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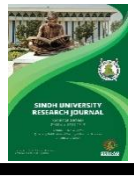

Genitalic Studies of Four Species of Family (Sphingidae: Lepidoptera) from Sindh, Pakistan

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Abstract:

Spinix moths are commonly known as Hawk moths because of their wing pattern and fast flight which resembles with hawk. Family *Sphingidae* is divided into two subfamilies, *Sphinginae* and *Macroglossinae*. Four known species of family *Sphingidae* are first time reported from Sindh, Pakistan on the basis of genital characters. Main purpose of this study is to dissect their genitalia for showing relationship among them. Four species of hawk moth (*Psilogramma increta*, *Acheronta styx*, *Hyles livornica*, and *Dephnis neri*) belonging to two sub families *Sphinginae* and *Macroglossinae* were selected for genital dissection. Specimens of these four species of family *Sphingidae* were collected and deposited in Laboratory of Entomology, Department of Zoology University of Sindh Jamshoro. For dissection, abdomen of moths was removed from body and boiled in 10% KOH solution for 30 minutes for softening, then soften abdomen was washed in distilled water. All scales of moths were removed by using camel brush and male and female genital organs were separated by fine forceps and needles. Genitalia were examined under Stereo zoom Binocular microscope with the attachment of digital camera, for their identification. The result showed that there were differences and similarities found in genital structures of these four species. Two species of Subfamily *Sphinginae*, *Psilogramma increta* and *Acherontia styx* shows similarity in genital structures, while *Hyles livornica* and *Dephnis. neri* of subfamily Macroglossinae also share some genital characters. These similarities in genital structure show taxonomic relationship among these species because of same habit and habitat.

Keywords: Hawk moth, *Sphinginae*, *Macroglossinae*, Sindh, Pakistan, Habitat, Taxonomic relationship

Introduction

The name of the "Sphinx" moth was coined by Linn in 1758, it is based on the rising behavior of larvae when threatened, giving it the appearance of the Egyptian Sphinx, while the name of the hawk moth family was coined by Samoel in 1819. The Streamlined shape, slender wings and fast flight of these moths resembling with hawks, hence their name kept as "Hawk Moth". “Hawk moth” also known as “humming bird moth” because during sucking flower saps their hovering behavior shows similarity with humming bird flight. The wingspan of the hawk moth ranges from a little over 2 cm to nearly 25.0 cm (Scobie, 1992).

The family *Sphingidae* is divided into two subfamilies, five tribes and 203 genera (Bridges, 1993). In 1986, 205 genera and 1050 species of the hawk moths were reported (Abrera, 1986). Family *Sphingidae* is divided into two subfamilies, *Sphinginae* and *Macroglossine*. Subfamily *Sphinginea* included 116 genera and *Macroglossinae* subfamily contains 89 genera worldwide (Messenger, 1997).

In addition, there are numerous spontaneous records of hawk moths found in Pakistan, edited primarily by workers conducting a broader study of the insect fauna they overviewe *Macroglossum sp*, from forest insect fauna of Pakistan, *Theretra oldenlandiae*, *Daphnis neri*, *Macroglossum nycteris*, *Acherontia lachesis*, *Clanis phalaris*, and *Sataspes scotti* were identified (Chaudhry, *et al.*, 1966). Sphingid moth species, *Acherontia lachesis*, *Hippotion celerio*, and *Hyles euphorbiae* reported from Peshawar Pakistan (Chaudhry, *et al.*, 1970). *Hyles livornica* (as *Hyles lineata*), *Nephele hespera* (as *Nephele didyma)*, *Theretra alecto* and *T. oldenlandia* reported from Rawalpindi Pakistan (Mohyuddin, 1987).

15 species from 13 genera of hawk moths are reported from Punjab (Mehmood, 1996). The distributional range and cladistics analysis of family *Sphingidae* from Pakistan with their morphological and genital components has been described (Kamaluddin, *et al.,* 1999). In the Checklist of moths in Pakistan, 14 species of 14 genera of family Sphingidae are listed in addition to their distributional range and host plants mentioned (Kamaluddin, *et al.,* 2007). Checklist of *Lepidoptera* from Pakistan, presented by Hashmi and Tashfeen, reported 53 species of *Sphingidae* and preserved them in museums of Pakistan (Hashmi,*et al.,* 1992). External genital morphology of seven species of Sphingid moths belonging to subfamily *Sphinginae* (Sphingidae), *Psilogramma increta, (Walker) Psilogramma menephron* (Crammer), *Acherontia styx,*(westwood), *Agaricus convoluli*, (Linneaus) *Leucophelbia emittens*, (walker), *Polyptychus trilineatus*, (Moore) and *Marumba dyras* (Walker) studied from North-western India (Sood, *et al.,* 2006). Study of external genitalia of four species of genus *Psilogramma, Rothschild & Jordan, menephron* (Gramer) the type species, *Casuarinae, increta* Walker, and *Orientalis* brechlin have been reported from India (Singh, *et al.,* 2012). For decades, insect genital features have been utilized to restrict species and in other domains of systematic. The widespread usage of genital traits is owing to their efficacy of specie differentiation. Genitalia are the only way to distinguish between species, which are similar in morphological view. In Sindh, Pakistan previously moth fauna is poorly reported and hawks moth are not reported previously. Present study focuses on new record of Spinix moth from Sindh, Pakistan with the help of male female genitalia because of same habitat and feeding preferences of Adult moth and larvae.

