

Differences in genitalia structure and function between subfamilies of longhorn beetles (Coleoptera: Cerambycidae)

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Abstract Beetle genitalia are usually described only for taxonomic purposes without considering the possible function of structures. Exceptions are sporadic detailed studies on single species. We studied genital structures in the subfamilies of Cerambycidae and outlined assumptions on the function of these structures and the implications for the phylogeny of the Cerambycidae. We found that male genitalia in particular are taxon-specific on a higher taxonomic level; e.g., the parameres are widely variable in Cerambycinae, while in most Lamiinae species they appear relatively uniform and differ from those of the Cerambycinae. Internal sac structures are very different among the various subfamilies. Small backwards-pointing spines are the most common armature of the internal sac. The female genitalia are less variable, although ovipositor morphology may differ among subfamilies. In most species, the connection between the mates during copulation is achieved by the long internal sac and the ovipositor only, whereas the median lobe and parameres are in contact with the female abdomen only at the beginning of copulation. Cerambycinae and Lepturinae have a basal swelling of the endophallus to prevent it from sliding back into the male abdomen during copulation. The long internal sac functions in connecting the mates and guaranteeing the sperm transfer.

Keywords Parameres · Endophallus · Ovipositor · Copulation · Functional morphology

Introduction

Because Cerambycidae are often very colourful and their external morphology is usually species-specific, there is no need to study the genitalia for taxonomical purposes in many taxa. Even in taxa, in which genital structures have been described, almost no ideas on their function are developed.

We tested the following assumptions on the functional roles of parameres and endophallus:

1. The parameres expand the female genital opening.
2. The parameres assure a solid mechanical grip between the mates.
3. The endophallus fixes the male genitalia in the female ovipositor.
4. The larger spines of the endophallus, found in some species, penetrate the female genitalia.
5. The basal swelling of the endophallus in many longhorn beetles serves to prevent the internal sac from sliding back into the male abdomen during copulation.

Phylogenetic relationships between the 13 subfamilies of longhorn beetles accepted by Lawrence and Newton (1995) are not sufficiently resolved yet. The most recent phylogenetic analysis of Cerambycidae subfamilies is Napp (1994). She compared 11 of the cerambycid subfamilies accepted by Lawrence and Newton (1995) with 128 morphological characters of adults and larvae, but only few characters of the genitalia. Cerambycinae have been very well represented in this study by 86 species, but there have been only 1–3 species of the other groups. Hence another aim of the present study was to gather phylogenetically promising characters of the genitalia for future phylogenetical analyses of Cerambycidae.

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Materials and methods

Specimens of 138 species of cerambycids of the subfamilies Cerambycinae (36 species studied), Disteniinae (1), Lamiinae (56), Lepturinae (29), Parandrinae (2), Prioninae (9) and Spondylidinae including Aseminae (5) have been collected in 13 countries all over the world, but mainly in Central Europe, dissected or studied under SEM. Specimens were preserved in 70% ethanol until dissection. To gather data on the other subfamilies, which have not been collected for this study, several references, most of them taxonomical, were surveyed, and the results were tabulated.

Additional copulating pairs of 23 species of the subfamilies Cerambycinae, Lamiinae, Lepturinae and Spondylidinae, mainly collected in Germany, have been frozen with ice spray in the field and dissected or studied histologically (stained with Richardson's solution). The advantage of ice spray compared to fluid nitrogen is, that it is easier to obtain and to transport.

Results

Differences of genital structures between the subfamilies of longhorn beetles

The genitalia of the four subfamilies comprising the majority of species (Prioninae, Lepturinae, Cerambycinae and Lamiinae) share common characters in all substructures on the tribe rather than the subfamily level. Table 1 gives an overview of character states of the median lobe, parameres and endophallus in males, as well as of the ovipositor and spermatheca in females. Two of these characters, the male parameres or lateral lobes and the female ovipositor, are described here in detail.

