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Corrected Version

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Advisor: Prof. Dr. Marcelo Duarte da Silva.

ABSTRACT

Hawkmoths occupy all regions of the globe, except Antarctica and Greenland. The family has 210 genera and about 1500 species, with about a third of the taxa registered for the Neotropical region. In Brazil, 33 genera and 196 species are known. They are classified into three subfamilies, with Macroglossinae being the most diverse lineage. They play an key in pollination. In this process, sphingids usually have contact with pollen grains in various parts of the body, particularly in the antennae. To clean them, these moths use the epiphyses that are in the foretibiae. In addition to being important for the biology of lepidopterans, the epiphysis also represents one of the synapomorphies that support the monophyly of these insects. However, few studies detail this structure morphologically. In this study, we seek to understand the possible variations in the epiphyses of representatives of Dilophonotini and Philampelini aiming to contribute to the discussions on the structure morphology, sexual dimorphism and the relationship with other body structures. For this, 19 genera and 59 species of both sexes (564 individuals in total) were studied. The extracted legs were placed in 10% KOH and taken to a water bath for four minutes to remove the scales. With the epiphyses cleaned, all individuals were photographed using a stereomicroscope and SEM to perform morphological descriptions of the epiphyses and measurements; after that, an allometric regression analysis was performed with other body structures (length and width of the epiphyses, position, length of the tibiae and length of the antennae); finally, an analysis of geometric morphometrics was conducted. As results, a new terminology was proposed for distinct parts of the epiphysis, 22 morphological characters were established from the epiphyses, greater morphological variation was found in the genera and subtribe levels, variations were found between males and females, a positive allometry was observed between the size of the epiphyses and the size of the antennae, and likewise, between the position of the epiphyses and the length of the tibiae. Morphometric analysis showed positive allometry, a homogeneous pattern with small variations between some genera. It was demonstrated that closely related genera have similar epiphyses, and others do not. This study is an important contribution to the knowledge of the Sphingidae morphology, with the proposal of new phenotypic

characters for the group. In addition to being a detailed study of the epiphysis, a structure that has been historically neglected, it highlights the need to expand the comparative analysis to other groups of Lepidoptera for a better understanding of the importance of using the epiphysis morphology in the taxonomy of these insects, as well as for a better understanding of the variation and evolution of the structure and its associated antennal grooming behavior.

RESUMO

Esfingídeos habitam todas as regiões do globo, exceto Antártida e Groenlândia. A família possui 210 gêneros e cerca de 1500 espécies, com cerca de um terço dos táxons registrados para a região neotropical. No Brasil, são conhecidos 33 gêneros e 196 espécies. Os esfingídeos estão classificados em três subfamílias, sendo Macroglossinae a linhagem de maior diversidade. Desempenham um papel importante na polinização. Nesse processo, costumam ter contato com os grãos de pólen em várias partes do corpo, particularmente nas antenas. Para limpá-las utilizam as epífises que estão localizadas nas tíbias anteriores. Além de ser importante para a biologia dos lepidópteros, a epífise também representa uma das sinapomorfias que sustentam a monofilia desses insetos. Porém, existem poucos estudos que detalham morfologicamente esta estrutura. Neste estudo, procura-se compreender as possíveis variações das epífises de representantes de Dilophonotini e Philampelini com o objetivo de contribuir para as discussões sobre a morfologia da estrutura, dimorfismo sexual e a relação com outras estruturas do corpo. Para isso, foram estudados 19 gêneros e 59 espécies, de ambos os sexos (564 indivíduos em total). As pernas extraídas foram colocadas em KOH a 10% e levadas a banho-maria durante quatro minutos para remoção das escamas. Com as epífises limpas, todos os indivíduos foram fotografados com auxílio de estereomicroscópio e MEV dando subsídios às descrições morfológicas das epífises e medições; na sequência, foi realizada uma análise de regressão alométrica com outras estruturas do corpo (comprimento e largura das epífises, posição, comprimento das tíbias e comprimento das antenas); finalmente, foi conduzida uma análise de morfometria geométrica. Como resultados, foi proposta uma nova terminologia para diferentes partes da epífise, foram estabelecidos 22 caracteres morfológicos nas epífises, foi encontrada maior variação morfológica nos níveis de gênero e subtribo, foram encontradas variações entre machos e fêmeas, foi observada uma alometria positiva significativa entre o tamanho das epífises e o tamanho das antenas, e do mesmo modo, entre a posição das epífises e o comprimento das tíbias. A análise morfométrica mostrou corroborou uma alometria positiva, um padrão homogêneo com pequenas variações entre alguns gêneros; foi ainda demostrado que gêneros proximamente relacionados podem ter epífises parecidas ou

pouca semelhança nas epífises. Esse estudo é uma contribuição importante para o conhecimento da morfologia dos Sphingidae, com a proposição de novos caracteres fenotípicos para o grupo. Além de ser um estudo detalhado da epífise, uma estrutura historicamente negligenciada, ressalta-se a necessidade de se ampliar a análise morfológica comparativa para outros grupos de Lepidoptera, no intuito de embasar o uso da descrição da epífise na taxonomia desses insetos, como também para melhor compreensão sobre a variação e a evolução da estrutura e do comportamento de limpeza das antenas.

