SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Comparative Anatomy of the Male Reproductive Internal Organs of 51 Species of Bees

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Anatomia Comparada dos Órgãos do Aparelho Reprodutor Masculino de 51 Espécies de Abelhas

RESUMO - A anatomia dos órgãos internos do aparelho reprodutor de machos (ARM) adultos e pupas foi comparada em 51 espécies de abelhas, incluindo representantes de seis famílias. Foram obtidos quatro tipos diferentes de ARM. O tipo I está presente em machos das famílias mais basais (Colletidae, Andrenidae e Halictidae) e é caracterizado por três túbulos seminíferos por testículo, o qual é quase totalmente envolvido pela membrana escrotal. O tipo II é um tipo intermediário entre os tipos I e III e está presente em Mellitidae e Megachilidae, como também em alguns Apidae estudados, sendo caracterizado por possuir dutos deferentes pós-vesiculares fora da membrana escrotal e possuir três ou quatro túbulos seminíferos por testículo, exceto *Apis mellifera* L., a qual possui secundariamente um número aumentado de túbulos. O tipo III foi achado somente nos Apidae estudados e é caracterizado por apresentar os testículos e dutos genitais (exceto o duto deferente pós-vesicular) encapsulados separadamente, as glândulas acessórias são bem desenvolvidas e o duto ejaculador é calibroso, apresentando fissuras em sua parede externa, as quais podem ocorrer também no tipo II. O tipo IV está presente exclusivamente na tribo Meliponini e é caracterizado pela ausência de glândulas acessórias.

PALAVRAS-CHAVE: Glândula acessória, genitália, morfologia

ABSTRACT - The anatomy of the internal organs of the male reproductive apparatus (MRA) of adults and pupae was compared among 51 species of bees, including representatives of six families. Four different types of MRA were found. The type I is present in males of the less derived families (Colletidae, Andrenidae, and Halictidae) and is characterized by three seminiferous tubules per testis, which are almost completely enveloped by the scrotal membrane. The type II is an intermediary between types III and I and is present in Melittidae and Megachilidae, as well as in some Apidae studied, being characterized by post-vesicular deferent ducts outside the scrotal membrane and by three or four seminiferous tubules per testis, except for *Apis mellifera* L., which has a secondarily increased number of tubules. Type III was only found in the Apidae studied and is characterized by separately encapsulated testes and genital ducts (except for the post-vesicular deferent duct). Accessory glands are well developed, and the ejaculatory duct is thickened with fissures in its wall, which may also occur in the type II. Type IV is present exclusively in the tribe Meliponini, and is characterized by the absence of accessory glands.

KEY WORDS: Accessory gland, genitalia, morphology

The male reproductive apparatus (MRA) of insects is constituted by a pair of testes connected to aedeagus through the genital ducts. The testes are formed by a variable number of solid filaments, known as seminiferous tubules. The seminiferous tubules are separated from one another by interstitial tissue; and individually encapsulated by a peritoneal membrane. The tubules are enveloped, as a group, by a scrotal membrane or capsule, forming a globular structure (Cruz-Landim 2002). Inside these tubules, spermatogenesis proceeds in synchrony within the cysts. Thin and short prolongations, known as efferent ducts, arise from the extremity of each seminiferous tubule and join in a single common duct, the deferent duct, which show apical dilation that forms the seminal vesicle, dividing the deferent ducts into a pre- and post-vesicular portion. The male reproductive apparatus of most insects possesses accessory glands, the

mesadenial or ectadenial glands, which open at the deferent ducts or at the ejaculatory duct, respectively (Snodgrass 1935, Chapman 1998).

In *Apis mellifera* L., the reproductive apparatus consists of the testes, the efferent ducts, the pre- and post-vesicular deferent ducts, the seminal vesicles, and a pair of accessory glands, the mucus glands. In this species, as in most insects, spermatogenesis occurs during the pupal stage, and the testes display their maximum development between the 5th and the 6th day of pupation (Bishop 1920). Therefore, in newly emerged adults, the testes are already undergoing degeneration, appearing as yellowish flat bodies (Snodgrass 1956). Yet it is only around the 12th day of adult life that a drone is mature for mating (Snodgrass 1956), when all the spermatozoa is contained in the seminal vesicles and ready to be ejected in the female during mating (Bishop 1920).

There are few comparative studies concerning to the male reproductive apparatus in bees, especially on solitary and stingless species. Therefore, it was done a comparative anatomical study of the internal genital organs of adult males of 51 bee species. Pupae of males of *Scaptotrigona postica* Latreille, *Melipona quadrifasciata* Lepeletier, and *Tetragonisca angustula* Latreille were also analyzed.

