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Biology of *Braueriella phillyreae* (Diptera: Cecidomyiidae) in Libya

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Abstract. *Braueriella phillyreae* (Löw, 1877) is a Mediterranean species of a gall midge, its larvae cause pustule galls on the leaves of *Phillyrea media* L. which belongs in the plant family Oleaceae. Attacked leaves were collected from *P. media* in 2017 and 2018 in the Sports City area near Bayda, Al-Jabal Al-Akhdar Region, Libya. *Braueriella phillyreae* is univoltine species, only one generation develops per a year. It overwinters as the larva of the first instar in the gall on the leaf of *P. media*, and pupation takes place in the galls in the spring, first adults were observed in April. Antennae of adults are composed of with 2+12 antennomeres. Female flagellomeres are cylindrical, male flagellomeres are bimodal. The wing vein R5 is joining C slightly beyond wing apex. Tarsal claws are curved without teeth. The ovipositor is not protractil. Morphological characters of larvae, pupae, and adults of *B. phillyreae* are shown in figures.

Key words. Life cycle, gall midge, distribution, Diptera, Cecidomyiidae, *Braueriella phillyreae,* Libya, Palaearctic Region.

INTRODUCTION

Till this time, seven species of gall midges were known to occur in Libya (Gagné & Jaschhof 2021). *Asphondylia ononidis* Löw, 1873, causing galls on *Ononis spinosa* (Fabaceae) occurs in many countries of Europe and its galls were found also in Libya; *Houardiella salicorniae* Kieffer, 1912, inducing galls on *Salicornia fruticosa* (Chenopodiaceae), occurs in Spain, Italy, Morocco, Tunisia and Libya; *Jaapiella parvula* (Liebel, 1889), causing galls on *Bryonia dioica* (Cucurbitaceae), occurs in the United Kingdom, the Netherlands, France, Portugal, and Libya; *Psectrosema tamaricinum* (Kieffer, 1909), causing galls on *Tamarix africana* and *T. nilotica* (Tamaricaceae). Galls were found in Egypt, Israel, and Libya; *Rhopalomyia producticeps* Kieffer, 1912, causing galls on *Artemisia herba-alba* (Asteraceae). Galls were found in Algeria and Libya; *Rhopalomyia tubifex* (Bouché, 1847), causing galls on *Artemisia campestris* (Asteraceae), its galls were registered in many countries of Europe and in northern Africa (Algeria, Tunisia, Libya); and *Stefaniola dupla* Möhn, 1971, causing galls on *Haloxylon salicornium* (Chenopodiaceae). The type locality of the latter species is Uadi Tininai, Libya.

The plant *Phillyrea media* L. belonging in the family Oleaceae is native to the Mediterranean region. It is one of the plant species in the Al-Jabal Al-Akhdar region in north-eastern Libya. It is an evergreen shrub, up to five metres high, the leaves are in opposite pairs, small, leathery, ovate to lanceolate, the flowers are small, greenish white, produced in short clusters. The fruit is a bluish-black drupe containing a single seed (Jafri & El-Gadi 1977). It is the host plant for several specialized herbivores insects such as *Psylla* sp. of the order Hemiptera.

Three gall midge species are associated with the host plants of the genus *Phillyrea*: *Probruggmanniella phillyreae* (Tavares, 1907), *Dasineura rufescens* (de Stefani, 1898) and *Braueriella phillyreae* (Löw, 1877). Characteristics of galls are given Roskam (2019). The larvae of *P. phillyreae* (Tavares, 1907) cause gall on fruits. The fruit is disfigured, swollen, slightly elongated, usually curved. In the gall a single yellowish larva develops. It occurs in Portugal, France, Italy, Croatia, Greece, Turkey, Israel, and Algeria. The larvae of *D. rufescens* (de Stefani, 1898) cause multi-chambered swellings on young twigs. Galls were found in Europe – in Sicily, southern Greece, Corfu and Samos, in western Asia – in Turkey, and in Algeria in North Africa. The larvae of *Braueriella phillyreae* (Löw, 1877) cause irregular blister-shaped swellings on underside of leaf blade. Till the present time the galls of *B. phillyreae* were found in several countries around the Mediterranean Sea - in Greece, Italy, Turkey, Corsica, Tunisia, and Algeria.

Our research was directed to the study of the life cycle of *Braueriella phillyrea* on its host plant *Phillyrea media* in Libya.