In the South American Oxypeltinae, parameres are strongly reduced (Fragoso 1985b) and in the genus *Stenhomalus* (Cerambycinae: Obriini) they are entirely absent (Napp 1994), while—as far as known—all other longhorn beetles have well developed parameres that are usually divided. We know only few tribes of Cerambycinae (Hyboderini, Molorchini, Obriini, Rhopalophorini and Stenopterini) containing at least one species with undivided parameres (own observations and Fragoso 1985a; Mermudes and Napp 2004). Promising characters other than the length of the divided part, are paramere width, shape (divergent, convergent or parallel), and number and position of setae. Some examples for parameres with different character states are depicted in Fig. 1.

The shape and sclerotization of the ovipositor are strongly correlated with ecological habits. For example, wood-boring species lay their eggs under the bark or in crevices of trees, whereas species feeding on herbs do not

need to penetrate rigid tissues with their ovipositors. Therefore, it is possible to detect differences in the structure of the ovipositor between different subfamilies: in longhorn beetles, which lay their eggs beneath the bark, like *Scatopyrodes beltii*, ovipositors are much heavier sclerotized than in other species (Fig. 2). Oxypeltinae are unique for lacking an ovipositor, and the 8th sternite is instead modified for oviposition (Fragoso 1985b). As far as known, all other subfamilies of longhorn beetles have an ovipositor, which varies in length relative to the body length, degree of sclerotization, size and position of the styli (apical or lateral to the coxites), and length of the coxites. While in some subfamilies the structure of the ovipositor is highly variable among tribes, in others, such as the species-rich Lamiinae, it is rather uniform: the styli form a small group and are situated apical to the long coxites (e.g., *Parachalastinus rubrocinctus* in Fig. 2).

Function of certain structures of the genitalia

In the species we observed, the only connection between male and female genitalia during copulation is the insertion of the male endophallus into the ovipositor. In many Cerambycidae this connection can be seen outside the male and female abdomina. The parameres are in contact with the female genital opening or abdomen only at the very beginning of the copulation if at all, when the male abdomen touches the female abdomen. However, because the parameres are hidden beneath the male pygidium, we could not detect their exact position and movement. Shortly after the first contact, the ovipositor is pulled out of the female's abdomen by the endophallus (Fig. 3), a scenario which we observed in various species of Cerambycinae and Lepturinae. In these groups, the time from the beginning of copulation until the ovipositor is pulled out lasts not longer than a few seconds. In contrast to the Cerambycinae and Lepturinae, in the Lamiinae studied the male and female abdomina are close to each other probably throughout the copulation (Fig. 4).

All Cerambycinae and Lepturinae studied have a basal swelling on their inflated endophallus (Fig. 5), whereas the Lamiinae studied do not have such a swelling, or a very small one (Fig. 6). This bulge is bordered by two sclerites of the basal part of the endophallus.

The middle and apical parts of the endophallus bear backwards-pointing spines of species-specific size and number (Figs. 7, 8). However, even in species with large spines on the endophallus (e.g., *Chlorophorus sumatrensis*), we did not observe their penetration into the ovipositor at 500× magnification. Similarly, we observed the endophallus inside the ovipositor in histological sections of a copulating female's abdomen, but although spines were clearly visible, there were no signs of penetration into or