Palavras-chave: "esfingídeos". "órgão de limpeza". "alometria". "dimorfismo sexual". "morfometria geométrica".

1. INTRODUCTION

1.1. Sphingidae Latreille, 1802

Lepidoptera comprises butterflies and moths with approximately 160,000 described species (Van Nieukerken *et al.*, 2011). Moths with nocturnal habits represent >75-85% of lepidopterans species diversity (Kawahara *et al.*, 2018). Bombycoidea is one of the most diversified and species-rich lineages of the clade Macroheterocera (Mitter *et al.*, 2017; Kitching *et al.*, 2018). This superfamily includes 10 families, among them, we highlight Sphingidae Latreille, 1802 for its species richness, relatively stable taxonomy, and its importance in studies of pollination and biological conservation (Kristensen, 1999; Van Nieukerken *et al.*, 2011; Kitching *et al.*, 2018).

Sphingidae comprises 210 genera and more than 1500 species (Van Nieukerken *et al.*, 2011; Kawahara and Barber, 2015; Kitching *et al.*, 2018); therefore, it constitutes the most species-rich group of Bombycoidea (Van Nieukerken *et al.*, 2011). Hawkmoths have a worldwide distribution, and are absent only in Antarctica and Greenland (Kitching and Cadiou, 2000; Moré *et al.*, 2014). In the Neotropical region, they represent approximately one third of the global diversity (Heppner, 1991; 1998). In Brazil, 33 genera and 196 species are known (Haxaire and Mielke, 2020).

The monophyly of Sphingidae is supported by synapomorphies of larvae (1), pupae (1) and adult (7) (Minet, 1994). Currently, it is also corroborated by molecular and morphological data (Kawahara *et al.*, 2009). Several authors classify Sphingidae in three subfamilies: Macroglossinae, Sphinginae and Smerinthinae (Kitching and Cadiou, 2000).

Initially, few researchers explored morphological data in the construction of a relationship hypothesis for Sphingidae. Rothschild and Jordan (1903) showed a comparative morphological study based on adults, however lacking a methodological standardization. These authors divided the family into two groups: "Semanophorae" and "Asemanophorae" by the presence or absence of microtrichia patch on the internal surface of the first segment of the labial palps.

Hodges (1971) changed the names of the groups recognized by Rothschild and Jordan (*op.cit.*) to Macroglossinae and Sphinginae, respectively, based on the genus type of each subfamily. On the other hand, Minet (1994) indicated that the absence of the modification in the labial palp was plesiomorphic, therefore, it could not be used as an autapomorphy of Sphingidae *sensu* Rothschild and Jordan (1903). However, Kitching and Cadiou (2000) indicated that among the three proposed subfamilies (Sphinginae, Smerinthinae and Macroglossinae), only Macroglossinae showed an exclusive apomorphy (modification of the labial palp).

Of all the implications for the classification of Sphingidae, Nakamura (1976) (*apud* Kawahara *et al.*, 2009) made the major contribution to the systematics of the family, grouping Smerinthini and Sphingini in a clade based on larval and pupal characters, but it is important to emphasize that only Japanese representatives were examined. Posteriorly, Regier *et al.* (2001) worked on the first molecular phylogeny for the hawkmoths. They employed only two genes (EF-1α and DDC) and low representation of taxa (18 genera; 14 of Sphingidae, two of Brahmaeidae and two of Saturniidae); despite that, their results corroborated those proposed by Rothschild and Jordan (1903) and Nakamura (1976). However, it was evident that the number of genera might be expanded and three important tribes that were not previously considered (Acherontiini, Ambulycini and Sphingulini) in those works might be added in future analyses.

The study of Kawahara *et al.* (2009) supported what was found by previous authors, grouping Smerinthinae plus Sphinginae, and confirmed Macroglossinae as a monophyletic group. In the latter, three groups are recognized: Hemarina, Dilophonotina *sensu lato* and Macroglossini *sensu stricto* (Kawahara *et al.* 2009). The "Dilophonotina *sensu lato*" groups exclusively representatives from the Neotropical region and includes Dilophonotini (except Hemarina), Philampelini and *Proserpinus* group with *Amphion* and *Sphecodina* (Kitching and Cadiou, 2000; Kawahara *et al.*, 2009). Implications from this phylogeny include the closest relationship of Hemarina and Dilophonotini, and the inclusion of the genus *Enyo* in the tribe Dilophonotini.