Material and Methods

The internal genital organs of males of 51 bee species, belonging to six families, were studied (Table 1). All the specimens were adults, except for the species *Scaptotrigona postica* Latreille, *Melipona quadrifasciata* Lepeletier and *Tetragonisca angustula* Latreille.

The species were identified by Prof. Pe. Jesus Santiago Moure (Departamento de Zoologia, Universidade Federal do Paraná), being most of the specimens identified in the Museu de Zoologia, Collection of the Department of Zoology, Universidade Federal do Paraná (Brazil).

After removing the genital organs in saline solution, these were schematized under a stereomicroscope with aid of a Zeiss Winkle camara lucida.

Results and Discussion

The results allowed separating the 51 species of bees studied into at least four types of organization of the male reproductive apparatus (MRA).

Type I was found in almost all species studied of the families Colletidae (except for *Tricholletes venustus* Smith), Andrenidae, Halictidae, and Megachilidae (except for *Anthidium manicatum* L., *Stelis aliena* Cokerell) (Table 1, Fig. 1A-F). It is characterized by testes, seminal vesicles, and genital ducts totally or almost encapsulated by the scrotal membrane, and forming a globular unit (Fig. 1A, C, E). In all species, two portions compose the accessory gland: a long tubular duct, and a dilated loop. This later portion usually presents anatomic variation among the species. The accessory gland loops can be developed (Fig. 1C), although they are often small (Fig. 1A), and the ejaculatory duct is often thin and simple, but occasionally may also be short or long (Fig. 1A, D, E), three short seminiferous tubules per testis (Fig. 1B), the seminal vesicle diameter is often not very different from the deferent ducts (Fig. 1A, B, D, F), and the post-vesicular deferent ducts always opens at the initial portion of the accessory glands. Among these families, the Colletidae presents the simplest anatomical pattern of MRA (Fig. 1A), and as for other characters can be considered basal (Michener 2000).

Type II was found in the studied representatives of the families Melittidae (*Heperaspis carinata* Steven), Megachilidae (*A. manicatum*, *S. aliena*), and Apidae (*Bombus atratus* Franklin, *Centris violacea* Lepeletier, *Mesoplia friesei* Ducke) (Table 1, Fig. 2A-D). It is characterized by very long post-vesicular deferent ducts that are located outside the scrotal membrane (Fig. 2A, C, D). The remaining internal organs of the MRA, except for the ejaculatory duct and the accessory glands, also form a globular unit encapsulated by the scrotal membrane, which may be elongated (Fig. 2A), with a median constriction (Fig. 3A), "U" or "S" shaped (Fig. 3C, D). In this type, the accessory glands are generally well developed (Figs. 2A, D; 3A, C). The ejaculatory duct is thicker, and, in some species, it may even present a complex pattern, such as longitudinal fissures (Figs. 2A-D; 3A-D). In the Megachilidae there are three seminiferous tubules per testis (Fig. 2B), while in the Type II of the Apidae (excluding *Apis mellifera* L.) there are four (Fig. 3B). In the Apidae with MRA of the Type II, the seminal vesicles differ only slightly from the pre- and post-vesicular deferent ducts, and the post-vesicular ducts open at the final opens at the final portion of the accessory glands, next to the ejaculatory duct (Figs. 2D; 3D). In the Megachilidae and Melittidae, these ducts may open at the same site as seen in the Apidae (Figs. 2A, D; 3D), or enter at beginning of the accessory glands, as in the Type I MRA, or even at an intermediary position (Fig. 2C).

Type III found in almost all representatives of the Anthophorini (except for *C. violacea*, *M. friesei*) and of the Apinae (except for *Euglossa cordata* L., *B. atratus*) is characterized by the presence of two units that are separately encapsulated by the scrotal membrane (Fig. 4A-F). Each contains one testis and one seminal vesicle (Fig. 4B, C). As in type II, the post-vesicular deferent ducts are not encapsulated. The accessory glands are well developed (Fig. 4E, F) with thick ejaculatory ducts presenting median longitudinal fissures and, in some species, also lateral fissures (Figs. 4A, C, E, F), and four seminiferous tubules per testis, which are longer than those of the Type I and than those with three seminiferous tubules in the Type II (Fig. 4B, C). With the exception of *Xylocapa* (Fig. 4D), which has an intermediary portion, the opening of the post-vesicular deferent duct is located at the final portion of the accessory gland (Fig. 4B, F). In the Type III, the seminal vesicles are easily distinguishable from the deferent ducts by being longer and thicker and because they are folded inside the scrotal membrane, forming a globular unit (Fig. 4B, C, E, F).

Type IV is similar to Type I, but it is only found in all

Table 1. List of the species studied and collecting sites.