MATERIAL AND METHODS

Leaves of *Phillyrea media* attacked by the gall midge *Braueriella phillyreae* were collected at the locality the Sports City area near Bayda in Libya (geographical coordinates: 32°47'11" N, 21°46'02" E). Twigs with attacked leaves in plastic bags were brought every ten days from March 2017 to May 2018 in the laboratory. Twigs with galled leaves were placed in the glass jar with water and covered with a thin screen until adults emerged.

Galls were dissected under a stereoscopic microscope to obtain the larvae and pupae. The reared specimens were preserved in 70% ethanol and then mounted on microscope slides in Canada balsam, following the methods outlined by Gagné (1994). Descriptions and measurements of galls from the start of infestation until the mature galls before the emergence of the adult were performed by digital caliber; the diameter of the adult exit hole was also measured by a micrometric slide. Some morphological characters and measurements were recorded for all the insect life stages (from eggs to adults) using a micrometric slide. All photos of diagnostic characters were taken by a digital camera fixed on the microscope. In addition, all phases from the egg to the adult were described, and the emergence dates of the adults were recorded in the field.

RESULTS

Braueriella phillyreae (Löw, 1877)

Description

Egg. The eggs are elongate elliptical, pale yellow translucent, the mean length is 0.09 mm, the mean width 0.02 mm.

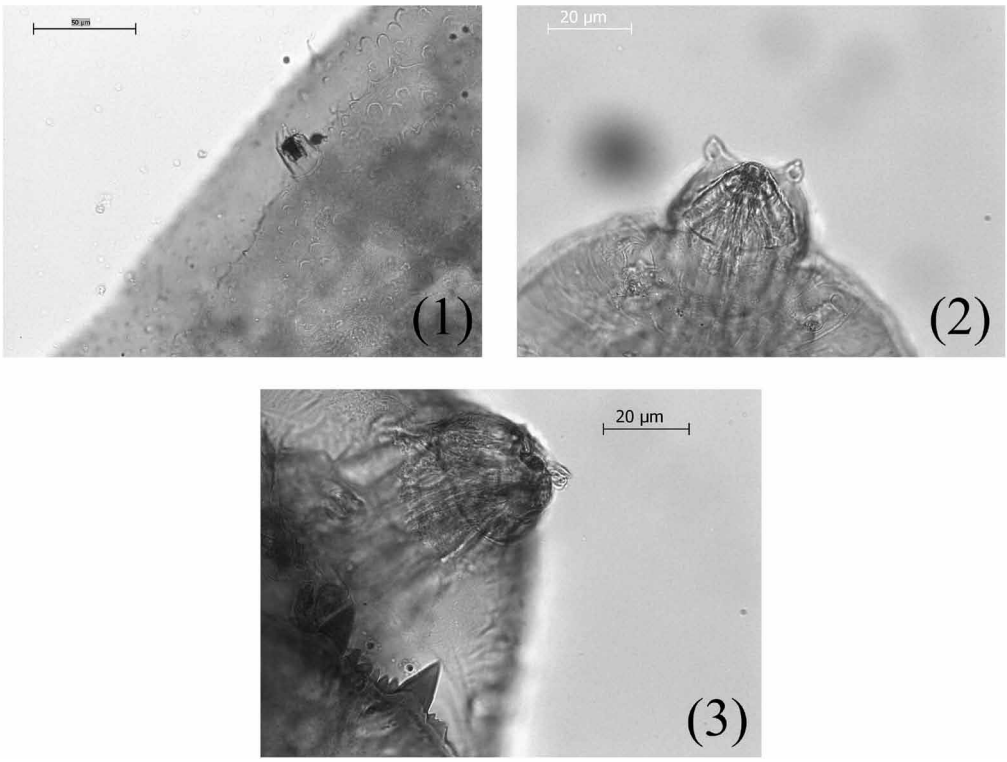
Larva. There are three larval instars. Larva of the first instar has a smooth and transparent cuticle. Body is orange coloured, slightly flattened, 0.1–0.7 mm long (n=12), 0.06–0.2 mm broad (n=10), translucent spiracles are present only on prothorax and eighth abdominal segment. Head capsule is lightly sclerotized, with mean length 20.01 μm and mean width 23.38 μm. Mouthparts and other head structures are reduced and difficult to distinguish, spatula absent. Larvae of this instar grow very slowly. The duration of this instar is the longest of the three instars. Larvae of this instar overwinter in the galls.

Larva of the second instar has the body wider and flatter than the larva of the first instar. Its body is 0.9–1.5 mm long (n=20), yellow colored, the periphrastic respiratory system is composed of nine pairs of spiracles, one pair on the prothorax and the eight pairs on the eight abdomen segments, integument is smooth. The head capsule is lightly sclerotized, with an average of 29.56 μm and width 54.38 μm. Mouthparts are reduced and difficult to distinguish; spatula absent.

