Morphological characters of fig wasps (Hymenoptera: Chalcidoidea) associated with *Ficus nitida* (Moraceae) in Qalyubia Governorate, Egypt.

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**Abstract:**
Pollinator and non-pollinator fig wasps (Hymenoptera: Chalcidoidea) and its host *Ficus nitida* have a specific mutualism relationship. *F. nitida* is an Asian fig tree belonging to the cultivated Moraceae family in Egypt and many countries. In this study, we focused on morphological characters and descriptions of the pollinator and non-pollinator fig wasps associated with fig tree *F. nitida* from three cities in Qalyubia Governorate, Egypt. This work was carried out from September 2013 to August 2016. We recorded two main species of pollinator fig wasps associated with *F. nitida*, *Eupristina verticillata* Waterston and *Eupristina spp.* (Agaonidae). In addition, non-pollinators *Philotrypesis spp.*, *Odontofroggatia spp.*, *Micranisa spp.*, and *Walkerella spp.* were also recorded in this survey. Morphological characters were illustrated by photographs scanned using Electron microscope (SEM) and high power light microscope.

**Keywords:** *Ficus nitida*, pollinator and non-pollinator fig wasps, scanning electron microscope, syconium.

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1. Introduction

The *F. nitida* is one of the traditional medicinal plant and food additives around the world[1]. The species name *F. microcarpa or nitida* refers to the species’ small-sized figs, about 8 mm in diameter[2]. The name has been applied to *Ficus retusa* and *Ficus nitida*. These species reportedly all have the common name ‘laurel de la India’[3]. Fig wasps are a group of wasps living within fig trees. They are related to order Hymenoptera, superfAMILY Chalcidoidea. Some are pollinator fig wasps and others are non-pollinator living in figs as phytophagous, galler or parasitoid[4, 5]. The pollinator fig wasp associated with *F. microcarpa* belonging to family Agaonidae, subfamily Agaoninae, while non-pollinator fig wasps classified under family Peromalidae divided to three subfamilies (Otitessellinae, Sylocyrcticinae, and Epichrysomalinae)[6-8]. *Eupristina Verticillata* Waterston considers as the specific pollinator wasp of *F. microcarpa (nitida)*. Fig wasps help figs in seed formation and fig ovaries be its shelter[9]. At first male pollinator fig wasp emerges and chews the wall of syconium ovaries to help female to go out after matting with it. Then females collect pollen grains from the mature anthers and stored them in pollen pockets which located in the ventral surface of mesosoma. Finally, the female flies away through the ostiole to pollinate another immature syconium and lay eggs. Female searches for figs only for two days then died[10].

Non-pollinator fig wasps (NPFWs) are responsible for the declining number of pollinator wasps and seeds in syconium. NPFWs divided into main two types, phytophagous and parasitoids. Phytophagous contain species that feed on seeds that called seed predators and the other species feed on syconium tissue[11]. Another type of parasitoid that kills other wasps is hyper-obligatory parasitoid[12]. But others can be parasitoids and also feed on plant tissue called secondary galler[10]. Genus *Odontofroggatia* that belonging to subfamily Epichrysomalinae considers as gall maker. The female lays eggs on fig ovaries and larvae grow in galled ovules feed on seeds. Figs with *Odontofroggatia* sp contain fewer seeds and pollinator wasp offspring but it in total rarely inhibit them[13]. Species of *Philotrypesis* (Subfamily: Sylocyrcticinae) are parasitoids and a secondary galler non-pollinator wasps[14]. Subfamily Otitessellinae has two main phytophagous non-pollinator genus *Walkerella* and *Micranisa*. Females of this subfamily lay eggs from the outside of fig in the syconia female florets[15].

This study focused on morphological characters and descriptions of the pollinator and non-pollinator fig wasps associated with *F. nitida* in three cities in Qalyubia, Governorate, Egypt. Morphological characters and description were photographed by using a high power light microscope. Several ultrastructural details of the external surface of PFWs and NPFWs were revealed by SEM, allowing us to compare the morphology of these species.