Families	Species	Collecting sites
Colletidae	*Colletes rufipes* (Smith)	Rio Claro, SP, BR
	Tricholletes venustus (Smith)	Ipswich, Queensland, Australia
Andrenidae	*Oxea flavescens* Klug	Rio Claro, SP, BR
	Psaenythia atriventris Gerstaecker	Rio Claro, SP, BR
Halictidae	*Megalopta sp.* (Smith)	Barro Colorado, Island, Panama
	Nomia melanderi Cokerell	Utha, USA
	Pseudaugochloropsis graminea Fabricius	Rio Claro, SP, BR
Megachilidae	*Anthidium manicatum* (L.)	Rio Claro, SP, BR
	Coelioxys pirata Holmberg	Rio Claro, SP, BR
	Megachile (Austromegachile) susurans Haliday	Rio Claro, SP, BR
	Stelis aliena Cockerell	Rio Claro, SP, BR
Melittidae	*Hesperapis carinata* Steven	Utah, USA
Apidae (Anthophorini)	*Centris (Hemisiella) tarsata* Smith	Rio Claro, SP, BR
	C. (Paramisia) fuscata Lepeletier	Rio Claro, SP, BR
	C. violacea Lepeletier	Rio Claro, SP, BR
	C. vittata Lepeletier	Rio Claro, SP, BR
	Epicharis flava (Friese)	Rio Claro, SP, BR
	E. bicolor Smith	Rio Claro, SP, BR
	E. cockerelli Friese	Rio Claro, SP, BR
	Exomalopsis auropilosa Spinola	Rio Claro, SP, BR
	Melitoma segmentaria (Fabricius)	Rio Claro, SP, BR
	Mesoplia friesei (Ducke)	Rio Claro, SP, BR
	Peponapis fervens (Smith)	Cuernavaca, Mexico
	Thygater analis (Lepeletier)	Rio Claro, SP, BR
	Xylocopa frontalis Olivier	Rio Claro, SP, BR
	X. hirsutissima Maild	Rio Claro, SP, BR
	X. nogueirai Hurd & Moure	Rio Claro, SP, BR
	Xylocopa sp.	Rio Claro, SP, BR
Apidae (Apinae)	*Bombus atratus* (Franklin)	Rio Claro, SP, BR
	B. transversalis (Olivier)	Manaus, AM, BR
	B. lapidarius L.	Sunninghill, London, UK
	B. agrorum Schrank	Sunninghill, London, UK
	B. lucorum L.	Sunninghill, London, UK
	B. terrestris L.	Sunninghill, London, UK
	Euplusia violacea (Blanchard)	Rio Claro, SP, BR
	Euglossa cordata (L.)	Rio Claro, SP, BR
	Eulaema nigrita (Lepeletier)	Rio Claro, SP, BR
	E. meriana (Olivier)	Manaus, AM, BR
	Psithyrus compestris (Panzer)	Sunninghill, London, UK
	P. sylvestris (Lepeletier)	Sunninghill, London, UK
	P. rupestris (Fabricius)	Sunninghill, London, UK
Apidae (Meliponini)	*Lestrimellita limao* (Smith)	Três Lagoas, MT, BR
	Melipona bicolor (Lepeletier)	Viçosa, MG, BR
	M. quadrifasciata anthidioides Lepeletier	Rio Claro, SP, BR
	M. seminingra merrilae Cockerell	Manaus, AM, BR
	Nannotrigona testaceicornis (Lepeletier)	Rio Claro, SP, BR
	Partamona pearsoni (Schwarz)	Cristalina, GO, BR
	Plebeia droryana (Friese)	Rio Claro, SP, BR
	Scaptotrigona postica (Latreille)	Rio Claro, SP, BR
	Tetragonisca angustula (Smith)	Rio Claro, SP, BR
	Trigona spinipes (Fabricius)	Rio Claro, SP, BR

meliponines studied. It is characterized by the absence of the accessory glands and by seminal vesicles that is much thicker than the deferent ducts (Fig. 5A-D); these vesicle s also form globular units that are encapsulated by the scrotal membrane (Fig. 5B). The post-vesicular deferent ducts are joined and open directly at the ejaculatory duct, which is short and simple (Fig. 5D). This Type of MRA has four seminiferous tubules per testis (Fig. 5A, D), which are long as those of the type III.