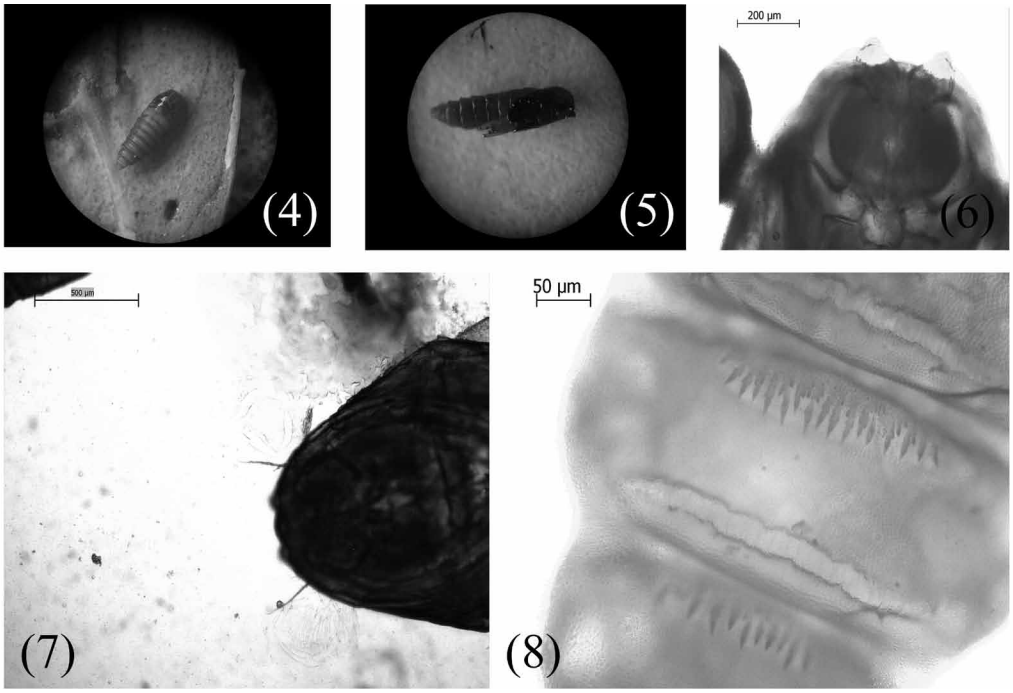
Larva of the third instar is highly mobile, capable of crawling long distances. The body is pale yellow, covered with convex verrucae, deeply segmented. Larvae develop very quickly. They

doubled their size in a short time. Body is 1.5–5.2 mm long, cylindrical, tapering posteriorly. The periphrastic respiratory system is composed of nine pairs of spiracles on the prothorax and the eight abdomen segments. The mean diameter of spiracle is 17.84 µm (15.8–19.4 µm; Fig. 1). The head is hemispherical, more elongated than in the head of the second instar, lightly sclerotized, somewhat conical; the mean length 70.13 µm; width 52.24 µm. Antennae are 16.29 µm long. The mean width of mouthparts is 28.8 µm (23.5–34.1 µm; Fig. 2). The spatula is bidentate, brown color; represented by a sclerotized transverse band, armed with two triangular teeth separated. The average distance between them is 21.94 µm (18.0–25.4 µm). The average height of right tooth is 9.63 µm (7.0–12.6 µm), and the average of the left tooth height is 9.89 µm (6.2–12.9 µm) between, and outside of which there are a variable number of short teeth, ranginng between three and five (n=5), between the two triangular teeth are located the two sternal papillae. Mean length of spatula 215.6 µm (195.0–231.4 µm), mean width of spatula head 111.65 µm (98.7–118.3 µm; Fig. 3). The prothoracic segment with pair of spinules lateral to the sternal spatula. The anal segment is weakly bilobed with two lateral papillae, the average diameter of the anus is 4.51 µm.