2. Materials and Methods

2.a. Collecting the fig wasps:
Fig wasp samples associated with the fig tree *F. nitida* were collected from three different cities (Benha, Kafr Shukr, and Shibin El Qanater). The three cities are located in Qalyubia Governorate, Egypt. Twenty mature syconia were collected haphazardly at late C or early D phase, according to Galil and Eisikowitch[16] from three trees per each city. Syconia were collected periodically form *F. nitida* in each city every two weeks, during the period between September 2013 to August 2016. The contents of each syconium were recorded after they were sliced into quarters and softened by being soaked in water for 10 minutes. Using a binocular microscope, all fig wasps
inside fig samples were identified using mainly Chen et al.[17] and Feng and Huang[18].

2.b. High power light microscope:

Fig wasp species were distinguished on the basis of their morphology and identified by using high power light microscope according to the methods of Chen et al.[17] and Feng & Huang[18]. The fig wasps were stored in 95% ethanol for long-term preservation. The contents of each syconium were recorded after they were sliced into quarters. The numbers of seeds were counted in each quarter and then the total number of seeds in each syconium was calculated.

2. c. Scanning Electron Microscope (SEM):

For more details, and ultrastructure of fig wasps, SEM was carried out at Faculty of Agriculture, Mansura University. Specimens that were stored in alcohol prepared using the heat assisted acetone drying procedure, as described below. Wasp specimens were mounted on brass stubs using chloroform based adhesive, drying with Tousimis Autosamdri – 815 Coater, sputter coated with gold using SPI Module – Sputter Carbon / Gold Coater, prior to observation and photography using a JEOL JSM-6510 LV SEM.

2. d. Heat assisted acetone drying process:

1. Specimens stored in alcohol were placed on filter paper, which was in turn placed on cotton wool soaked in acetone in a glass dish with a lid.

2. Acetone was allowed to gradually replace the alcohol present in the specimens for three hours, after that the fig wasp specimens were removed and placed under an electric lamp for 30 minutes to evaporate the acetone. The above heat assisted drying air and remove acetone from the specimens[19, 20].

Female and male pollinator and NPFWs were identified, measured, and counted. The body length of each fig wasp in both sexes, the length of female's antennae and the ovipositors, as well as the length, males head capsule were measured (3replicates / specimen); Specimens were measured by using SEM.

3. Results

There are two species of *Eupristina* associated with *F. microcarpa*, *E. verticillata* the main pollinator female fig wasp and another *Eupristina sp* which considers as secondary pollinator made a mistake and entered the wrong kind of fig. In addition, four NPFWs were recorded, secondary galler parasitoids wasp genus *Philotrypesis*, phytophagous genus *Micranisa* and *Walkerella*, as well as the phytophagous gall maker genus *Odontofroggatia*.

1. Description of PFW subfamily Agaoninae, genus *Eupristina*:

The two sexes of genus *Eupristina* are different in body size and structure form (Fig.1.a) *Eupristina verticillata* (Fig.1.a, A) smaller than *Eupristina sp* (Fig.1.a,B ) in body size. Both species are winged. The females are dark brown body color; have well-developed head, thorax and abdomen slightly covered with setae; the female's body length mean is measured 1.06 ±0.08mm (Fig.1.b). It has flattened semi rectangular prognathous head slightly covered with short setae; the head relatively large and obvious compound eyes are located in the posterolateral position. The anterior dorsal part of the head is occupied by two deep grooves that may serve to hold the flagellum when the pollinators pass through the syconium. The well-developed chewing mouth parts are located ventrally with a serrate, blade–like mandibles extending beneath the head (Fig.1.c, d). A pair of 3 segmented geniculate antennae is located on the frontal surface; the flagellum with long extruded spine, multiparous plate sensilla (MPS) is *Elisabethiella baijnathi* (E.b) type; antennal length is 0.33±0.02mm; the scape is large and triangular in shape jointed with flagellum by short pear- shaped pedicile; the scape has two deep grooves on the ventral side that may serve to hold the flagellum when the pollinators pass through the syconium; flagellum consists of 9 flagellomeres from the fifth onwards, more and more cup-shaped (Fig.1.e). The thorax has well-developed and distinct three segments with three walking legs; frontal tibia have two teeth in the dorso-apical comb. (Fig.1.f). The transparent fore wing is covered with setae and venation is reduced to only two veins near the costal margin; Pigmentation of wing venation ending with a knob on parastigma, rest of veins colorless, indistinct; so the venation appears incomplete: marginal, stigmatic and post marginal absent, hind wing is narrower and shorter than fore wing (Fig.1.g). The pollen pockets found on the lateral surface of mesopleural, carrying pollen grains, but lacks coxal comb (Fig.1.h). The ovipositor is shorter than the body and strongly arched dorsally with valves; exerted for distance equal to the length of metasoma; it has medium length about 0.56± 0.018 mm (Fig.1.i, j).
Male of genus *Eupristina* is apterous, yellowish brown color, U-shape, with mean body length 0.64±0.07 mm, with prognathous head strongly dorsoventrally flattened; small compound eyes; head length 0.21±0.004 with a medium groove extending from v-shaped facial cavity to posterior margin, the lateral margins rounded in dorsal view with large stout mandibles; antenna geniculate type, flagellum has 7 small flagellomeres with anellus; thorax is rounded in dorsal view with legs adapted for walking, fore and hind legs femur are larger than mid femur; the gaster segments telescoping, strongly tapered and normally curved forward beneath mesosoma (Fig.2.a, b).