Kawahara and Barber (2015) analyzed the evolution of anti-bat ultrasound in Sphingidae based on molecular and behavioral data and concluded that: 1) the subtribe Hemarina is phylogenetically close related to Macroglossini, 2) *Enyo* is placed in Macroglossina and 3) Philampelini comprise only one genus, *Eumorpha*. On the other hand, the global checklist of Bombycoidea proposed by Kitching *et al.* (2018) concluded that: 1) Hemarina is considered as tribe, 2) Philampelini is considered as subtribe of Dilophonotini including 11 genera (e.g. *Aleuron*, *Enyo*, *Unzela* and *Eumorpha*).

There are several conjectures about the Macroglossinae taxonomy. I decided to work with the classification of Kawahara *et al.* (2009), using the clade Dilophonotini *sensu lato* on which it is included the subtribe Hemarina (*Cephonodes* and *Hemaris*) without *Proserpinus* group.

1.2. Grooming in insects

Grooming is a normal and innate behavior in animals (Jander, 1966; Murray, 1987; Böröczky *et al.*, 2013). This behavior is performed frequently to avoid contamination on the body surface (Hlavac, 1975; Böröczky *et al.* 2013; Zhukovskaya *et al.* 2013). Thus, it helps maintaining the acuity of the sensory organs (Robinson, 1996; Böröczky *et al.*, 2013; Hackmann *et al.*, 2015), removing pathogens, fungi (Chouvenc *et al.* 2009; Yanagawa *et al.* 2014), dirt and pollen from the body (Jones and Jones, 2001; Hackmann *et al.*, 2015). Also, grooming prevents attacks by ectoparasites (Pettis and Pankiw, 1998; Léonard *et al.*, 1999; Leung *et al.*, 2001) and parasitoids (Vincent and Bertram, 2010). Additionally, parasitoids clean the antennae to remove compounds released by the hosts, without which it could compromise the location of new host and their own fitness (Corbet, 1973).

Another advantage of the grooming behavior is to help in locomotion (Wendler and Vlatten, 1993). Finally, grooming helps the distribution of hydrocarbons, brochosomes, antiseptic, antimicrobial and grooming secretions over the body surface (Kovac and Maschwitz, 1990, 1991; Lusebrink *et al.*, 2008; Böröczky *et al.*, 2013; Dong and Huang, 2013).

Grooming is, as seen, a crucial and beneficial behavior for the development of insects. The insects are numerous, a diverse group (Grimaldi and Engel, 2005; Trautwein *et al.*, 2012; Misof *et al.*, 2014), and they developed distinct types of adaptation for cleaning (Jander, 1966; Hlavac, 1971, 1975; Robbins, 1989; Hackmann *et al.*, 2015).

Based on the phylogenetic hypothesis of the insect evolution of Trautwein *et al.* (2012) and Misof *et al.* (2014), the antennal grooming in these animals is described in the following paragraphs.

Archaeognatha cleans the body with oral grooming; there are no reports about antennal grooming, instead they used only the mouthpiece (Valentine 1986, Basibuyuk and Quicke 1999), while *Thermobia domestica* (Packard, 1873) (Zygentoma) uses the first pair of legs and the oral apparatus (Jander, 1966; Valentine, 1986).

In Palaeoptera, the dragonflies (Odonata) clean head, antennae, and eyes using only their legs and post-tarsus (Jander, 1966; Leung, *et al.*, 2001). Jander (1966) tried to document the cleaning behavior in mayflies (Ephemeroptera) without success. She concluded that they possibly missed this behavior or it is unknown yet (Jander, 1966; Jander and Jander, 2011).

There are several studies of grooming in Polyneoptera. Many species of grasshoppers (Orthoptera) hold the antennae with the prothoracic legs and pull into the mouth to clean them (Jander, 1966; Lefebvre, 1981; Chapman, 1998). As opposed, *Diradius vandykei* (Ross, 1944) (Embioptera) does not use the legs to assist in cleaning, instead, it uses the basal musculature of the antennae. With this musculature, the antennae are bent and held by the mouthparts, and the flagellomeres are gradually cleaned (Jander, 1966; Valentine, 1986).