The MRA of *A. mellifera* could either be considered as a sub-group of the type III MRA, or even as an altogether different type of MRA. It is characterized by a
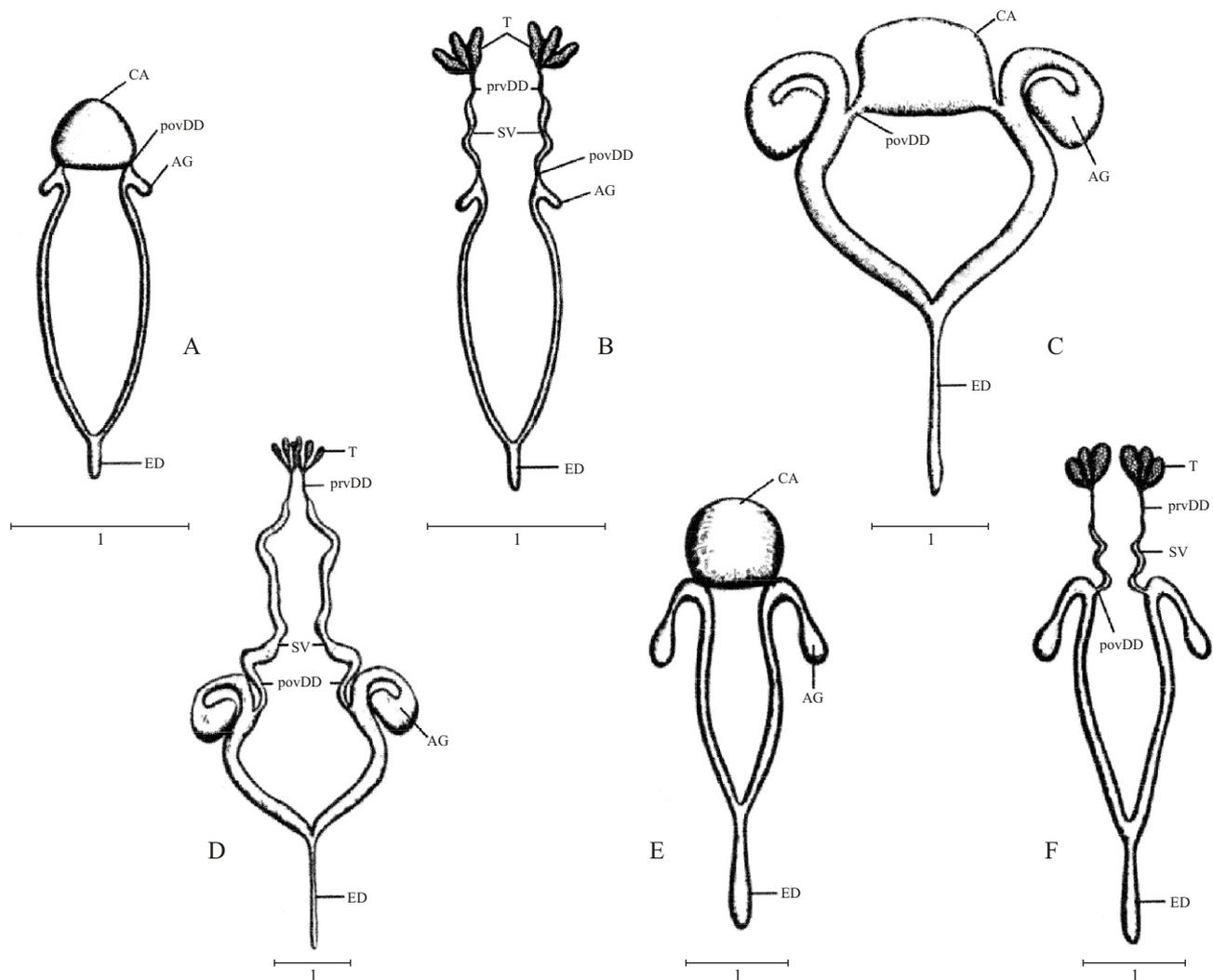
Figure 1. Scheme of the male reproductive apparatus (MRA) of the Type I. A. *Colletes rufipes* (Colletidae). B. *C. rufipes* without the scrotal membrane. C. *Oxaea flavencens* (Andrenidae). D. *O. flavencens* without the scrotal membrane. E. *Coelioxys pirata* (Megachilidae). F. *C. pirata* without the scrotal membrane. CA = scrotal capsule, AG = accessory gland, ED = ejaculatory duct, povDD = post-vesicular deferent duct, prvDD = pre-vesicular deferent duct, SM = scrotal membrane, SV = seminal vesicle, ST = seminiferous tubules. (scale = mm)

very high number of seminiferous tubules, about 250 per testis (Louveaux 1977).

All the species studied of the phylogenetically more basal families (Colletidae, Andrenidae, and Halictidae) presented Type I MRA. Most of the species studied of the Apidae, except for Meliponini, presented MRA of type III. The species that belong to the phylogenetical intermediate families, Melittidae and Megachilidae, and some species of Apidae, presented Type II, which showed intermediate or transitional anatomical characters. The Megachilidae also contain representatives with MRA of the type I in the meliponines. Whereas the tendency in the other families is towards a higher development of the accessory glands, separation of the units encapsulated by the scrotal membrane and an increase sizing and

complexity of the ejaculatory duct, the meliponines are characterized by the disappearance of the accessory glands and a general simplification of the genital ducts, including the outer genitalia.