Table 1 Genital characters of Cerambycidae subfamilies

	Median lobe	Parameres	Endophallus	Ovipositor and bursa copulatrix	Spermatheca
Vesperinae (after Saito 1990; Sama 1983)	Long, slender	Horseshoe-shaped	With flagellum of short length	Very long, no bursa copulatrix	Not clearly separated from spermathecal duct
Oxypeltinae (after Fragoso 1985b; Napp 1994)	Basal apophyses very thin, dorsal and ventral lobe grown together	Reduced or absent	Very long	Absent; 8th sternite reshaped for oviposition	Unknown
Disteniinae (after Ehara 1954; Saito 1990 and current study)	Long, slender; basal apophyses very short	Long and parallel	Two long chitinized rods (flagellum?)	Very long, styli apical; bursa copulatrix strongly reduced	No spermathecal duct, but spermatheca with coils
Anoplodermatinae (after Dias 1986)	Short; basal apophyses short and sturdy	Medium length, similar to Prioniinae	Unknown	Baculi strongly sclerotized; styli lateral and reduced	Unknown
Philiinae (after Saito 1990; Wu and Jiang 2000)	Medium length, slender; basal apophyses long	Medium length	With flagellum	Long, slender; styli apical; no bursa copulatrix	Membranous; short spermathecal duct
Parandrinae (after Saito 1990 and current study)	Apically pointed; basal apophyses wide	Short	Unknown	Similar to Anoplodermatinae; strongly sclerotized; dorsal and ventral part	Duct short; basal part of spermatheca wide
Prioniinae (after Edwards 1961; Kuboki 1980 and current study)	Usually short and wide; basal apophyses long	Usually short to medium length	Usually long, no real flagellum	Length variable among tribes; styli lateral; bursa copulatrix large	Duct short; spermatheca usually of medium size
Spondyliidinae (after Raske 1973 and current study)	Usually curved; basal apophyses usually long	Short to medium length	Unknown	Usually short to medium length; bursa copulatrix usually of medium size	Duct short to medium length; long and U-shaped
Apatophysinae (after Ohbayashi 2007; Saito 1990; Villiers 1982)	Madagascar: apically trilobate, very slender; Oriental: apically pointed, medium width	Madagascar: long, slender; Oriental: medium length	Medium length, only few sclerites, no flagellum	Madagascar: unknown; Oriental: long, strongly sclerotized	Madagascar: unknown; Oriental: duct short; spermatheca weakly sclerotized
Necydalinae (after Niisato 2004; Saito 1989)	Medium length, strongly curved	Long, slender	Unknown	Long; styli apical; bursa copulatrix almost totally reduced	Duct wide, short; spermatheca small
Lepturinae (current study)	Variable among tribes	Variable among tribes	Variable among tribes, no flagellum	Variable among tribes	Variable among tribes
Cerambycinae (current study)	Variable among tribes	Variable among tribes	Variable among tribes, no flagellum	Variable among tribes	Variable among tribes
Lamiinae (current study)	Variable among tribes	Variable among tribes	Variable among tribes, some with flagellum	Length variable among tribes, but styli always tiny and apical	Variable among tribes

Fig. 1 Variation in paramere morphology of selected species. From left to right: *Rutpela maculata* (Lepturinae), *Leptura quadrifasciata* (Lepturinae), *Spondylis buprestoides* (Spondylidinae), *Obrium brunneum* (Cerambycinae). From Hubweber and Schmitt (2006)