2. Description of phytophagous NPFWs subfamily Otitesellinae:

In the present study, two genera belong to non-pollinator putative phytophagous subfamily Otitesellinae were recorded. Genus *Micranisa* and *Walkerella*.

2.1 Description of genus *Micranisa*

Female of genus *Micranisa* is winged and the whole body is black with some metallic green-blue glosses; tibia and tarsi yellowish brown (Fig.3.a, b). Body length mean is 1.22±0.04 mm head and thoracic dorsum with very fine sculpture, lower face surface with conspicuous setiferous punctures, thorax often finely densely reticulate, pronotum shorter than mesocutum, propodum without medium carina (Fig.3.c). Hypognathous head, large compound eyes, mandible normal adapted for chewing (Fig.3.d). Antenna with mean length 0.28±0.017mm; 3 anelli; MPS is *Elisabethiella stickenbergi* (*E.s*) type; distance between toruli nearly as diameter of torulus (Fig.3.e). Laterally compressed gaster, apex of gaster and ovipositor are curved downward; with ovipositor length 0.22±0.02mm (Fig.3.f).
Fig (3): a & b) Female *Micranisa* *sp* under high power microscope a) dorsal view, the whole of its body black color with some metallic green-blue gloss and legs tibia and tarsi yellowish brown color. b) Lateral view. C-f) *Micranisa* *sp* female, under scanning electron microscope. c) Lateral view, thorax often finely densely reticulate, pronotum shorter than mesocutum, gaster laterally compressed. d) Head Hypognathous, mandible normal. e) Antenna with three anelli; MPS is Elisabethiella stuckenbergi type. f) Ovipositor curved downward from abdomen.
Male of genus *Micranisa* is apterous, yellowish brown color, body length is 1.25± 0.09 mm, head scape quadrangular or angularly expanded at base, Epistomal emargination without medium tooth, head length 0.51±0.02 mm; antennal toruli far apart much closer to the eyes than to each other and the space between them convex, scape foliaceous quadrate quadrangular, or angularly expanded at base, Epistomal emargination without medium tooth, Chewing mouthparts with very strong jaws. Thorax propodeum, mesocutum and mesonotum fused and telescoping segmented abdomen (Fig.4.a, b).

Fig (4): a) Dorsal view of *Micranisa sp* male under high power microscope, yellowish brown color. b) Dorsal view of *Micranisa sp* male, under scanning electron microscope, Head subquadrangular, antennal toruli far apart much closer to eyes then to each other, Epistomal emargination without median tooth. Propodeum, mesocutum and mesonotum fused mandibles usually slightly shorter than in alternate. Abdomen segments telescoping.