Pupa. The body of pupa is covered with pointed cylindrical, elongate verrucae. The pupa in early stages is yellow coloured, later the head, thorax, anterior part of antennae and wing sheaths turn dark

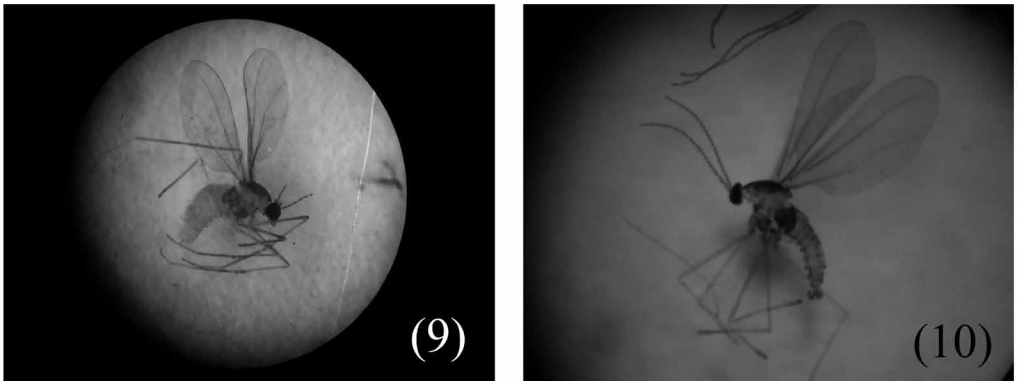


Figs 1–3. Larva of *Braueriella phillyreae* (Löw, 1877). 1 – larva of the third instar in the gall, 2 – head capsule showing mouthparts and short antennae, 3 – spatula.



Figs 4–8. Pupa of *Braueriella phillyreae* (Löw, 1877). 4 – in lateral view, 5 – in dorsal view, 6 – head with a pair of horns, 7 – two short and thin apical cephalic setae, 8 - dorsal spinules on abdominal segments.

brown, the rest part is orange (Figs 4 and 5). The body of the male is 2.6 mm long (2.3–3.5 mm). The body appendages are 2.0–2.5 mm long (n=7). The body of female is 3.6 mm (3.2–4.5 mm), the body appendages are 2.1–2.4 mm long (n=6). Two tiny angular horns are pointed, elongated,



Figs 9–10. Adults of *Braueriella phillyreae* (Löw, 1877). 9– female, 10 – male.

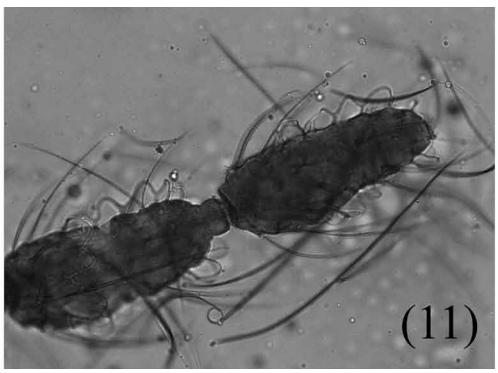
simple, and separated from each other. The mean distance between two horns is 234.72 μm (Fig. 6), and two apical cephalic setae are short and thin, pointed, and simple with a mean of 235.64 μm in length (200.0–257.5 μm; Fig. 7). The average distance between them is 594.3 μm. On the second to the seventh abdominal segment is a series of brown transverse dorsal spinules, the anterior spinules of each series are small and simple, those of the posterior rows are long and bearing smaller spinules (Fig. 8).

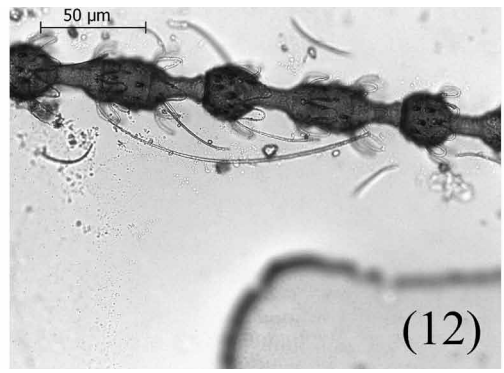
Adult. Females are generally larger than males; the body size is 2.5–3.8 mm, in females is on average 3.3 mm (2.7–3.8 mm; Fig. 9), in males 2.7 mm (2.5–2.9 mm; Fig. 10). The average length of the antenna in females is 1.5 mm (1.3–1.6 mm), in males 2.3 mm (2.2–2.3 mm). Adults were sexed based on antennal and genital morphology and the presence of eggs in the female.

The head has compound black eyes, broadly confluent vertex. Antennae are pale brown, the first two segments are unconnected, the scape is obconic, pedicel globose in both sexes. Antennae are composed of 2+12 antennomeres. The flagellum of female consists of twelve oval or cylindrical flagellomeres, barely narrowed in the middle, with two circumfila looped shorter than that of the male. They are connected by a looped longitudinal circumfila. The neck is transverse (Fig. 11), the average length of the flagellomere is 103.70 μm and the average length of the neck 23.48 μm. The first flagellomere of the flagellum is the longest and about 1.25× longer than the other flagellomeres. Antennae of male are composed of binodal flagellomeres (Fig. 12).