Excluding the meliponines, and considering Type I as the ground plan of MRA, the first major modification of the MRA was the increased development of the accessory gland, which can already be observed in some representatives with Type I. The second modification was an increase in the number of seminiferous tubules per testis, or their widening, as well as an elongation of the post-vesicular deferent ducts. Next, there is a displacement of the opening site of the post-vesicular duct, tending to be nearer the ejaculatory duct, as well as an increase in the length and diameter of the ejaculatory duct.

The meliponines differ from the other bees by the loss of

the accessory glands. The presence of four seminiferous tubules per testis is the only factor that approximates them to the MRAs of Types II and III. In the rest of their characteristics however, they share anatomical elements with Type I, such as those present in Colletidae, which have very filiform accessory glands with a very small dilation portion. The greater development of the seminal vesicles may be an adaptation to the increase in the number and length of the seminiferous tubules and the absence of the accessory glands in stingless bees.

Apparently the tendency of the male reproductive tract of bees was towards an increase in number and length of the seminiferous tubules, which must have resulted in an increased number of spermatozoa production. There was also an increase in distance between the testes and the rest of the internal organs due to the elongation of the post-vesicular deferent ducts, increased production of accessory gland secretion, and a spermatozoa releasing closer to the ejaculatory ducts.

In *Xylocopa*, contrary to the observed in the other genera, the seminiferous tubules are straight and short, unfolded. This condition may be related and compensated by the continued spermatogenesis that extends into the adult life stage.

The function of the accessory glands is not well known in bees. In *A. mellifera* and *Bombus terrestris* L., these glands seem to be involved in the production of a mucous plug that prevents the reflux of sperm after copulation (Bishop 1920, Snodgrass 1956). Since mating has an effect on vitellogenesis, or on the maturation of oocytes in the ovary of insects (Chen 1984, Coleman *et al.* 1995), it is possible that these glands may produce substances that regulate fertility and the sexual behavior of the females (Dallacqua & Cruz-Landim 2003). According to Colonello & Hartfelder (2003), the protein content increases in males of *A. mellifera* from newly emerged immature drones to six-day old drone. After this period, the number of protein bands in electrophoretic gels reduce, when at eight days old drones
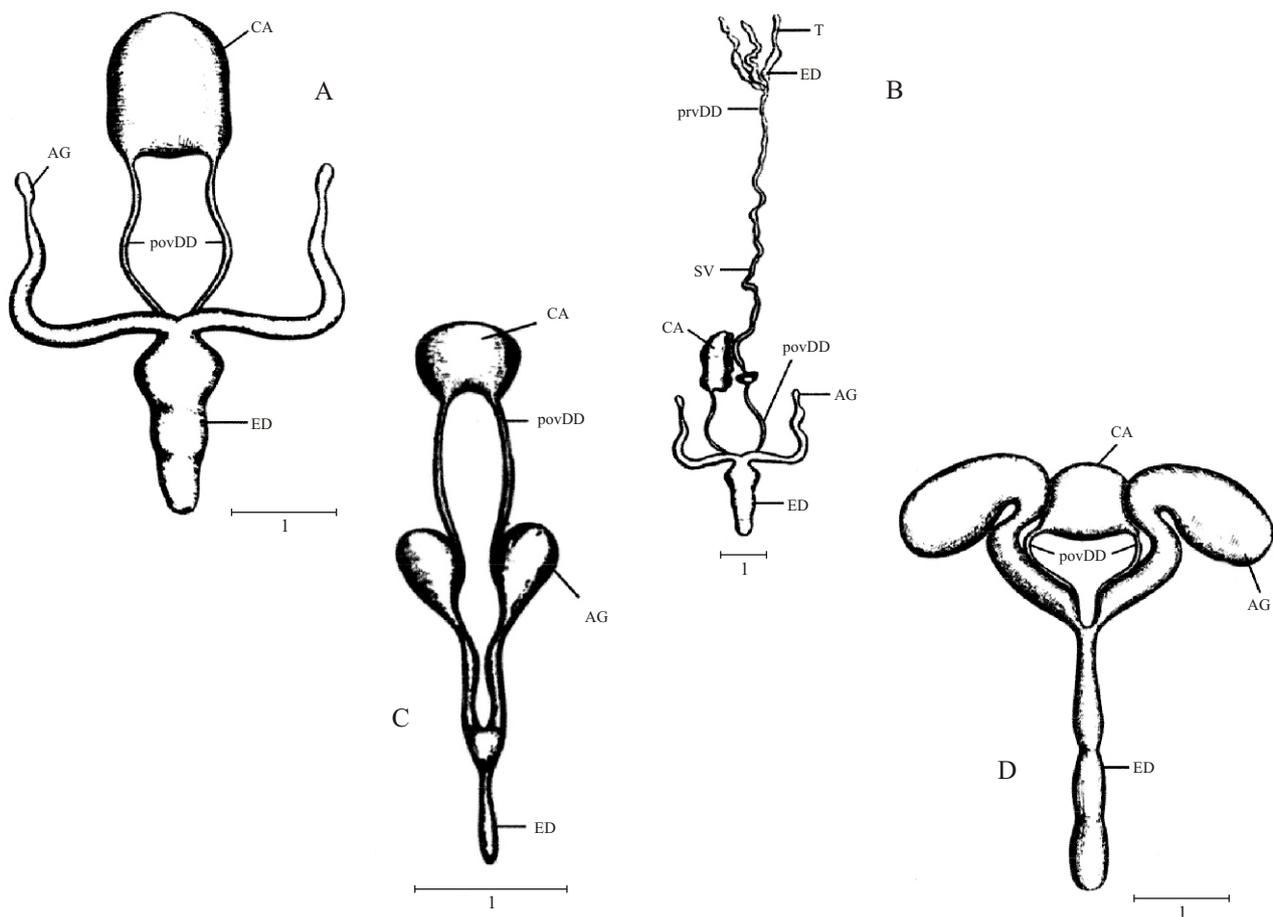