2.2 Description of genus *Walkerella*:

The body of female *Walkerella* with black color, gaster slight metallic cast (Fig.5.a), the body length is 1.73± 0.08 mm, Head and thoracic dorsum with sculpture has fine reticulation, lower face surface with inconspicuous setiferous punctures, dorsum of mesosoma with strongly impressed, pronotum shorter than mesoscutum (Fig.5.b), hypognathous head with obvious compound eye and three ocelli (Fig.5.c), geniculate antenna with length 0.45± 0.31mm showing radicular, scape, pedicel, anelli, funicle 9 antennomer, antenna have two anelli distance between toruli less than diameter of torulus (Fig.5.d), Fore wing postmarginal vein longer than stigmal vein at angle much less than 90 (Fig.5.e, f). Gaster compressed, posterior segments and ovipositor sheaths horizontally extending straight behind or slightly downward; with ovipositor length 0.31±0.024mm (Fig.5.g, h).
Fig (5): a) Dorsal view of *Walkerella* sp female on syconium male under high power microscope, the whole of body black color, Gaster with slight metallic cast. b, h) *Walkerella* sp female under scanning electron microscope. b) Lateral view of  female, dorsum of mesosoma with strongly impressed, pronotum shorter than mesoscutum and have uniformly reticulate sculpture. c) Hypognathous head with obvious compound eye and three ocelli. d) Female antenna with Two anelli; MPS is *Elisabethiella stuckenbergi* type.e &f) Female wing, postmarginal vein longer than stigmal vein with angle less than 90°.g &h) Female posterior abdominal segments, gaster not compressed; cover with several long setae, ovipositor sheaths extending straight from the end of the abdomen.
Male of genus *Walkerella* is apterous yellowish brown color (Fig. 6.a); Body length is $1.65 \pm 0.19$ mm, head is sub quadrangular dorsally depressed; antennal toruli as much apart as distance from eyes, escape flat and clavat tapering at its base; Thorax propodum and mesocutum only fused and the abdomen segments telescoping (Fig. 6.b).

Fig (6): a.) Dorsal view of *Walkerella sp* male, under high power microscope, body wall yellowish brown color. b.) Dorsal view of *Walkerella sp* male small form, under scanning electron microscope.

3. Description of secondary galler parasitoid NPFWs subfamily Sycoryctinae:

Two main species of genus *Philotrypesis* were recorded, female of sp. A with black body, while sp. B. with orange brownish body color and black bands on dorsal surface of abdomen (Fig. 7.a). The mean body length of female genus *Philotrypesis* is $1.8 \pm 0.09$ mm (Fig. 7.b); the female with hypognathous head; large compound eye, three ocelli (Fig. 7.c), chewing mouthpart; mandibles with 2 or 3 teeth; large scythe-like jaws; labial palpus 2 segments and maxillary palpus 4 segments, antennae geniculate differ from above with elongated proximal scape, short triangular pedicel, the elongated flagellum comprised 7 flagellomeres similar in size; antenna with length $0.4 \pm 0.06$ mm (Fig. 7.d,e,f). Wings are hyaline, stigmal vein without knob; fore femur only moderately swollen cover with setae (Fig. 7.b); metasoma without petiole, mesocutum with shallow notauli, thorax dorsal surface cover with setae, metathorax have pair of spiracles and also the first abdominal segment (Fig. 7.g); long ovipositor ($1.2 \pm 0.024$ mm) (Fig. 7.h,i).
Fig (7): a) Lateral view of Philotrypesis sp female under light microscope; sp. A, Black color, sp. B, Brownish orange color with dark bands on gaster. b, i) Female under scanning electron microscope. b) Lateral view of Philotrypesis sp A c) Female head hypognathous; mandibles normal; antenna different from above with 3 anelli. d) Head dorsal view, large compound eye and three ocelli, MPS sensilla is Elisabethiella stuckenbergi type. e) Head and prothorax, frontal legs coxae articulate strongly with prothorax. f) Chewing mouthpart, mandible with 3 teeth. g) Dorsal view of female thorax and first abdominal segment, dorsal surface of thorax cover with setae, both metathorax and first abdominal segment have pair of spiracles. h, i). Ovipositor, ovipositor longer than body cover with bristles.

