medioventral carinae tuberculate. Apices of femora, and dorsal carinae of tibiae dentate. Note: left mesothoracic leg missing from specimen.

Mesothorax broadens posteriorly. Notum developed into a V-shaped crest of spines. Specimen appears “hunchbacked” in lateral view. Pleura spinose. Two pairs of closely set spines, posteriomedially on metanotum, demarcate anterior boundary of abdominal segment I. Row of six spines demarcates posterior boundary.

Abdominal tergites V to IX dentate, VI and VIII markedly so; III to VII flanged laterally. Epiproct prominent. Operculum strongly concave, projecting, apex acute.

Measurements: body length 72 mm, head 5.9 mm, antennae 48.9 mm (not complete), pronotum 5.1 mm, mesonotum 16.9 mm, metanotum 6.1 mm, femora: prothoracic 15.0 mm, meso- 12.2 mm, meta- 15.1 mm, tibiae: prothoracic 15.1 mm, meso- 12.1 mm, meta- 15.5 mm, tarsi: pro- 8.8 mm, meso- 8.9 mm, meta- 9.3 mm, epiproct 2.0 mm.

Holotype label data: ♀. Puerto Rico: Rio Grande, Luquillo Experimental Forest, El Toro trail, ca. 3000 feet, dwarf forest, 29/July/1996, coll: G. Camilo, on *Miconia* sp. (ANSP).

Etymology: Latin, “misty,” referring to the climate of the dwarf forest where this species is found.

Description of paratypes

Females: three specimens. Similar to Holotype in overall appearance, but vary in coloration and strength of spines. One specimen lime green with powerful spines, one light brown with weaker spines, one reddish brown with spines reduced and many tubercles absent.

Measurements: body lengths 63 to 72 mm. Smallest specimen: head 5.1 mm, antennae 35.1 mm (not complete), pronotum 5.1 mm, mesonotum 14.9 mm, metanotum 5.9 mm, femora: prothoracic 14.4 mm, meso- 11.0 mm, meta- 13.9 mm, tibiae: prothoracic 14.0 mm, meso- 11.3 mm, meta- 14.5 mm, tarsi: pro- 7.3 mm, meso- 6.1 mm, meta- 6.3 mm, epiproct 1.0 mm.

Males: two specimens. Body cylindrical, proportionally narrower than female; similar pattern of spination, but not rugose; lacking: V-shaped crest of mesonotal spines and lateral abdominal flanges. Body coloration brown, with faint patches of olive brown on femora.

Measurements: both similar size, one specimen measured: body length 66 mm, head 4.0 mm, antennae 31.2 mm (not complete), pronotum 3.9 mm, mesonotum 15.8 mm, metanotum 6.5 mm, femora: prothoracic 13.8 mm, meso- 12.3 mm, meta- 13.9 mm, tibiae: prothoracic 14.9 mm, meso- 11.9 mm, meta- 14.1 mm, tarsi: pro- 7.1 mm, meso- 5.5 mm, meta- 6.0 mm.

Paratype label data: Puerto Rico, Rio Grande, Luquillo Experimental Forest, El Toro trail, ca. 3000 feet, dwarf forest, 16/March/1993, on *Tabebuia rigida,* 1 ♂ (ANSP). Puerto Rico, Rio Grande, Luquillo Experimental Forest, El Toro trail, ca. 3000 feet, dwarf forest, 29/July/1996, coll: G. Camilo, on *Miconia*, 1 ♀ (GMNH). Puerto Rico, Rio Grande, Luquillo Experimental Forest, El Toro trail, ca. 3000 feet, dwarf forest, 16/March/1993, on *Tabebuia rigida*; 1 ♀ and 1 ♂ (GMNH). Puerto Rico: Caribbean National Forest, El Yunque, dwarf forest, G. Camilo, 13/June/1990, *Lamponius neblina* Camilo, det. G. Camilo, 1♀ (CUIC).

It should be noted that the specimens described by Moxey (1972) as *Lamponius clytaemnestra* were not reexamined for this study. They are believed to be *L. nebulosus* in spite of the males having stronger spines on the legs and body.