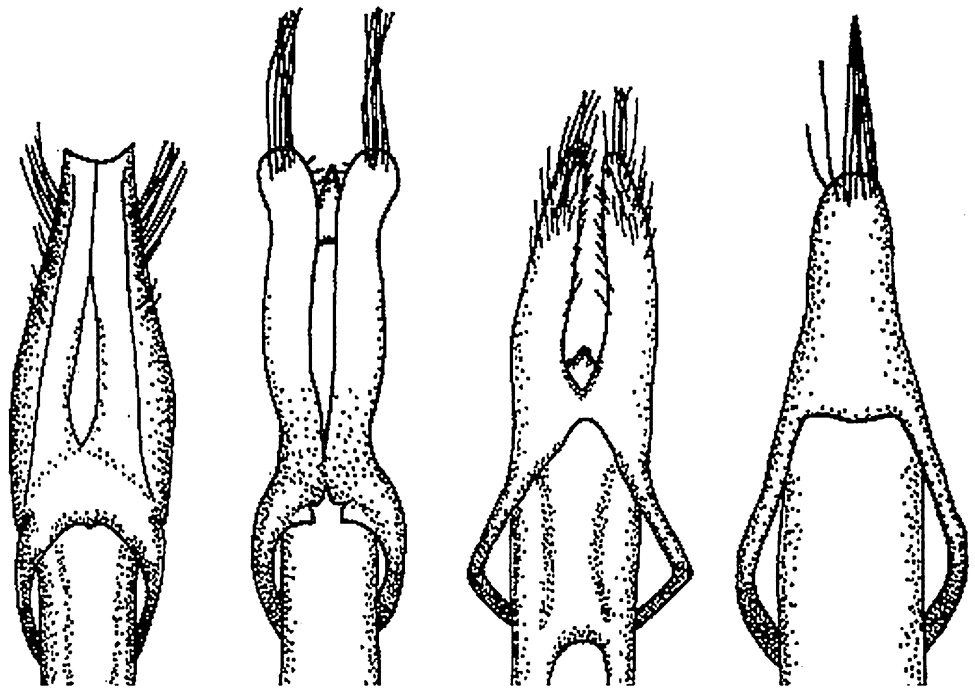


Fig. 2 Apical parts of the ovipositor. From left to right: *Anastrangalia dubia* (Lepturinae), *Chlorophorus sartor* (Cerambycinae), *Parachalastinus rubrocinctus* (Lamiinae) and *Scatopyrodes beltii* (Prioninae). Scales: 0.3 mm



Fig. 3 Mating pair of *Pyrrhidium sanguineum* (Cerambycinae) showing the only connection of genitalia between mates outside the male and female abdomens. Scale: 0.5 mm



Fig. 4 Mating pair of *Phytoecia cylindrica* (Lamiinae). The male is covering the female genital opening with its pygidium. Scale: 0.5 mm



Fig. 5 Aedeagus with basal part of the endophallus of *Chlorophorus sumatrensis* (Cerambycinae), showing the basal swelling just in front of the aedeagus. Scale 0.5 mm



Fig. 6 Aedeagus with basal part of the endophallus of *Phytoecia cylindrica* (Lamiinae), without any basal swelling of the endophallus. Scale 0.5 mm

damage of the ovipositor (Fig. 9), as could have been expected with respect to the large spines.

Discussion

In the present study we were able to test some of our assumptions regarding functional aspects of the genitalia of longhorn beetles:

Assumption 1 The function of the parameres is to expand the female genital opening.

The male pygidium covered the parameres during the first few seconds of copulation, when contact between the parameres and the female genital opening was possible. Thus, we cannot provide empirical data with regard to this assumption. However, the direct contact of the male and female abdomens at the beginning of the copulation is very brief, in contrast to the situation in carabids or scarabs, for example, in which males have been shown to trigger the opening of the female genitalia by stimulation with the parameres (Alexander 1959; Eberhard 1993). Moreover, the cerambycid parameres are much more delicate than the corresponding contact zone on the female abdomen, hence

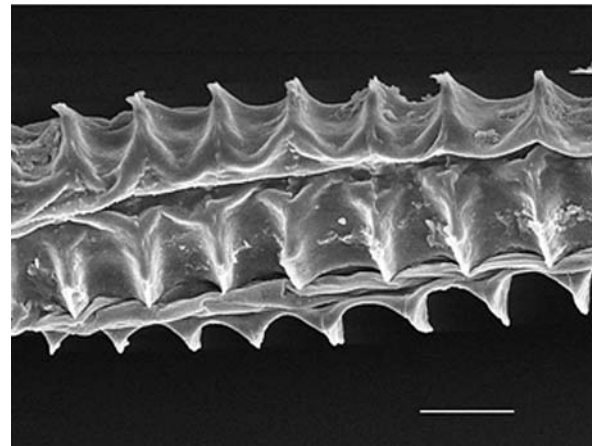


Fig. 7 Above distal part of the endophallus of *Chlorophorus sumatrensis* (Cerambycinae), bearing prominent spines. Scale 1 mm. Below spines on the middle part of the endophallus of *Chlorophorus sumatrensis* under SEM. Scale 0.1 mm

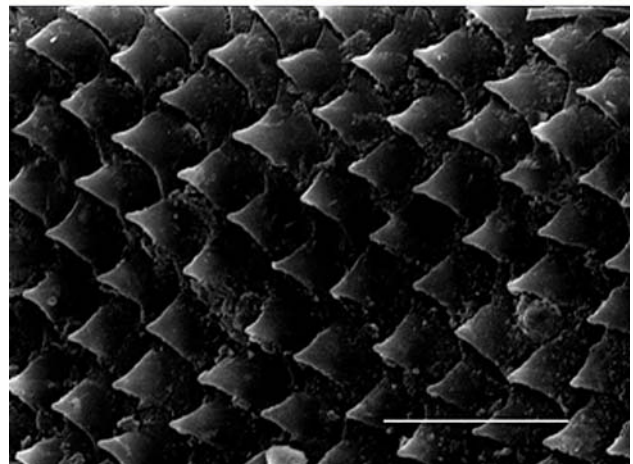


Fig. 8 Above distal part of the endophallus of *Alosteria tabacicolor* (Lepturinae), bearing tiny spines not visible at this magnification. Scale 1 mm. Below spines on the middle part of the endophallus of *Alosteria tabacicolor* under SEM. Scale 0.03 mm