Grylloblattodea also uses the front legs to bend the antennae towards the mouth to clean them as observed in Orthoptera (Kostromytska *et al.*, 2015). In *Aborolabis mauritanica* (H.Lucas, 1849) (Dermaptera) the antennae reach to the mouthpiece by a sudden movement, and there, they are cleaned piece by piece (Jander, 1966). We can also observe Dermaptera using the legs to place the antennae on the mouthparts (Valentine, 1986), thus, the grooming can be of two types in Dermaptera. *Zorotypus*

hubbardi Caudell, 1918 (Zoraptera) pulls the antennae with the prothoracic legs towards the mouthpiece, and remain there to hold the antennae, while they are cleaned by the mouthparts (Valentine, 1986), however, as mentioned by this author it may not be a standard behavior for the species, concluding that sampling was biased.

Within the Dictyoptera (Trautwein *et al.*, 2012), the praying mantises (Mantodea) use the first pair of legs to move the antennae to mouth, thus cleaning the flagellomeres with the mouthparts (Jander, 1966; Valentine, 1986; Basibuyuk and Quicke, 1999). Similarly, Blattodea also clean the antennae with the mouthparts (Robinson, 1996; Chapman, 1998). *Battela germanica* Linnaeus, 1767 uses the labium and maxilla for the antennal grooming (Robinson, 1996); however, some cockroaches clean the antennae with the mandibles (Chapman, 1998). This behavior in cockroaches may vary among families (Smith and Valentine, 1985). There are few studies with termites (Isoptera) on cleaning behavior; however, the grooming is like other Dictyoptera, they also employ the first pair of legs to place the antennae on the mouthparts and there the grooming is performed (Grassé, 1986). In summary, several studies within Polyneoptera show that these insects use the forelegs to bring the antennae to the mouthparts, except in *Diradius vandykei* (Ross, 1944) (Embioptera), which uses the basal muscles of the antennae.

Paraneoptera includes Psocoptera, Phthiraptera, Thysanoptera and Hemiptera. They all possess piercing-sucking mouthparts (Grimaldi and Engel, 2005; Trautwein *et al.*, 2012; Misof *et al.*, 2014), therefore they do not employ the mouthparts for the antennal grooming (Jander, 1966; Hlavac, 1975). Jander (1966) indicated that *Ectopsocus parvulus* Kolbe, 1880 (Psocoptera) might perform a derivative behavior using the prothoracic legs. Regarding the order Phthiraptera, no information about grooming is available. On the other hand, there are reports about wing cleaning in thrips (Thysanoptera) (Ellington, 1980; Mound and Walker, 1982; Moritz, 1999), but, again, no data on the antennal grooming was found in the literature.

Hemiptera is the most diverse order of hemimetabolous insects (Grimaldi and Engel, 2005). Heteropterans use a comb located at the apex of the foretibia to clean the

antennae, and it has been observed in *Nezara viridula* Linnaeus, 1758 (Pentatomidae), *Oncopeltus fasciatus* (Dallas, 1852) (Lygaeidae) and others species (Hlavac, 1975; Barão *et al.*, 2013; Rebora *et al.*, 2019). Other structures may also be found on the tibia of Asopinae (Pentatomidae). Foretibial expansion with a channel on the ventral surface and the foretibial apparatus have been reported, but the function of both structures is unknown (Brugnera *et al.*, 2018). In summary, paraneopterans do not use the mouthparts for antennal grooming, which is done by the first pair of legs.

Holometabola or Endopterygota is a well resolved phylogenetically group and the most diverse lineage of living being on the planet (Grimaldi and Engel, 2005; Trautwein *et al.*, 2012; Misof, *et al.*, 2014). There is wide grooming diversity in this group.

Many hymenopterans employ a specialized tibia-tarsal complex (named as *strigilis* by some authors, Schönitzer and Lawitzky, 1987) on the prothoracic legs for cleaning the antennae. The tibia-tarsal complex is composed of a notched (on the inner surface of the proximal end of the basitarsus) and a movable tibial spur (Thelen and Farish, 1977). Several authors have studied the behavior and the structure involved in the grooming (Schönitzer and Renner, 1984; Schönitzer and Lawitzky, 1987; Hackmann *et al.*, 2015). Bees (Apidae) have a tibial-tarsal complex with comb in the notched (Schönitzer and Renner, 1984). Ants (Formicidae) have a spur (called *strigilis*) with comb and bristles, and a notched with comb, bristles and brush (Schönitzer and Lawitzky, 1987; Hackmann *et al.*, 2015). Mutilids (Mutillidae) have a velum with a smooth edge and two rows of teeth in the apex, a notched deeper than ants; tiphiid wasps (Thiphiidae) show diversity in the antenna cleaner (Schönitzer and Lawitzky, 1987) and paper wasps (Vespidae) do not have a specialized tibia-tarsal complex for the antenna grooming (Sumana and Starks, 2004).