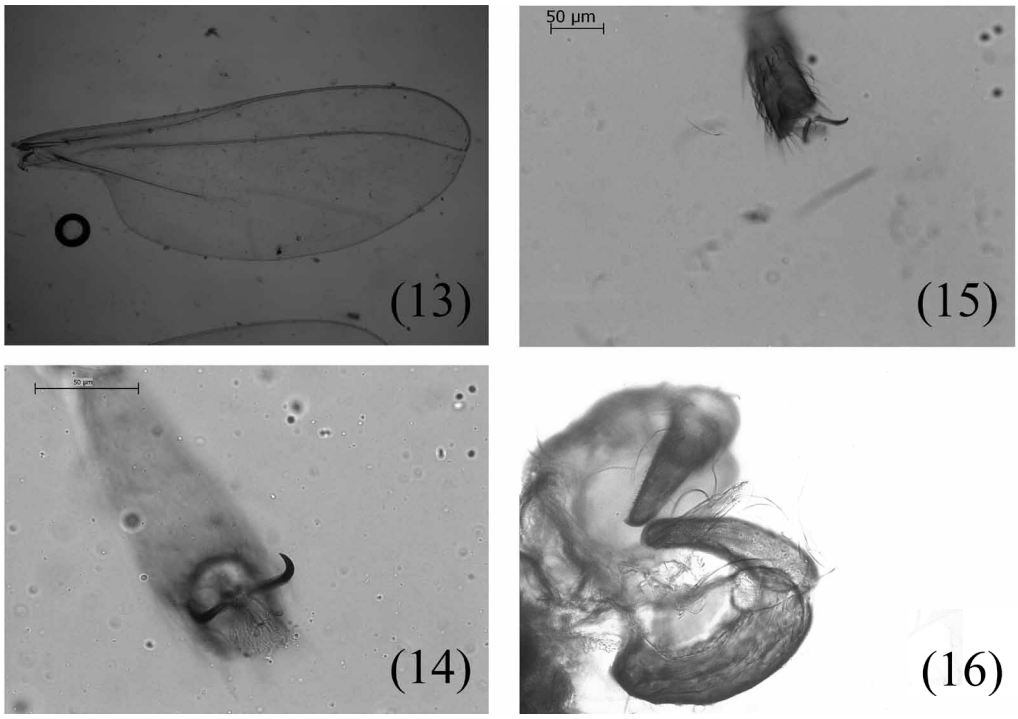
The first flagellomere is the longest of the other flagellomeres. The mean length of the node is 47.09 μm, and the mean length of the binode is 75.64 μm. The flagellum starts with a node and ends with a binode. The simple node bears one looped circumfilum, and the binode bears two looped circumfila. The three circumfila are convergent; the neck of the binode slightly shorter than the neck of the node; the average length of the neck for both node and binode is 28.36 μm. The last flagellomere bears an apical process in both sexes. The flagellomeres are covered with microtrichia and bear several large sensorial pores and long, strong setae in both sexes.

The wing (Fig. 13) is relatively large. Mean length of wing is 2.95 mm (2.6–3.2 mm) in male and 3.3 mm (3.1–3.5 mm) in female. The vein R5 joining C slightly beyond wing apex, the Rs is missing. The wing fold is clear, the vein Cu is forked at half of the wing length. The vein Cu2 is stronger than Cu1. Wing folds are weak.





Figs 11–12. Antennae of *Braueriella phillyreae* (Löw, 1877). 11– terminal part of female antenna, 12 – male flagellomeres.



Figs 13–16. *Braueriella phillyreae* (Löw, 1877). 13 – wing; 14 – tarsal claws, 15– claws with empodium in lateral view. 16 – male terminalia in frontal view.