we speculate that the parameres serve for aiding physical orientation of the male genital structures rather than for any form of communication between the mates.

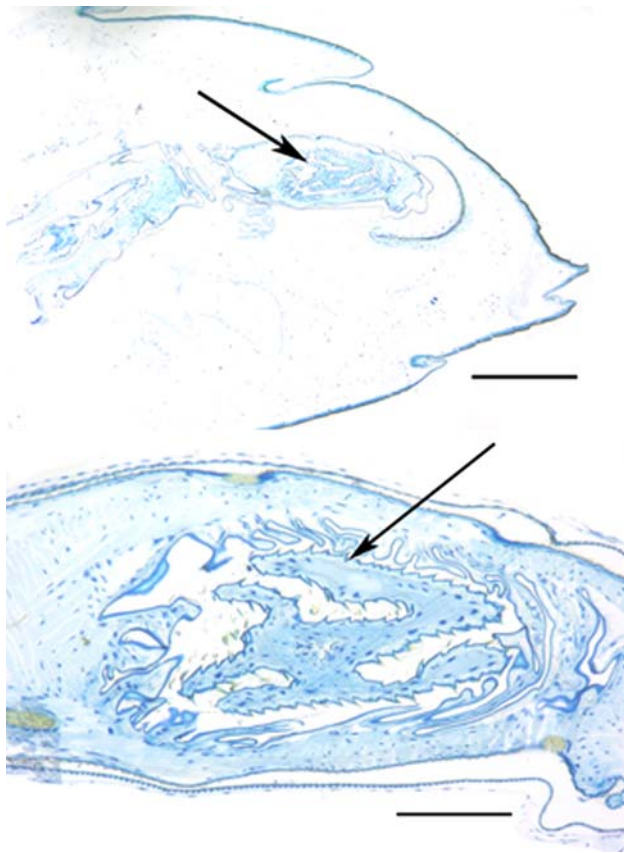


Fig. 9 Above section of a female abdomen of *Anastrangalia dubia* (Lepturinae) during copulation with the male endophallus (arrow) inside the ovipositor. The endophallus shrivelled during histological preparation. Consequently, its membrane does not fit to the wall of the ovipositor. Scale 0.5 mm. Below enlarged portion of Fig. 9 above. Endophallus (arrow) inside the ovipositor. Scale 0.1 mm

Assumption 2 The parameres assure a solid mechanical hold between the mates.

This assumption is refuted because copulating mates in Cerambycinae and Lepturinae are connected only by their endophallus and ovipositor during most of the copulation process, when the parameres are already retracted into the male abdomen. Even in species of other beetle families, such as most chrysomelids, in which the abdomens stay close together during the entire time of copulation, the parameres do not secure any mechanical connection between the mates (Düngelhoef and Schmitt 2006).

Assumption 3 The function of the endophallus is to fix the male genitalia in the female ovipositor.

This hypothesis has been confirmed, as the spines covering the surface of the endophallus are pointing backwards and so prevent the endophallus from slipping out of the ovipositor, as has also been found in previous studies on *Prionoplus reticulatus* (Prioninae) (Edwards 1961).

Assumption 4 The function of the larger spines of the endophallus, is to penetrate the female genitalia.