Hymenopterans pass the entire length of the antenna through antenna cleaner using one or two legs (Farish, 1972; Basibuyuk and Quicke, 1999). Finally, they clean the tibia-tarsus complex with the mouthparts (Jander, 1966) or rubs the prothoracic legs along the antenna (Vespidae) (Sumana and Starks, 2004). A more detailed review about the grooming in hymenopterans may be consulted in Farish (1972).

Strepsiptera has no information about the antenna grooming, even its biology is complex (Kathirithamby, 1989; Cook, 2014). Coleoptera that is the sister group of Strepsiptera is one of the most diverse groups in Holometabola (Trautwein *et al.*, 2012). Beetles have two types of grooming, one of them includes the forelegs, and in the other there is, an antenna cleaner (specialized structure for grooming) (Jander, 1966; Hlavac, 1971; Valentine, 1973). The grooming employing the forelegs can be "antenna-clean" or "antenna rub". The antenna-clean mechanism is when the foretibia or tarsus reaches the antenna and pulls it into mouth where it is cleaned with a chewing motion. It has been observed in 27 families of Coleoptera except in most weevils, Carabidae, Cicindelidae, and some Cerambycidae and Meloidae (Valentine, 1973). Antenna rub is when the foretibia or tarsus rub along of the antenna, starting at or near the base and progressing distally. After cleaning the antennae with the foretibia, these beetles clean the legs using the mouthparts, and this behavior has been recorded in 29 families, but it is absent in non-anthribid weevils and Cicindelidae (Jander, 1966; Valentine, 1973).

The antenna cleaners or grooming tools have been described in Carabidae and Chrysomelidae. These can be formed from setal aggregations on the foretibia (Carabidae), or can be comb-like array of cuticle outgrowths (Chrysomelidae) (Hlavac, 1971; Hosoda and Gorb, 2011). On the other hand, the loss of oral antennal cleaning in Adephaga indicates that this type of behavior can have independently evolved within the order (Valentine, 1986).

The grooming behavior is similar among the three orders in the clade Neuropterida. Neuropterans hold the antennae between both forelegs (Jander, 1966; Chapman, 1998). Jander's investigation describes antenna grooming in representatives of Chrysopidae, Hemerobiidae, Osmylidae and Myrmeleontidae. Chrysopidae, Hemerobiidae and Osmylidae rub the head with both tarsi of the forelegs, thus hold the antenna from the base with the crossed tarsi and they form a big loop that is cleaned from the base to the tip. Antlions (Myrmeleontidae) have short antennae therefore and may not loop them as in the other families mentioned previously. On the other hand, dustywings (Coniopterygidae) have a similar behavior but they bow their heads to the

side and cross both forelegs at the level of their tibiae and pull the antenna down towards the front. Finally, *Libelloides coccajus* (Denis & Schiffermüller, 1775) (Ascalaphidae) grooms the antennae using only one leg and the cleaning movements are slow compared to Chrysopidae.

The behavior cleaning in Megaloptera was observed in *Sialis fuliginosa* Pictet, 1836 (Jander, 1966). These insects employ the forelegs for antenna grooming. First, the head and thorax are moved down; after that, both antennae are cleaned together with the forelegs. In the same way, the grooming in Raphidioptera was described in Jander (1966). The species *Raphidia notata* Fabricius, 1781 and *Agulla* sp. clean the antennae using the forelegs. Both forelegs are held together and pass through both antennae, remarkably similar to the behavior of the megalopterans (Acker, 1966; Jander, 1966).

Amphiesmenoptera is well-established as a monophyletic group and includes Trichoptera and Lepidoptera (Kristensen *et al.*, 2007; Mitter *et al.*, 2017). Trichoptera has a report about cleaning behavior. *Phryganea striata* Linnaeus, 1758 and *Limnephilus flavicornis* (Fabricius, 1787) put both antennae horizontally forward in parallel and with both tarsi (forelegs) take both antennae to clean them (Jander, 1966).

Butterflies and moths (Lepidoptera) have two kinds of antennal grooming behavior: with or without a specialized grooming structure (Philpott, 1924; Jander, 1966; Robbins, 1989). In many lepidopterans, antenna cleaning is done with the epiphysis, a structure located in the foretibia (details below in section 1.3) (Philpott, 1924; Jander, 1966; Odell *et al.*, 1982; Robbins, 1989). However, other groups lack the epiphysis; they use other mechanisms for grooming. In Pieridae, grooming occurs with the movement of the foretibia and tarsus against the antennae. They may or may not possess a brush of long scales on the foretibia and a scale brush on the middle tibia (Robbins, 1989). The families Lycaenidae, Riodinidae and Nymphalidae do not have a brush on the foretibia or on the middle tibia. These butterflies clean the antennae by moving them between the basal middle tibia and distal midleg femur; or midleg tarsus and middle tibia (Jander, 1966; Robbins, 1989).