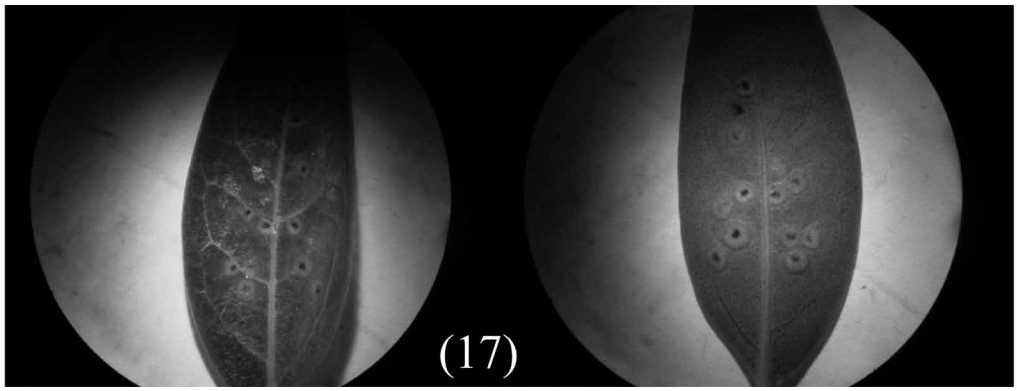


Fig. 17. Galls of *Braueriella phillyreae* (Löw, 1877) on leaves of *Phillyrea media* L., in the form of spots, in the upper surfaces and lower surfaces of the leaf.

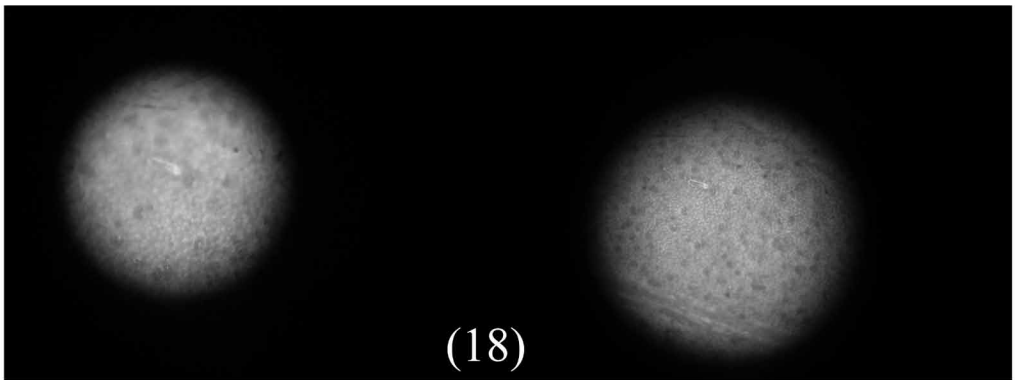


Fig. 18. The egg of *Braueriella phillyreae* (Löw, 1877) on the leaf of *Phillyrea media* L.

Legs are brownish, tarsal claws are sclerotized, curved at the basal third, without teeth (Fig. 14). Empodia are long and reach beyond the bend in claws. The average distance between claws is 46.2 μm (Fig. 15).