Even in species with very prominent spines on the endophallus, we could not detect their penetration through the ovipositor wall under a dissecting microscope (up to 500 times magnification). The structure of the endophallus differs from that of some other insects, such as the bruchid beetle *Callosobruchus maculatus* and the fly *Sepsis cynipsea*, which have been reported to penetrate the bursa copulatrix (Crudgington and Siva-Jothy 2000; Blanckenhorn et al. 2002). The most prominent spines of the endophallus in these insects are situated at the apex of the endophallus to penetrate the bursa copulatrix, whereas in longhorn beetles with long spines on the endophallus, these are situated on a more basal part, which lies inside the ovipositor during copulation. This assumption is therefore rejected and it appears that the only plausible function of the spines is to fix the endophallus inside the ovipositor during copulation, thus supporting Edwards' (1961) interpretation in the case of *Prionoplus reticulatus* (v.s.).

Assumption 5 The basal swelling of the endophallus in many longhorn beetles serves to prevent the internal sac from sliding back into the male abdomen during copulation.

The basal bulge is most prominent in species in which a membranous tube is the only connection during copulation. This swelling is formed by the endophallus and the ovipositor outside the male and female abdomina and is always situated just outside the male genital opening, hence this assumption is confirmed. Edwards (1961) suggested a different function of the basal sclerites of the endophallus, i.e., retaining the invaginated structure inside the median lobe. This could be an additional function of the sclerites.

Obviously, some characters of cerambycid genitalia bear phylogenetic signal, as has previously been indicated for both male and female genitalia (Ehara 1954; Saito 1993, respectively). Examples include differences in length, width, and shape of the parameres among subfamilies, although in some cases these characters are significant only on lower taxonomic levels. Napp (1994) used only two characters of the male genitalia in her phylogenetic study of 11 of the 13 cerambycid subfamilies recognized here, none of these characters pertained to the parameres or endophallus. She did not use more genital characters because these are said to be "too variable within the subfamilies". In our opinion, this statement holds true for the species-rich subfamilies Cerambycinae, Lepturinae and Lamiinae, whereas many characters of the parameres and endophallus appear to bear valuable phylogenetic signal for the relationships among the smaller subfamilies.

The ovipositor is likewise phylogenetically informative. Although characters of the ovipositor may depend on the

ecology of the species due to differences in oviposition sites and substrates and therefore are subject to certain constraints in morphology, characters such as the ovipositor length or the position and size of its styli vary among different subfamilies. Some character states are characteristic of certain groups, e.g., the ovipositor of Lamiinae with its long coxites and very small styli, or the lack of an ovipositor in the Oxypeltinae. Napp (1994) used five characters of the female genitalia in her phylogenetic analysis of Cerambycidae, three of which are variations of the same structure. Again, the phylogenetic information provided by these structures may be better suited for inferring relationships among the smaller subfamilies.

In copula spray-frozen insects tend to change the position of genital structures prior to dissection. However, the couples studied stuck tightly together when dissected. Even the histological sections demonstrate the stronghold of the endophallus within the ovipositor, although these specimens had to be dried for fixation with resin. We therefore conclude that the position of the genital structures of copulating cerambycid specimens generally do not change before dissection.

Although we did not reach a conclusive answer with regard to the possible function of the parameres, we have demonstrated that they do not serve to provide mechanical hold between the mates. The function of the spines on the endophallus in longhorn beetles is to fix the male genitalia inside that of the females in order to warrant proper sperm transfer, rather than to penetrate the female genital tract. We have also presented the first plausible explanation for the function of the basal endophallus swelling.