Mecoptera and Siphonaptera are sister groups closest relatives of Diptera in the clade Antliphora (Misof *et al.*, 2014). There are few records of the grooming behavior in Mecoptera (Setty, 1940; Jander, 1966). One of them described the antennal grooming of these insects (Jander, 1966). The genus *Panorpa* cleans the antennae with both forelegs; they grip the antenna from the base, and forming a concavity between the protarsus and the spurs of the foretibia through which the antenna passes to be cleaned (Jander, 1966), and the other author does not mention antennal grooming (Setty, 1940). For Siphonaptera, no information about grooming was found in the literature.

Diptera has few information about antennal grooming. There is one report of antennal grooming in Brachycera; *Musca domestica* Linnaeus, 1758 (Muscidae) does not use the mouthparts for antennal grooming, but instead it sweeps the forelegs for cleaning head appendages (Barber and Starnes, 1949). This behavior is similar to the specie *Volucella inflata* (Fabricius, 1794) (Syrphidae), this species usually rubs one antenna between the raised forelegs (Jander, 1966).

In contrast, mosquitoes (Culicidae) and biting midges (Ceratopogonidae) have a structure for grooming (Linley and Cheng, 1974; Walker and Archer, 1988). The females of *Ochlerotatus triseriatus* (Say, 1823) (Culicidae) bear tibial combs that function as grooming organs. They grasp the antenna with apices of both foretibia with both legs working synchronously (Walker and Archer, 1988), and *Culicoides melleus* (Coquillett, 1901) and *C. furens* (Poey, 1853) (Ceratopogonidae) have comb with spines on the fore and hind legs, and the grooming organs are sexually dimorphic. There is a similarity between the antennal grooming of Diptera (Muscidae and Syrphidae) and Mecoptera due to use of the forelegs (Barber and Starnes, 1949; Jander, 1966).

To briefly summarize, the wingless insects have an oral grooming with (Zygentoma) or without use of the legs (Archaeognatha). In Palaeoptera, oral grooming is lacking, and in Polyneoptera, except for Embioptera, it is regained. In Paraneoptera, the oral grooming is not observed due to the type of mouthpart, but in Hemiptera it is present as a structure for antennal grooming. Then, until Paraneoptera (except for Hemiptera), there was no specialized structure for the cleaning function. On the other

hand, certain holometabolous have a complex antenna cleaning, showing some exclusive and specialized structures for this purpose. Thereby, Hymenoptera, Coleoptera, Lepidoptera and Diptera have these grooming structures, which coincidentally are the most diverse groups of Insecta.

1.3. Epiphysis: Origin of term, description of structure, function, shape and importance

A historical review of term 'epiphysis' is provided in the present study, based on all bibliographic sources at our disposal. At the beginning of the 19th century, Fabricius (1807) published a glossary of entomological terms. The term *Pedes* is referred for the legs of the Lepidoptera, but in the description, there is no single allusion to the epiphysis.

Kirby and Spence (1826) mention that in the foretibia of Lepidoptera and Trichoptera possess two or three mobile spurs called *'Calcaria'*, which are positioned at the apex or in the middle tibia. This term would be what is known as *'epiphysis'* today. Burmeister and Shuckard (1836) also described spurs using the term *'Calcaria',* but these authors did not mention its presence in Lepidoptera. Thus, the descriptions of species were made without considering the epiphysis as a taxonomic character (Walker, 1869). A year later, Meyrick (1895, p. 4) mentioned that on the foretibia there is a *'median spine-like process, sometimes also with an apical hook'*. However, in that same year, the term *'epiphysis'* was already associated with a structure located in the foretibia of butterflies and moths (Smith, 1895). Four years later, Sharp (1899, p. 314) described the epiphysis as a '*peculiar mobile pad'* with peculiar scales, which could be used as comb organ.

In the early 20th century, Rothschild and Jordan (1903) used the term *'epiphysis'* as a spur of the foretibia, indicating to be homologous to the spur of the middle tibia. Thereafter, the term *'epiphysis'* appeared in glossaries as '*a movable process attached near the base of the inner side of the anterior tibia in many Lepidoptera'* (Smith 1906, p. 140). But in others glossaries, the term *'Calcaria'* appeared to refer to the tibial spurs (Jardine, 1913). Other terms are also found in the literature as *'strigils or strigilis'*

(Philpott, 1924; Costa Lima, 1945; Chapman, 1998) or *'fibula'* (Zombori and Steinmann, 1999).