Three different polymorphic species were recorded. All species yellowish brown color (Fig.8. a, b, c, d); sp A: is a winged form (Fig.8.a); sp. B is Apterous and head and notum without very long setae and also temple without seta, (Fig.8.b, c); spC is aperous, head and notum with very long setae, but temple with a series of seta (Fig.8.d). Generally, male of genus Philotrypesis, with and body length 1.18±0.1 mm.; head length 0.35±0.05 mm.; mandibles shorter than the head; eyes smaller than one fourth of head and black color; antenna toruli in lower face depression and close but separated by a keel; funicle normal, propodeum longer than mesocutum both separated by thin dark line and have smooth sclerites (Fig.8.e, f).
4. Description of phytophagous gall-maker NPFWs subfamily Epichrysomallinae:

Two main different species of *Odontofroggatia* both sexes were recorded, according to gaster petiole length. *Sp1* with long gaster petiole and *Sp2* short one: (Fig.9.a, b, respectively). Both male and female of genus *Odontofroggatia* are winged, color variable in both sex yellowish brown and black (Fig.9.a, b). Female body length of the two species is 2.6 ± 0.13 mm. From the same figures, the female has yellowish brown shiny color, transverse head; antenna length 0.73± 0.11; funicle 6 segments, sensilla plates longer than segment length, thorax with spare paired setae, tarsi 4 or 5 segments, stigmal vein as long as marginal vein and virtually at right angle to wing margin, postmarginal vein very short and stub-like; female ovipositor length 0.21± 0.002.

Male winged; body length 2.2± 0.12 mm; head length 0.72 ± 0.04 mm with small eye, long mandibles, antenna toruli very close to clypeus edge; funicle 5 segments, sensilla plates longer than segment length, tarsi 4 segments, metacoxa large and toothed, gaster petiolar longer (Fig.9.a,c,d); or with short petiole (Fig.9.b).
4. Discussions

Based on the morphological characters, nine species of fig wasps associated with *F. nitida* were recorded from the three cities in Qualiobia Governorate, Egypt during the survey period, two PFW species (Agaonidae), in addition to seven species of NPFWs, *Philotrypesis* spp, *Micranisa* spp., *Walkerella microcarpa* (Bouček), and two species of *Odontofroggatia*.

Similar studies on fig wasps associated with *F. nitida (microcarpa)* in several places were done. *E. verticillata* has been recorded in most sites where *F. nitida* has been introduced[4, 15, 17-28]. Besides the agaonid PFWs, at least 26 NPFW species have been recorded from the figs of *F. nitida* in the plant's native range, belonging to the families Pteromalidae, Eurytomidae and Ormyridae [17, 18, 29, 30]. *F. nitida (microcarpa)* pollinator wasps recorded as *E. verticillata* Waterston, but this taxon may be a complex of closely related species[31]. In this study, two PFWs species were found to be associated with *Ficus nitida*, the main pollinator *E. verticillata* and a secondary pollinator *Eupristina sp* which considers as made a mistake and entered the wrong kind of fig. The fig breeding system affects directly in the spread and diversity of pollinators[32]. Pollinators in monoecious *Ficus* travel long distances to find their host trees makes them more likely to meet hosts of fig species. For this reason, they may be more likely to make errors in identifying the host, and thus it is predicted that the female wasp switches is common in monoecious fig pollinators [33-35].

The life style and reproduction behavior of fig wasps indicated that males and females show sexual dimorphism externally. The females of *Eupristina* are winged, long multi-segmented antennae provided with large number of sensillae, large compound eyes. Joseph[36] stated that these features enable the female to travel long distance and recognize their host tree easily and to move through the inside the fruit. The flattened semi rectangular prognathous head force themselves through the very narrow ostiolar canal. The female loses its wings and its antennae in this process. She moves through the syconium hollow to the female flowers and dusts them with the pollen that she brought with her from the fig in which she
grew up. The figs have three types of flowers: short female flowers, long female flowers and male flowers. The length of female ovipositor enables it to reach only to the ovary of the short female flower and so she deposits her eggs there. In a day or two after the pollination and oviposition had taken place the female dies inside the syconium.