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References

- Alexander RD (1959) The courtship and copulation of *Pasimachus punctulatus* Haldeman (Coleoptera: Carabidae). *Ann Entomol Soc Am* 52:485
- Blanckenhorn WU, Hosken DJ, Martin OY, Reim C, Teuschl Y, Ward PI (2002) The costs of copulating in the dung fly *Sepsis cynipsea*. *Behav Ecol* 13:353–358
- Crudginton HS, Siva-Jothy MT (2000) Genital damage, kicking and early death. *Nature* 407:855–856
- Dias MM (1986) Revisão da subfamília Anoplodermatinae. Parte II. Tribo Anoplodermatini. Gêneros: *Acanthomigdolus* Bruch, 1941, *Paramigdolus*, gen. n., *Anoploderma* Guérin-Méneville, 1840, *Sypilus* Guérin-Méneville, 1840 (Coleoptera, Cerambycidae). *Rev Bras Entomol* 30:115–139
- Dtingelhoef S, Schmitt M (2006) Functional morphology of copulation in Chrysomelidae—Criocerinae and Bruchidae (Insecta: Coleoptera). *Bonn Zool Beitr* 54:201–208
- Eberhard WG (1993) Copulatory courtship and genital mechanics of three species of *Macroductylus* (Coleoptera: Scarabaeidae: Melolonthinae). *Ethol Ecol Evol* 5:19–63
- Edwards JS (1961) On the reproduction of *Prionoplus reticularis* (Coleoptera, Cerambycidae), with general remarks on reproduction in the Cerambycidae. *Q J Microsc Sci* 102:519–529
- Ehara S (1954) Comparative anatomy of male genitalia in some Cerambycid beetles. *J Fac Sci Hokkaido Uni. Ser 6/Zool* 12:61–115
- Fragoso SA (1985a) The terminalia as a basis for the classification of Cerambycidae (Coleoptera) subfamilies. Part I Terminology and genital morphology of *Cerambyx cerdo* L. *Rev Bras Entomol* 29:125–134
- Fragoso SA (1985b) The terminalia as a basis for the classification of Cerambycidae (Coleoptera) subfamilies. Part II, Oxypeltinae. *Rev Bras Entomol* 29:165–168
- Hubweber L, Schmitt M (2006) Parameres—similarities and differences in Chrysomelidae and Cerambycidae (Coleoptera). *Bonn Zool Beitr* 54:253–259
- Kuboki M (1980) Notes on the female genitalia in some Parandrinae and Prioninae (Coleoptera: Cerambycidae). *New Entomol* 29:69–75
- Lawrence JF, Newton AF Jr (1995) Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). In: Pakaluk J, Slipinski SA (eds) *Biology, phylogeny, and classification of Coleoptera. Papers celebrating the 80th birthday of Roy A Crowson, vol 2*. Muzeum i Instytut Zoologii PAN, Warszawa, pp 779–1006
- Mermudes JRM, Napp DS (2004) Comparative morphological study of the neotropical Cleomenini genera and their transference to the tribes Rhopalophorini Blanchard and Rhinotragini Thomson (Coleoptera, Cerambycidae, Cerambycinae). *Rev Bras Entomol* 48:251–272
- Napp DS (1994) Phylogenetic relationships among the subfamilies of Cerambycidae (Coleoptera—Chrysomeloidea). *Rev Bras Entomol* 38:265–419
- Niisato T (2004) Two new *Necydalis* (Coleoptera, Cerambycidae) from Sichuan and Guangxi, southwest China. *Elytra* 32:425–435
- Ohbayashi N (2007) A revision of the genus *Formosotoxotus* (Coleoptera, Cerambycidae, Apatophyseinae), with description of a new species from Sikkim. *Elytra* 35:194–204
- Raske AG (1973) *Tetropium parvulum* elevated to species rank and contrasted to *T. cinnamopterum* in morphology and host preference (Coleoptera: Cerambycidae). *Can Entomol* 105:745–755
- Saito A (1989) Female reproductive organs of Lepturine Cerambycid beetles from Japan, with reference to their taxonomic significance (Part 2). *Elytra* 17:201–233
- Saito A (1990) Female reproductive organs of Cerambycid beetles from Japan and neighbouring areas. I. Philini through Atimiini. *Elytra* 18:231–260
- Saito A (1993) Female reproductive organs of Cerambycid beetles from Japan and the neighbouring areas. V. General consideration. *Elytra* 21:199–216
- Sama G (1983) *Vesperus conicicollis* Fairmaire e Cocquerel, 1866 specie nuova per la fauna Italiana e note sulle specie italiane del genere (Coleoptera, Cerambycidae). *Fragm Entomol* 17:139–150
- Villiers A (1982) Démembrement du genre *Mastododera* Thomson [Coleoptera, Cerambycidae, Lepturinae]. *Ann Soc Entomol Fr N.S* 18:129–162
- Wu W-W, Jiang S-N (2000) A taxonomic study of the male genitalia of some Philid beetles with one new species in China (Coleoptera: Cerambycoidea). *Acta Entomol Sin* 43:78–87