Materials and methods

Study area

Moth specimens of four species were collected from four different areas of Sindh, Pakistan (Hyderabad, Jamshoro, Tandojam and Larkana) because these areas are popular for their rich flora. There are number of variety of crops cultivated here. Study was carried out from March 2021 to December 2021.

Data Collection

Moth specimens were collected by applying different methods, Insect net, light trap, and light sheets. These methods are very common for moth collection. Some moths were captured during day time by usage of insect net, while Pennsylvanian light trap and light sheets were used in night time. Light trapping acquiesces a huge number of specimens with a minimum of exertions (Holloway, *et al.,* 2001, Fiedler, *et al.,* 2004). Light trap was run for 4 hours in consecutive periods. By usage of light source, especially mercury bulb, moths were attracted to the trap because moths are nocturnal insects and they are directly attracted towards light.

Material examined:

Different numbers of four species of family *Sphingidae* were collected for genital dissection. 2 Males, 3 Females of *Psilogramma increta*. 3 Males, 5 Females of *Acherontia styx*. 10 Males and 12 Females of *Hyles livornica* and 3 Males, 2 Females of *Dephnis nerii* were collected for genital dissection from studied areas. Specimens were deposited in Entomology laboratory, Department of Zoology, University of Sindh Jamshoro.

Genital dissection

For 30 minutes, the abdomens of moth specimens were boiled in a 10% KOH (potassium hydroxide) solution. The softened abdomen was cleaned with distillated water after boiling in KOH, and all scales or hairs were removed from the abdomen before it is placed in a prepared glass with water. With the use of preparatory needles, fine forceps, and clean hairs, the male and female genital capsule or Bursa copulatrix removed from the abdomen and the genitalia recognized under a binocular microscope (ST 40-2L Optica Binocular Dissecting Microscope).

Identification

Identification has been done on genital morphology. Adult moths were identified with the help of literature, from different research papers which were confirmed by Kitching, (research entomologist British Museum of Natural History London) and also by authentic keys taken from different research articles of hawk moths (Younus, *et al.,* 2014). They implemented common method for genital study (Eberhard, 1985).

Illustration and preservation of moth's genitalia:

Adult moths and their male and female genitalia were photographed by attaching digital camera with binocular microscope (ST 40-2L Optica Binocular Dissecting Microscope), and then genitalia were stored in a tiny flask or minute bottles with a drop of glycerin. By using ocular graph, illustrations of male and female genitalia of each member of four species were drawn.

Results

Psilogramma increta, (Walker, 1865)

Male genitalia

Uncus well developed long and simple deep bifurcation erected, at the side of tip it's acuminate and ventrally curve, at apex side its sclerotize. Analis present. Gnathos shortly bifid or have sinous weakly. Broad tugumen which are weakly sclerotized. V shaped viniculum present, sinus nearly to absent, elongated valve and sole shaped from middle to apex it's thickly scetosed. Sclerotized costa having straight margin and broad apex. Well-developed succulus. Aedegus faintly developed, having moderate length

slender and straight, at apex wedge shaped fork present (Figure 1).

Female genitalia

Psilogramma increta have similar lobes of ovipositor as sphinix. size of posterior apophysis smaller then anterior apophysis, both apophysis are long and rod shaped, ostium bursae with free angular plate, posterior part of plate sclerotized and partially moon shaped. With the elevation of lateral margins ductus bursae short weakly sclerotized thick and curved. Ductus seminalis entering just about the last part of ductus bursae extended and oblong (Figure 3).

***Acherontia styx*, (Westwood, 1848)**

Male genitalia

Oval shaped tegumen present, large bulb like saccus present which are without saccular process, uncus is curved inwardly and larger in size, bearing sharp tip, gnathos are compact and membranous, larger paramere and rectangular in shape possess minute l scales, and 3 thorn like process present at the ventral basal margin. Aedeagus are rod shaped narrowed at distal side, membranous flap enclosed median portion (Figure 5).