Figure 2. Scheme of Type II MRA. A. *Anthidium manicatum* (Megachilidae). B. *A. manicatum* without the scrotal membrane. C. *Hesperapis carinata* (Melittidae). D. *Mesoplia friesei* (Apidae). CA = scrotal capsule, AG = accessory gland, ED = ejaculatory duct, povDD = post-vesicular deferent duct, prvDD = pre-vesicular deferent duct, SM = scrotal membrane, SV = seminal vesicle, ST = seminiferous tubules. (scale = mm)
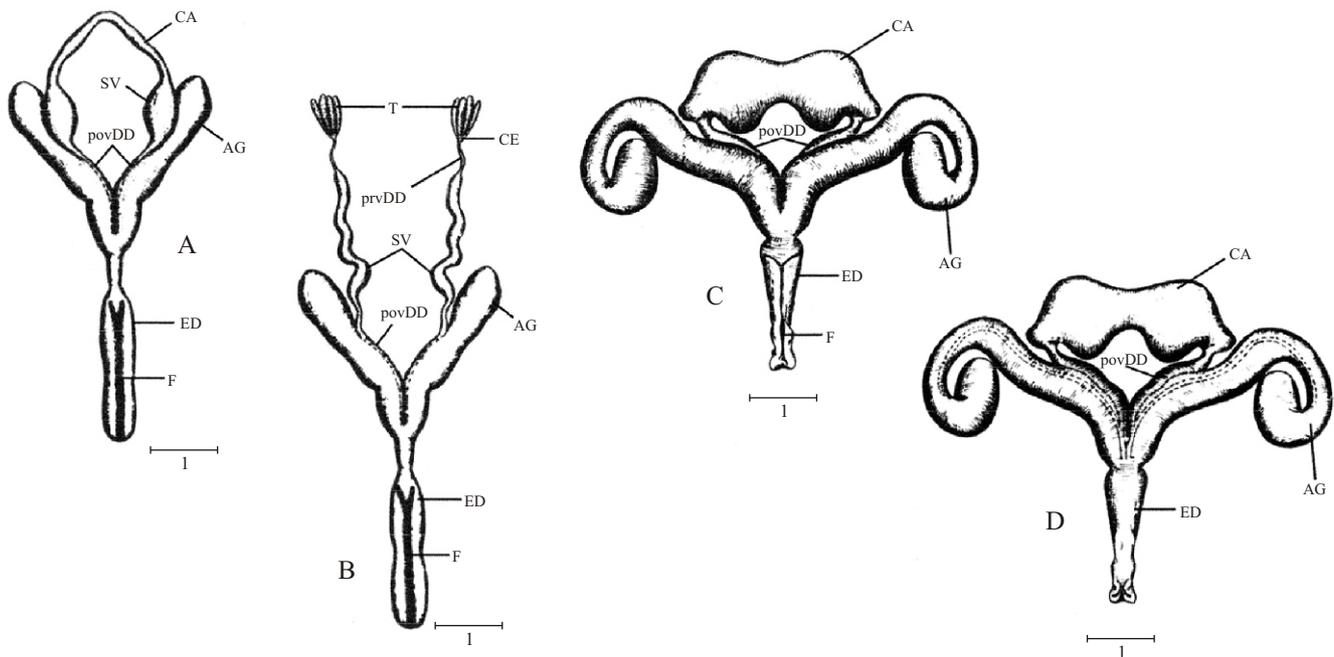
Figure 3. Scheme of Type II MRA. A. *Centris violacea* (Apidae, Anthophorini). B. *C. violacea* without the scrotal membrane. C. *Bombus atratus* (Apidae, Bombini), dorsal view. D. *B. atratus*, ventral view. CA = scrotal capsule, AG = accessory gland, ED = ejaculatory duct, F = constriction or fissure in the wall of the ejaculatory duct, povDD = post-vesicular deferent duct, prvDD = pre-vesicular deferent duct, SM = scrotal membrane, SV = seminal vesicle, ST = seminiferous tubules. (scale = mm)
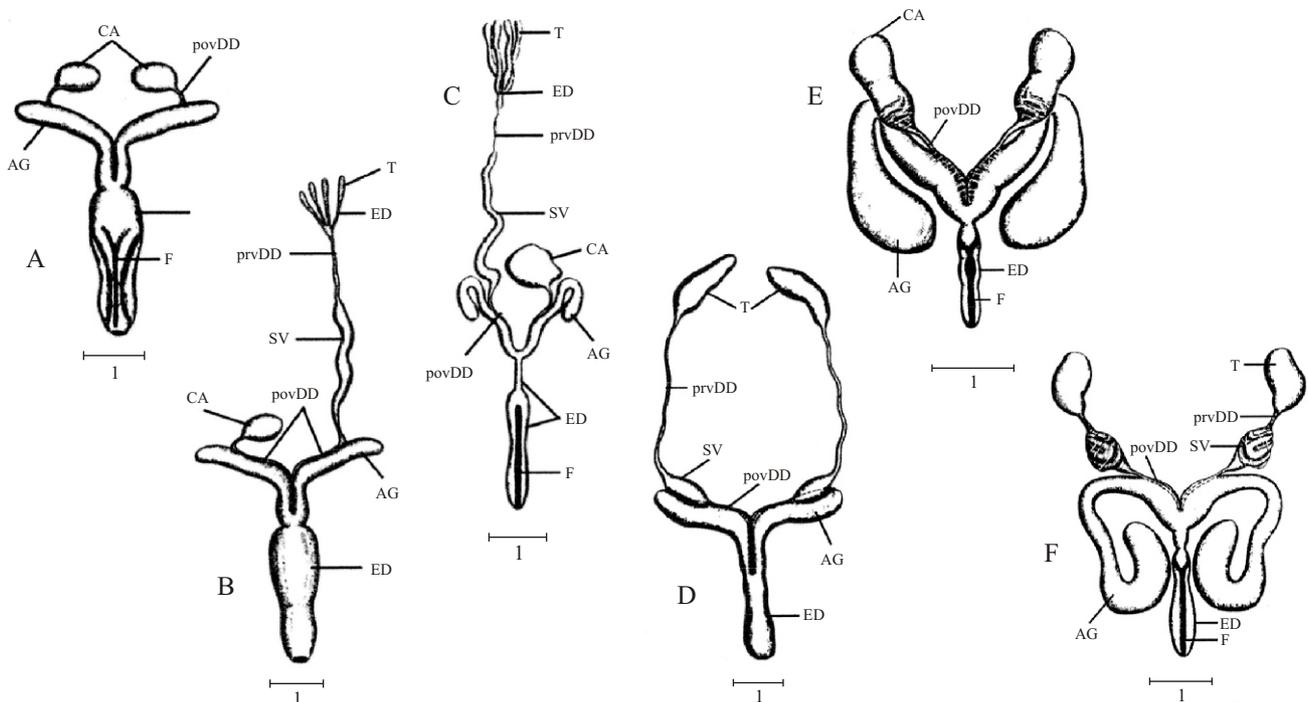