Of all the terms proposed in the literature to name the cleaning structure present in Lepidoptera (*'Calcaria'*, *'Epiphysis'*, *'Strigil'*, *'Fibula'*), the most used is *'epiphysis'*; which I will use throughout the current research, due to the others terms are obsolete and *'strigilis'* is used for the cleaning structure in ants.

The epiphysis is an synapomorphy of lepidopterans (Common, 1975; Kristensen, 2003; Kristensen *et al.*, 2007), but it may be reduced or absent in some families (e.g. Acanthopteroctetidae, Lophocoronidae, Palaeosetidae, Anomosetidae, Cyclotornidae, Megalopygidae, Dalceridae, Pieridae, Nymphalidae and Lycaenidae). Whether present or reduced, it is located on inner surface of the foretibia (Philpott, 1924; Callahan and Carlysle, 1971; Odell *et al.*, 1982; Robbins, 1989; Kristensen, 1999, 2003; Kristensen *et al.*, 2007).

Epiphyses are multicellular outgrowths, which may be derived from the unpaired spur present in the same location in the Amphiesmenoptera ground plan (Whalley, 1986; Kristensen, 2003; Kristensen *et al.*, 2007). This specialized spur is a movable process (Rothschild and Jordan, 1903; Richards and Richards, 1979; Odell *et al.*, 1982; Kristensen, 2003; Triplehorn and Johnson, 2005) and it is covered by a cuticular protuberances called acanthae (Richards and Richards, 1979; Odell *et al.*, 1982; Gorb, 2001).

Acanthae are projections of unicellular composition that have as morphological characteristic the lack a socket and a sensory cell (Richards and Richards, 1979; Gorb, 2001). Those projections are present in various structures of many insects and can to be various shapes (bifurcated, fimbriate, composed pectines or combs, with straight or bent tips) (Richards and Richards, 1979; Gorb, 2001). In Lepidoptera, they have a spatulated-shaped and have a mechanic function on the epiphysis (Odell *et al.*, 1982).

Due to the presence of acanthae covering the structure, the function of the epiphyses as an antenna cleaning structure is not debatable. Several authors indicate that butterflies and moths clean themselves using the epiphyses, which is a very efficient

mechanism for the grooming (Philpott, 1924; Costa Lima, 1945; Callahan and Carlysle, 1971; Odell *et al.*, 1982; Robbins, 1989; Kristensen, 2003; Sambaraju *et al.*, 2016). The antennae are cleaned with the legs of its corresponding side, for that, the antenna is put in the angle between the appendage and the foretibia, doing this repeatedly (Jander, 1966; Robbins, 1989; Kristensen, 2003; Sambaraju *et al.*, 2016).

On the other hand, this cleaning structures vary in shape, size and origin. Such variation is widespread in several families, genera and species (Philpott, 1924) and some examples may be found in the following species: *Helicoverpa zea* (Boddie, 1850), *Lymantria dispar* (Linnaeus, 1758), *Manduca sexta* (Linnaeus, 1763), various species of Papilionidae (*Papilio glaucus* Linnaeus, 1758, *Baronia brevicornis* Salvin, 1893 and *Parnassius clodius* Ménétries, 1855) and Hesperiidae (*Erynnis juvenalis* (Fabricius, 1793), *Poanes zabulon* Boisduval & LeConte (1837), *Pyrrhopyge araxes* Hewitson, 1867, *Megathymus yuccae* (Boisduval & Leconte, [1837]), *M. ursus* Poling, 1902 and *Carterocephalus palaemon* (Pallas, 1771) (Callahan and Carlysle, 1971; Odell *et al.*, 1982; Robbins, 1989; Kent and Griffin, 1990). Descriptions of the epiphyses in the works abovementioned were poorly detailed.

For all the above, the importance of the epiphysis is highlighted by its role as antennal cleaner and as informative character for taxonomists. First, the structure is important for butterflies and moths, which need to clean the antennae regularly for maintaining the fitness (details below in section 1.4). Second, these structures are useful for taxonomists. In the literature, there are several scientific articles considering the epiphysis as diagnostic character for the description of species (Philpott, 1924; Burrows, 1932; Lemaire and Wolfe, 1988; Schoorl, 1990; Minet, 1994; Schmitt *et al.*, 1996; Mielke *et al.*, 2008; Mielke and Drechsel, 2009; Pinheiro *et al.*, 2011; Mielke and Casagrande, 2013; Castro-Torres and Llanderal-Cázares, 2016; Perini *et al.*, 2019).

In our group of study, there is little information on the epiphyses. Hawkmoths have prominent and well-developed epiphyses (Philpott, 1924; Madden, 1944; Kristensen, 1999, 2003). These structures may vary in length, position (Rothschild and Jordan, 1903; Philpott, 1924), and they also may vary between sexes (Kent and Griffin,

1990) with a comb on the margin of inner fold (Philpott, 1924; Madden, 1944). These characteristics are taxonomically informative in Sphingidae; nevertheless, in addition to the characters already mentioned (length and position), there may exist others features potentially important for taxonomic purposes.