Fig wasps belonging to galler parasitoids wasp subfamily Sycoryctinae are the largest NPFWs associated with fig trees in Old World regions[37]. The female Philotrypes can easily be assigned to Philotrypesini because of the presence of subquadrate narrow pronotum and tubular urogomites of the terminal segments (7th and 8th segments) ovipositor. The extraordinarily long ovipositor not only functions as an egg-laying apparatus but is also capable of piercing the fig wall [38].

Two Otontofroggattia female species are characterized by short or long petiole. The interaction between F. microcarpa (nittida) and the fig wasp Odontofroggattia galili has been studied by Galil and Copland (1981)[39]. They mentioned that the distinct petiole of the female is an important modification in a unique oviposition behavior. The female twists the long ovipositor shaft within the petiole. The ovipositor shaft is gradually ejected from the petiole and injected into the fig tissue by a rotary movement and telescoping of the proximal end of the inner ovipositor plate independent of the petiole tip. This mechanism enables the tip of their ovipositor shaft curves into a barb within the fruit tissue.

Two genera of phytophagous NPFWs of subfamily Otitesellinae (Chalcidoidea, Pteromalidae) were recorded in this study, Genus Micranissa and Walkerella. Walkerella is an Old World genus of non-pollinating fig wasps in the subfamily Otitesellinae. It is the most widely distributed genus of the subfamily, although there are only six known species in the world[40]. Females of Otitesellinae are characterized by ovipositor clearly shorter than the gaster; In the Otitesellinae sexual dimorphism is strongly expressed. The pale, flightless males apparently do not normally leave the syconium in which they develop. Bouček[41] stated that Walkerella females oviposit from the outside of small fig syconia and larvae develop in galls formed from female florets (ovaries). The ovipositional habits of this species do not appear to have been determined[15, 40].

Based on our observations, the male wasps of all species were characterized by small strong apterous pale-coloured body, large stout head, well-developed anterior and posterior legs and telescopic abdomen. According to Susheela et al. (2016) [21] these features enable the males to live and move easily inside the syconium in order to search for the fig ovaries containing their females for copulating with them and for facilitating their function during copulation. Micranissa female; Color mostly black, gaster with the slightly metallic cast; dorsum of mesosoma with strongly impressed, uniformly reticulate sculpture; gaster not compressed; posterior segments and ovipositor sheaths extending straight behind or slightly downward and male Micranissa; Antennae widely separated, closer to the compound eye than to midline of the head, entirely pale; sides of the head from above semicircular in outline[15]. Excluded from these, males of Odontofroggattia, these males are fully winged. According to early study by Galil and Copland[39], Odontofroggattia male is free living unlike the apterous species males of other Agonidae as copulation takes place outside the syconium. Also we observed that female body size of PFW and NPFWs was larger than male except in phytophagous genus Micranissa the male size mean is slightly larger than female size. In most chalcids an increase in a male's size may have little impact on their reproductive successes, which probably explain why male chalcids are generally smaller than female[42].

Male Sycoryctinae exhibit diverse adaptations in their morphology, including winged dispersers and wingless fighters[43]. Generally Philotrypes sp males exhibit square-shaped head, which is quite different from other Sycoryctinae males[38]. Both winged and flightless polymorphic Philotrypes males were observed in this study. The flightless males were themselves polymorphic. Vincent & Compton[44] stated that polymorphic Philotrypes species, winged males were found to be rare at high densities, but common at low densities. Also he detailed that no species had only winged males. Enquist & Leimar[45] observed that mating sites are thus the primary determinants of male morphology and behaviour. In fighting species males were larger than their females, whereas pacifists and aggressors were equal in size or smaller than conspecific female. The most characteristic feature of fig wasps that fight is their large mandibles. Fig wasp mandibles also serve purposes apart from fighting, such as chewing into galls containing females. Walkerella males characterized by large quadrate head and stout mandibles scythe-like jaws. Wang et al.[46] noticed that Walkerella males fight using their mandibles and can kill each other. Males damage their mandibles during fights more frequently than other parts of the body, but heads were never detached. More studies are needed in this area; especially this is the first recording of the fig wasp fauna in Egypt.

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