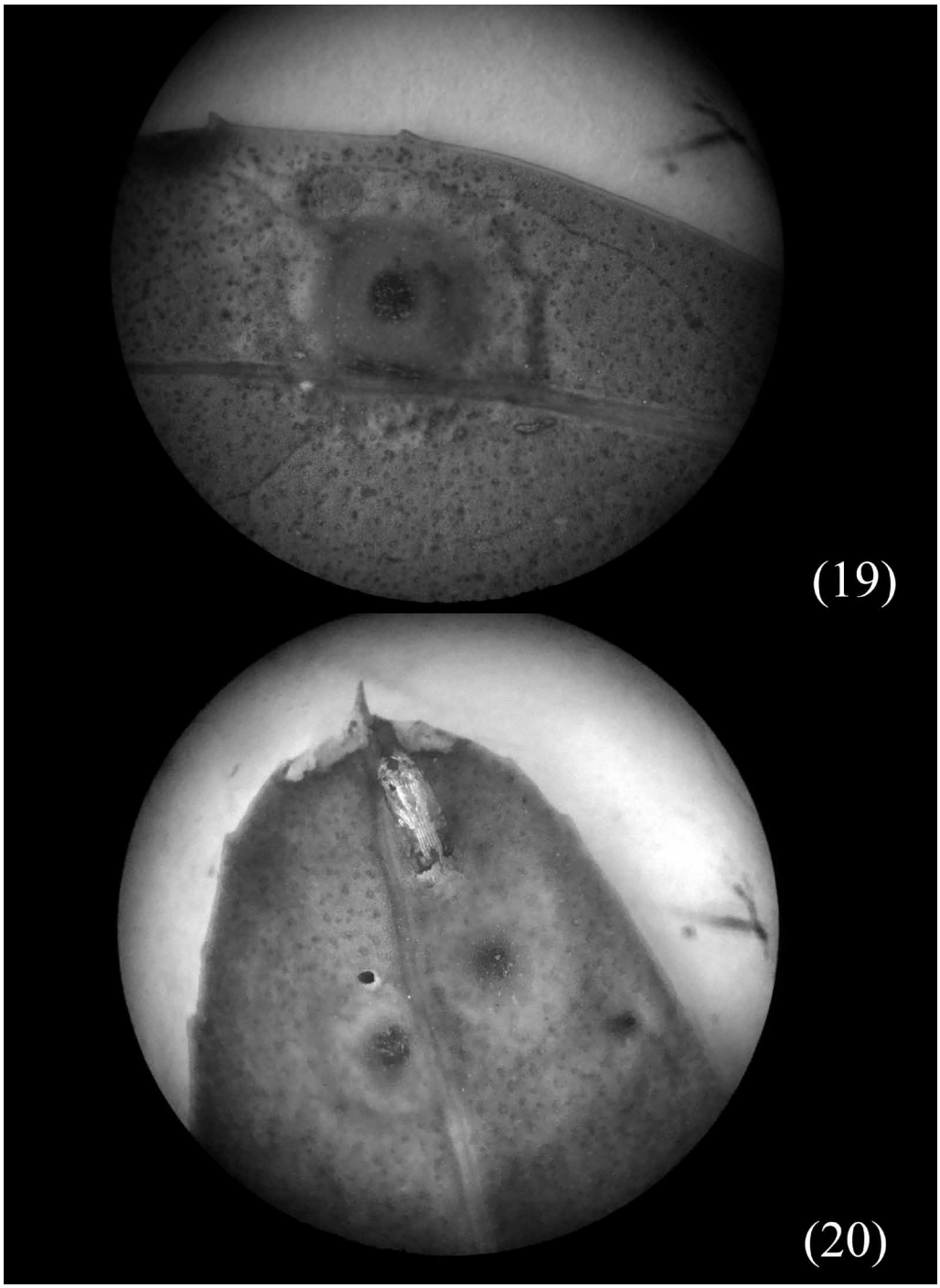
Terminalia: ovipositor of female is not protractible, cerci are ovoid, separate, and setose bilobed hypoproct with microsetae. Male terminalia: gonocoxite is splayed with the average length 166.42 μm; the average length of the gonostyle is 143.09 μm, sclerotized, enlarged in proximal portion tapering to apex, apex with a black nail; the average of the aedeagus length is 119.90 μm (Fig. 16).

Gall. The development of the gall starts as a halo that is pale green to yellow and light brown in the middle. There is a dark brown spot in the upper surfaces of the leaf, black in the lower surface of the leaf (Fig. 17). It increases in diameter and size by increasing the larval stages. At the beginning, after the appearance of the spot, and about two weeks after the exit of the adult, the diameter of the gall is 0.86 mm. The height is 0.3 mm, and at the beginning of April, at any time of the pupal stage, the diameter ranged from 3 to 6 mm, with an average diameter of 4.6 mm and the height 1.1–1.6 mm (mean 1.29 mm). The gall has lenticular shape, rising slightly above the leaf surface at the top and more at the bottom. The galls may be found on young or mature leaves. Only one larva develops in the gall. Two or more galls are frequently fused when there are more than three galls on the same leaf. The number of galls per leaf was variable, it was possible to find from one gall up to thirty-six galls per one leave.

Life cycle

Only one generation of *Braueriella phillyreae* develops per year. There are no field observations of eggs being deposited into or onto the leaves of *Phillyrea media.* Still, we observed one female deposit an egg onto the leaf from galls brought to the laboratory (Fig. 18).

Hatched larvae are small, as long as the eggs. There is no observation of the duration of eggs, but the symptoms were observed after two weeks from the emergence of adults. The symptoms were observed in the middle of May. The black spot in the middle of the gall, on the lower surface of the leaf, was noticed, and the presence of the first instar larva was under this spot. The larva remains there approximately twelve months from the end of April or the beginning of May to mid-April. Larvae of the first instar spend summer, autumn and winter in the gall. Larvae of the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Figs. 19, 20. *Braueriella phillyreae* (Löw, 1877). 19 – the mature gall of with closed opening (adult emergence hole) made by larva of the third instar before entering in the pupal stage. 20 – exuvia of the pupa protruding from the exit hole on the leaf of *Phillyrea media* L. after emergence of the adults.
←

second instar were observed at the end of December, while the larvae of the third instar at the end of February, until the end of March. Larvae of the third instar are highly mobile. Larvae of the third instar prepare an opening closed only with a delicate cuticle of the gall before entering the stage of the pupa (Fig. 19). The diameter of the opening is 0.9–1.2 mm. Larvae pupate in the galls. The head of pupa is situated near the opening to facilitate the exit from the gall.