1.4. Antenna: Function, shape, importance and its relation with the epiphysis

Antennae are appendages of the head; they are movable olfactory sensors found in all insects (Callahan, 1975; Snodgrass and Eickwort, 1993; Chapman, 1998). Due to the high presence and variety of sensilla, they are sensory structures (Snodgrass and Eickwort, 1993; Chapman, 1998; Field and Matheson, 1998), and function olfactory, gravitational, thermosensory and thermo-hygrosensory (Trouvelot, 1877; Chapman, 1998; Field and Matheson, 1998; Shields and Hildebrand, 1999a, 1999b; Böröczky *et al.*, 2013).

These sensorial structures are quite variable in shape among insect groups; and sexual dimorphism is common (Chapman, 1998; Triplehorn and Johnson, 2005). Moths and butterflies have morphologically distinct antennae, and it is often possible to observe sexual dimorphism in these structures (Chapman, 1998; Symonds *et al.*, 2011; Li *et al.*, 2018). Having the hawkmoths as the object of our study, they have antennae often lamellate ventrally and roughly filiform, clavate, setiform or pectinate (bi-, tri- or quadripectinate) and apex usually hook-shaped (Schreiber, 1978; Kristensen, 1999). Furthermore, there may have differences between male and female (Madden, 1944; Schreiber, 1978; Kristensen, 1999). Males have two rows of long trichoid sensilla, while in females these sensilla are absent or shorter (Madden, 1944; Kristensen, 1999).

The association between the antennae and epiphyses is indisputable; because the only function of the epiphysis is to clean the sensory organ (Philpott, 1924; Jander, 1966; Callahan and Carlysle, 1971; Odell *et al.*, 1982; Kent and Griffin, 1990). Philpott (1924) (p. 215) indicated: *'Species with the most plumose antennae have the most highly developed strigilis'*. He concluded that the development of the epiphysis could to be

associated with shape of the antennae. However, the variation of the epiphysis may also be correlated with other parameters not yet studied.

To summarize, there are still discrepancies in the taxonomy of Sphingidae, and to clarify or resolve some uncertainties of a taxonomic nature, more and better characters are needed. Epiphyses as a synapomorphy of moths and butterflies can be one of those key characters; however, the details of such characteristics have been little explored. On the other hand, as it is a potential antenna cleaning organ in Lepidoptera, it is presumed that its morphology is associated with the shape of the antennae and other parameters. For all the reasons, the main objectives of this study are:

−   To present a detailed morphological study of the epiphyses of representatives of the tribes Dilophonotini and Philampelini.

−   To describe the morphological variation among species of Dilophonotini and Philampelini.

−   To quantify the size and shape variation of the epiphyses of Dilophonotini and Philampelini.

−   To clarify the correlation between the epiphysis (epiphysis length, epiphysis width, position of the epiphysis) and others parameters (antenna length and foretibia length).

5. CONCLUSIONS

For the first time, the morphology of the epiphyses of 59 species of Dilophonotini and Philampelini were examined and described in detail using different tools such as steromicrocospe, SEM, and geometric morphometrics. Furthermore, statistical analyzes were carried out to show relationships with other body measurements including antenna length, tibia length and epiphysis position. New terminologies were proposed to standardize future studies aiming a better understanding of the structure.

All the species examined showed typical epiphyses with comb on the inner margin, which appears to be a common feature of several lepidopteran groups, and it is presumed to be a character that independently evolved in various lineages or/and that has been lost several times. Additionally, 22 characters are established to assist in the description and comparison of the epiphyses among species, genera and tribes. Nevertheless, variation of these characters is more evident in genera and tribe levels.

A significant positive allometry demonstrated a relationship between the position of the epiphyses and the foretibia length; in the same way, between the antennae length and the epiphysis length. Epiphyses are sexually dimorphic structures in various characters, including those related to size. Most males have longer epiphyses than females, which may be associated with the antennae length rather than body-size, the variation between males and females are showed in the antennae length.

Geometric morphometrics showed that some species are more similar to other genera than those that are currently classified. Evolutionary allometry is not an important factor in the evolution of our group of study, and some closely related genera do not have similar epiphyses shape, which might indicate rapid radiation events or strong selective pressures on this structure.

This study is an initial contribution to a more comprehensive understanding of the evolution of the lepidopteran epiphyses, with detailed and illustrated descriptions. We claim for other studies that can reveal new morphological patterns in other moths and butterflies.

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