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Female genitalia

Lobes of ovipositor are like typical Sphingidea type. Number of papillae makes outer lobes, each emitting spines which are very thin, and lower spines longer, inner lobes smaller. Posterior apophysis longer than anterior apophysis both are rod shaped. Ostium bursae have proximal transverse flap, which is shallowly sinuate from mesially. Ostium bursae is narrow and rounded having eight sclerotized sternite which encircling it. Ductus bursae elongated from anteriorly its weakly sclerotized. Ductus seminalis entering near corpus bursae. Corpus bursae elongated have globular pouch which is wrinkled anteriorly. Membranous sigma missing (Figure 7).

***Hyles livornica*, (Esper, 1780)**

Male genitalia

Oblong tugumen, saccus is like cup-shaped lacking process of saccular, tapered uncus, internal middle surface intensely created peak incurved and pointed, somewhat longer than the wide gnathos, later it becomes incurved among piercing apex, large theca is tubular which is divided into two identical parts by a pair of sclerotized thecal appendages bearing 5–6 dentitions on one side and a simple dentation with a broad membranous conjunctival lobe exclusive of cornuti on the other (Figure 9).

Female genitalia

Moderate papillae anales, bearing besets which are bean-shaped with large thick hairs, posterior apophysis is greatly longer than anterior apophyses which is narrowed apically, plate like lobus vaginalis, large ductus bursae present, corpus bursae are tubular, cornuti are balloon like with U shaped extended (Figure 11).

***Dephnis neri*, (Linnaeus)**

Male genitalia

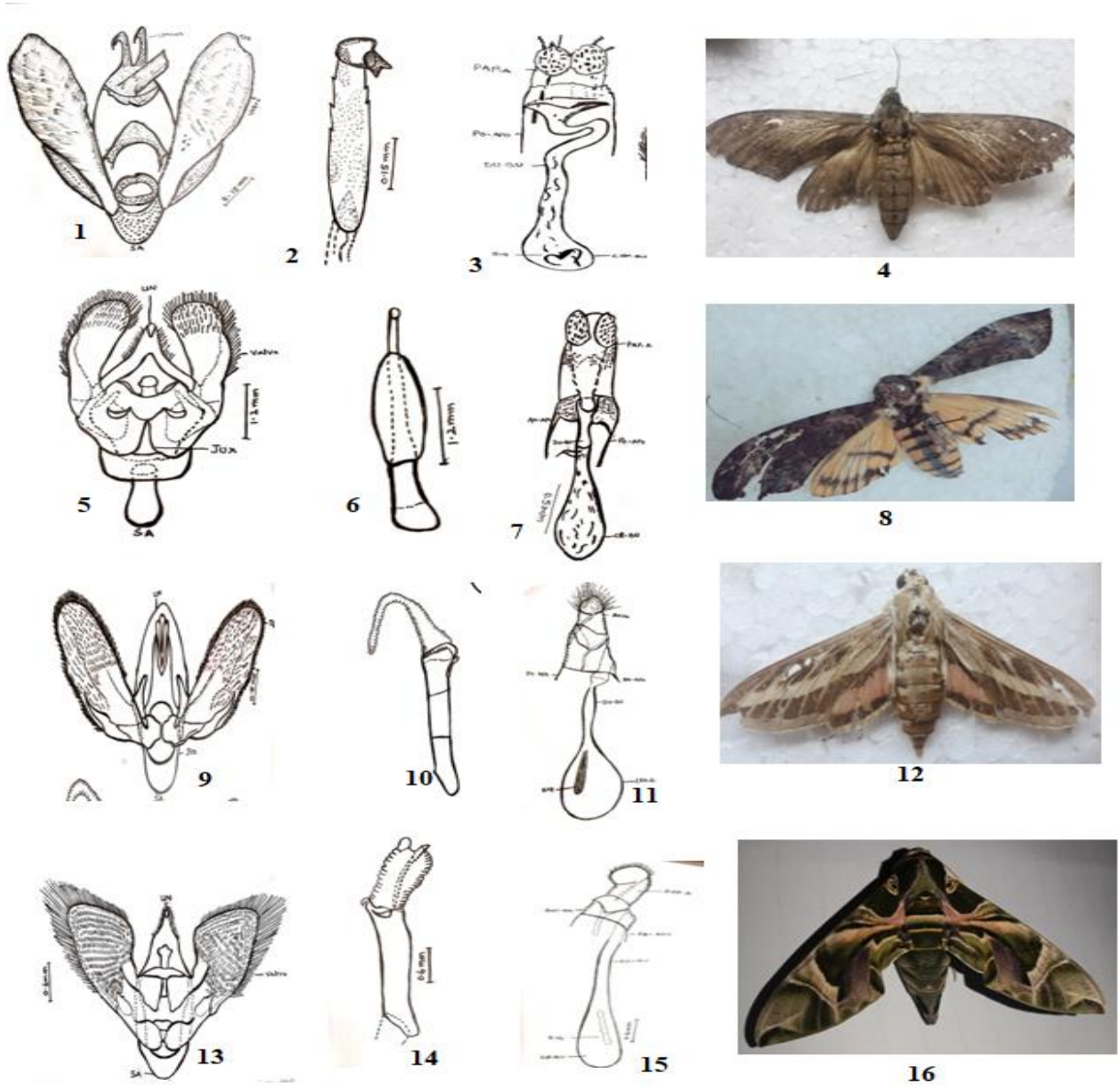
Uncus is wide by base side, slowly becoming narrow at tip side, quite sclerotized, having blunt, tip, gnathos are triangular in shape, saccus well sclerotized and lid like at distal side, broad tegumen, to some extent also sclerotized, almost as the same lengthwise of vinculum; gradually vinculum extended and becomes rounded, a little hair like sclerotized, juxta have the shape of bean but possess tapered ends, vaguely sclerotized. Valva somewhat wide, outside its well reaching the position of uncus; costa are semi sclerotized, sacculusare wide little bit sclerotized. Aedeagus, is slender, and it have moderate length partially sclerotized, distal end of aedeagus on one side having a highly sclerotized spur and a slightly sclerotized bicuspid plate present at the end of distal side, vesica without any framework (Figure 13).