Figure 4. Scheme of Type III MRA. A. *Centris fuscata* (Apidae, Anthophorini), dorsal view. B. *C. fuscata* with one of the testis without the scrotal membrane, ventral view. C. *Epicharis cockerelli* (Apidae, Anthophorini), with one of the testis without the scrotal membrane. D. *Xylocopa sp.* (Apidae, Xylocopini). E. *Bombus agrorum* (Apidae, Bombini). F. *Psithyrus silvestris* (Apidae). CA = scrotal capsule, AG = accessory gland, ED = ejaculatory duct, F = constriction or fissure in the wall of the ejaculatory duct, povDD = post-vesicular deferent duct, prvDD = pre-vesicular deferent duct, SM = scrotal membrane, SV = seminal vesicle, ST = seminiferous tubules. (scale = mm)
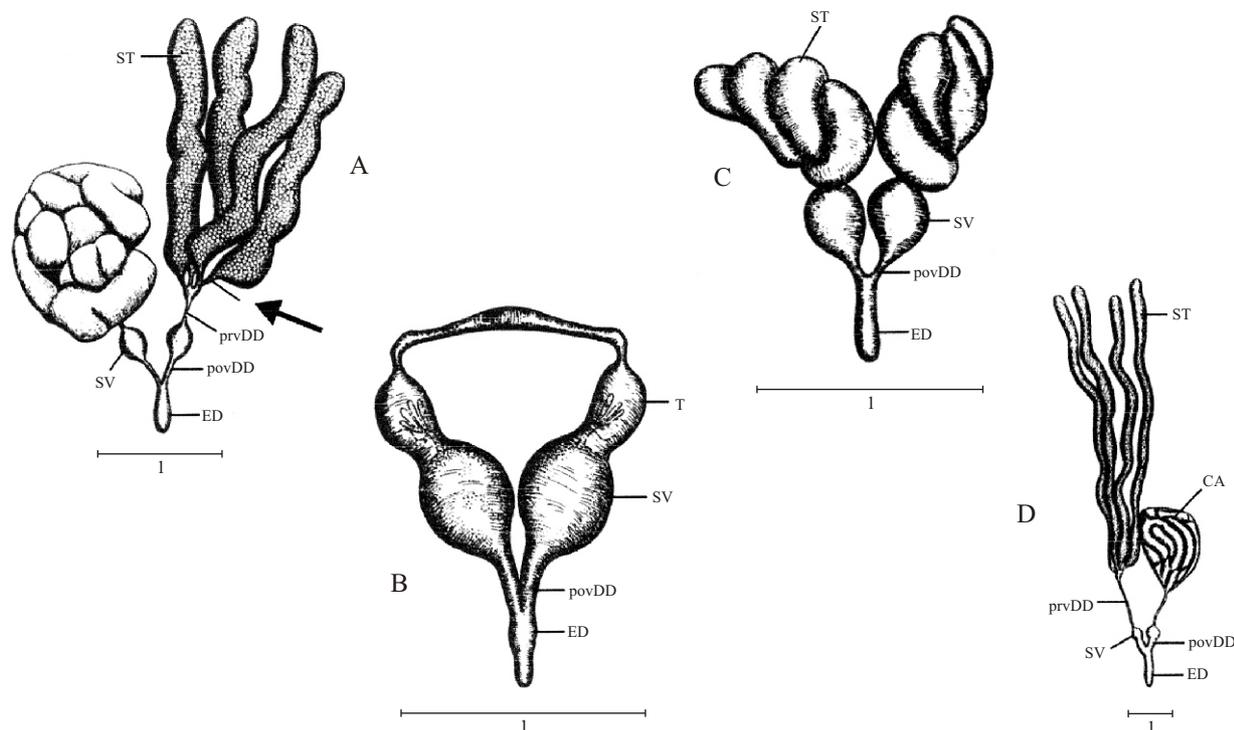
Figure 5. Scheme of Type IV MRA. A. Pink-eye pupa of *Scaptotrigona postica* (Apidae, Meliponini). B. Adult male of *S. postica*. C. Pink-eye pupa of *Tetragonisca angustula* (Apidae, Meliponini). D. Pink-eye pupa *Melipona quadrifasciata* (Apidae, Meliponini). CA = scrotal membrane, ED = ejaculatory duct, povDD = post-vesicular deferent duct, prvDD = pre-vesicular deferent duct, SM = scrotal membrane, SV = seminal vesicle, ST = seminiferous tubules. (scale = mm)