Type depositories

ANSP- Academy of Natural Sciences, Philadelphia, Pennsylvania, USA
GMNH-Georgia Museum of Natural History, Athens, Georgia, USA
CUIC-Cornell University Insect Collection, Ithaca, New York, USA

Discussion

Unlike *Lamponius portoricensis* Rehn, which can attain high population densities (Willig et. al 1985, Willig and Camilo 1991), *L. nebulosus* is a rare species (Camilo pers. obs.). It is only known from the cloud forest habitat of the Luquillo Experimental Forest (altitude above 762m) and based on our observations is endemic to this habitat. Its range does not overlap with that of *L. portoricensis*, which is found at lower elevations in the Tabonuco forest ecosystem.

Known host plants of *L. nebulosus* include *Miconia* sp. (Melastomataceae) and *Guzmania* sp. (Bromeliaceae) and it may feed on *Tabebuia rigida* Urban (Bignoniaceae) and *Calycogonium squamulosum* Cogniaux (Melastomataceae).

The primary defense of *L. nebulosus* against predators is its remarkable camouflage. The spiny cuticle serves to break up the body outline. Females look like sticks overgrown with lichens or moss. This type of mimicry is relatively common for Phasmida inhabiting cloud forest ecosystems, but has probably evolved by convergence (see Moxey 1971). If crypsis fails to protect the insect, secondary defenses include catalepsy, and/or the regurgitation of fluid from the mouth. Although defense glands are present, the emission of a defensive spray has not been observed.

Van Den Bussche *et al.* (1988) questioned the taxonomic placement of *L. nebulosus*, but did not propose an alternative classification. Mr. P. Brock suggested that a comparison of the eggs of *L. nebulosus* with other *Lamponius* might resolve the taxonomic status of this species. Based on the overall similarity of the adults of *L. nebulosus* to other *Lamponius*, its generic placement seems justified, until an alternative hypothesis is presented and supported.

Acknowledgements

We are grateful to Paul Brock, Philip Bragg, Tatiana Kiselyova and Joseph McHugh for providing insightful reviews of the manuscript. We thank James Liebherr, E. Richard Hoboeke, Cecil Smith, Daniel Otte, Rosser Garrison, and Michael Willig.

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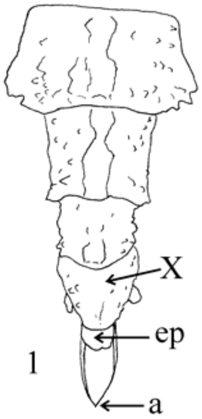
Figure Legends

Figure 1. *Lamponius nebulosus*, apex of female abdomen. Abbreviations: a- apex of operculum, ep- epiproct, x- tergum ten.

Figure 2. *Lamponius portoricensis*, apex of female abdomen.



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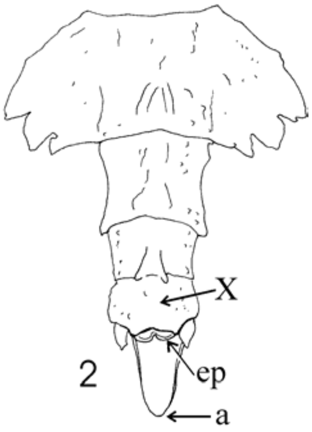

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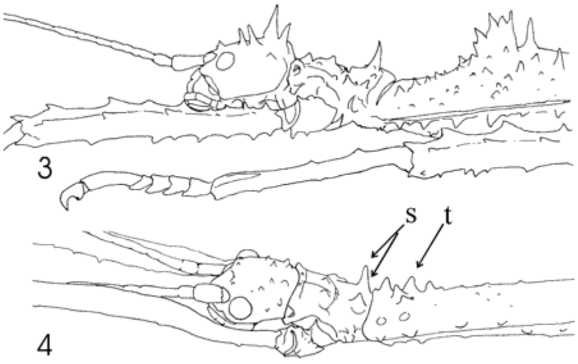
Tilgner et al. 2000

Figure 3. *Lamponius nebulosus*, female, lateral view of head and anterior portion of thorax.

Figure 4. Male, same view. Abbreviations: s- paired prothoracic spines, t- tubercles. Note: figures 3 and 4 not to same scale.



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CHAPTER 5

IS MANTOPHASMATODEA A NEW ORDER?[1]

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[1] Tilgner, E.H. Submitted to Science, 5/6/02.

Klass *et al.* (2002) linked an extinct fossil taxon *Raptophasma* to newly described species of an extant taxon *Mantophasma*, hypothesizing that they form a monophyletic group. This result is interesting and uncontroversial. However, I doubt the claim that *Raptophasma* and *Mantophasma* represent a new order of Insecta, Mantophasmatodea, and feel that these insects are aberrant members of the order Orthoptera (Ensifera: crickets, katydids, etc., Caelifera: grasshoppers, etc).