Female Gennitalia

Corpus bursae is campanulate, both walls of Corpus bursae are protruding in the side of middle, and its membranous, signum is vase-like and longitudinal, ductus bursae moderate, and in the middle elbow-shaped, basal half of ductus bursae is guarded by well sclerotized genital plate, from the anterior end of genital plate ductus seminalis originating well before. Anterior apophysis is shorter in size than posterior apophysis, which is expanding towards the tip. The posterior apophysis is long and narrow, having blunt apices, papilla analis deltoid, fringed with micro and macro setae (Figure 15).

Discussion

The reprehensive family *Sphingidae* is most diverse. Present work is based on male and female genitalia of four species. Previously no record of *Sphingidae* reported from Sindh Pakistan. So there is great need to work on these valuable creatures. Genital dissection has been done in order to describe species on genital characteristics from other parts of country (Gilgit Baltistan Khyber Pakhtunkhwa, Islamabad, Kashmir, Punjab, Peshawar, and Rawilpindi). 60 species of Sphingidae fauna reported only on the basis of morphological Characteristics (Rafi, *et al.*, 2014, Kamaluddin, *et al.*, 2007).



Figs.1-4 *Psilogramma increta;* 1, Male genitalia; 2. Aedeagus; 3. Female genitalia; 4. Adult. Figs.5-8 *Acherontia styx;* 5, Male genitalia; 6. Aedeagus; 7. Female genitalia; 8. Adult. Figs. 9-12 *Hyles livornica* 9. Male genitalia; 10. Aedeagus; 11. Female genitalia; 12. Adult, Figs.13 -16. *Dephnis neri* 13, Male genitalia; 14. Aedeagus; 15. Female genitalia; 16. Adult.

Presently Four sphingid species, *Psilogramma increta*, *Acherontia styx*, *Hyles livornica*, and *Dephnis neri* dissected. All species show difference and quite similarities in genitalia. Male having different valve shape, tegument, and Aedeagus which show difference in all species of corpus bursae, ductus bursae, signum, and, papilla analis. *Psilogramma increta* and *Acherontia styx* belongs to subfamily Sphinginae, which shows variations in their ductus seminalis, which was originated from Ductus bursae in *Acherontia styx*, while in *Psilogramma increta* it was originated from Ostium bursae. *Hyles Hyles livornica* and *Dephnis neri* belongs to subfamily Macroglossinae therefore both of these species shows slightly same characters, in both Female genital plate reduced, which is main character of subfamily Macroglossinae. Many taxonomists have worked on genitalia they adopted term lock and key hypothesis (Fiedler, & Schulze, 2004) Evaluate the post Darwinian which explains that divergence of genitalia is a type of the characters of dislocation, which is mostly determined by sexual selection (Younus, *et al.*,2014).

Conclusion

Four species of family Sphingidae *Psilogramma increta*, *Acherontia styx*, *Hyles livornica*, and *Dephnis neri* identified on the basis of genitalia and first time reported from study localities of Sindh, Pakistan. Similarities in genital structure of female genitalia were found, while male genitalia structure was quite different in each species. The similarities in female genitalia showed taxonomic relationship in these species because of same habitat and feeding preferences of larvae and adult.

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