is present only three dominant polypeptides.

In solitary species, such as *Anthidium maculosum* L., the males are territorial and the females mate multiple times (Alcock *et al.* 1977). In *A. manicatum* males it was observed in the present study a large diameter of the ejaculatory duct. A thicker ejaculatory duct allows a higher adhesion to the female during copulation, in addition, the secretion of the accessory glands increases even more the diameter of the ejaculatory duct at the moment of mating and might serve, as in *Bombus*, as a plug that prevents the reflux of sperm or even improves the viability of the sperm posterior to mating (Bishop 1920, Snodgrass 1956).

The similarity of the MRA of meliponines to that of the basal solitary bees is not the only similarity between these two groups of bees. As in most solitary species, the queens of meliponines seem to mate with a single male (Peters *et al.* 1999, Strassman 2001). Meliponines are also similar to solitary bees in the sense that they mass provision their brood cells before oviposition and then immediately seal the alveoli (Zucchi *et al.* 1994). Contrary to the solitary bees, however, the meliponine queen produces a much higher number of eggs and is long lived. Therefore, there appears to be a selective pressure towards and increased production of spermatozoa by the meliponine males, thus showing a larger size of the testes. Taking into account the possibility that the accessory gland secretion inhibits further matings, the absence of these glands in meliponines might be explained by the species being monoandric.

Alternatively, the remainder of the ruptured male genitalia in the female tract may function as a plug. Furthermore, it is possible that a secretion similar to the accessory gland is being produced in other parts of the male genital tract (Dallacqua & Cruz-Landim 2003).

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