Klass *et al.* state that "while wingless and non-jumping Orthoptera can be modified almost beyond recognition, the large prothoracic pleuron unconcealed by pronotal lobes and the lack of an anterior intervalvula in the ovipositor exclude *Mantophasma* from this clade." A fully formed pleuron does not exclude *Mantophasma* from Orthoptera, however, since this condition is found in at least one family, Proscopiidae.

The presence of anterior intervalvula has never been demonstrated to be a synapomorphy of Orthoptera (some Phasmida, e.g. *Timema*, also possess it). Regardless, if the absence of the structure were an autapomorphy it would not exlcude *Mantophasma* from Orthoptera anyway. Similar arguments apply to the "lack of a longitudinal series of denticles in the proventriculus."

Klass *et al.* state that having five tarsomeres is incompatible with *Mantophasma* being subordinate in crown-group Orthoptera. However, the first three tarsomeres of *Mantophasma* are synsclerotic, making the tarsi identical to those of Orthoptera: Caelifera.

If Klass *et al.* were to present a cladistic analysis that included *Mantophasma*, showing that it is not phylogenetically subordinate to an already recognized major monophyletic lineage, it would be a sound basis for these authors to pose the hypothesis that *Mantophasma* and *Raptophasma* are a new order of Insecta. As it appears, support for their hypothesis is in the form of subjective opinions about morphological character polarity, and standard methods of phylogenetic analysis (e.g., simultaneous, unconstrained parsimony analysis performed with a computer using a data matrix that is published) were not used.

CHAPTER 6

CONCLUSIONS

This dissertation advances our knowledge about the phylogeny, fossil history and taxonomy of the insect order Phasmida. It is concluded that Phasmida and Euphasmida are monophyletic groups and the genus *Timema* is sister group to the Euphasmida clade. Contrary to preconceived expectations, *Orthomeria pandora* (Westwood) and *Dajaca monilicornis* Redtenbacher formed a clade at the base of the Euphasmida tree, one node below *Agathemera maculafulgens* Camousseight which was presumed to be the most basal Euphasmida taxon. The relationships of the basal part of the Phasmida tree were well supported, but relationships of the crown group were weakly supported. Based on the results obtained in the phylogenetic study the taxonomic groupings Areolatae, Anareolatae, Pseudophasmatidae, Bacillidae, Phasmatidae, Heteronemiidae, Pseudophasmatinae, Bacillinae, Cladomorphinae, Phasmatinae, Necrosciinae, Lonchodinae, *sensu* Bradley & Galil were not monophyletic, but Heteronemiinae were monophyletic. Given the low Bremer support values for the Euphasmida crown group and the relatively small percentage of taxa studied (less than 1% of all described species) the author felt uncomfortable proposing a new taxonomic scheme to reflect the phylogeny hypothesis. This is in spite of the strong support for the basal part of the phylogeny.

Based on a study of the fossil evidence, Phasmida cannot be considered an ancient group of Insecta, since their fossil record only dates back only to the middle

Eocene. The fossil species all belong in the Euphasmida clade, but their exact placement is uncertain. The oldest documented Euphasmida fossils are 44-49 million years old indicating that molecular clock dating provided by Sandoval *et al.* (1998) underestimates the origin of their sister group *Timema* by at least 24 million years. The author believes that during the Cenozoic era, or possibly the Cretaceous period, Euphasmida underwent an evolutionary radiation exploiting the new resources and niches provided by the leaves of angiospermous plants. Their amazingly cryptic appearances seem to be the result of natural selection imposed by vertebrate predators, e.g., birds that use vision as the primary means to hunt for these insects.

A new endemic species of Phasmida was discovered in Puerto Rico and it was named *Lamponius nebulosus*. In addition to the description of the species, information was provided about its host plants, defensive behavior, and distribution on the island.

Finally, an argument is made against the claim that Mantophasmatodea represents a new order of Insecta. The evidence provided by Klass *et al.* (2002) does not necessarily exclude Mantophasmatodea from the Orthoptera and they may be highly aberrant members of this taxon. Although not included in the letter to the editor of Science, the author notes that at least one morphological character, antennae inserted high on the frons, is characteristic of crown group Orthoptera suggesting that Mantophasmatodea may be subordinate to this taxon.

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