The pupa moves from side to side frequently. The pupae break through the side thin epidermis by their two horns and push themselves as far out of the gall as the development of the midge requires. After the emergence of adults, the brown pupal skin remains stuck in the opening of the gall. Empty galls remain attached to the leaf. The first pupa was found on 20 March 2017, and the first emergence of adults was observed on 20 April 2017. Adults emerged in April, leaving the pupal exuviae at the gall (Fig. 20). The stage of pupa is estimated at one month.

DISCUSSION

There are only few works dealing with the biology of *Braueriella phillyreae*. Löw was the first who described this species under the name *Diplosis phillyreae*, on the basis of material that was emerged from the galls discovered by J. E. Bergenstamm at the castle of Miramare at Trieste (Skuhravá et al. 2007). Kieffer (1896) designated the genus *Braueriella* with the type species *Diplosis phillyreae.* Later Kieffer (1913) determined the diagnosis and descibed the male and female of *Braueriella phillyreae* in detail.

The present study is the first of the biology of *B. phillyreae* in the environs of Bayda, Al-Jabal Al-Akhdar Region. This work includes the description of galls, eggs, three developmental stages of larvae, description of pupa and adults. Larval instars are distinguished by their size, color and some morphological characters. The width of the head capsule at the first instar is almost as wide as the body segments. In contrast, the width of the head capsule in the second of larva is distinctly smaller in proportion to the body segments compared to the first instar. The ventral side of the prothorax of the third instar of *B. phillyrea* has a sternal spatula formed of two prongs which is a characteristic feature of the third instar of cecidomyiid larvae. Mamaev & Krivosheina (1992) suggested that phytophagous species utilize the spatula to puncture or extend apertures in plant tissue. Among the cecidomyiid species variety, this happens fundamentally within the shape of the summit, which can have one, two, or three prongs. Möhn (1961) stated that full□grown larvae, that pupate in the soil, mainly exhibit orange or reddish body color, while those that pupate in the galls are whitish or yellowish. Full□grown larvae of *B. phillyreae* that pupate in the galls, are usually pale yellow. The antennal horns in the pupa and the spatula of the third instar larva are alternations often found in the gall midges of several taxa that live in forests. Species that pupate in the galls usually possess pupal antennal horns (Gagné 1994). Adult midges range in size from 2.5 to 3.8 mm depending on the nutrient availability to the developing larvae (Rowley et al. 2016). Females are generally larger than males (Sirjani & Lewis 2020). The male antenna of *B. phillyreae* is composed of 2+24 flagellomeres; alternatively, a node and a binode bear three circumfilum with loops. Harris (1966) indicated the characteristics of the supertribe of the Cecidomyiidi, including the absence of cross-vein (Rs), that is usually associated with binodose male antennal segments, with two or three sets of long, looped circumfila. The vein R5 joined C shortly after the wing apex; antennal segments of female elongate, cylindrical, with relatively

short necks, circumfila simple but short loops like in some genera in supertribe Cecidomyiidi (Harris 1966). The antenna of the male is longer than the antenna of the female. This adaptive significance of the longer male antennae might be the more surface area of sense organs that may help detect sex pheromones (Dweck 2009, Schneider 1964). Larvae of *B. phillyreae* cause pustule galls on leaves of *Phillyrea media*. The insect infests only the leaves of various species of the genus *Phillyrea* (Skuhravá & Skuhravý 2002, Skuhravá et al. 2006). The majority of gall inducers are specific to their host plant, galling organ and their gall type in general. Their adaptive mechanisms and selection pressures from the environment and natural enemies may be linked to this phenomenon (Stone & Schönrogge 2003). The galls are green at first and become pale green and yellow. Mendonça & Romanowski (2002) noted that gall tissue lacks all chlorophyll, giving the gall a pale green color.

The life cycle of *B. phillyreae* was little studied. Two studies were done long ago by Löw (1877) and Kieffer (1913). It was difficult to follow the deposit of eggs in the field, but according to one note in the laboratory, the female deposits an egg on the surface of the leaf. The ovipositor of *B. phillyreae* is not protrusible, so this species probably lay their eggs on the leaves. In a study by Yukawa (1974, cited in Tokuda 2012) for some species that do not possess needle□like ovipositors, females deposit eggs on the host tissues. The current study did not report the eggs stage, but in Cecidomyiinae, the egg stage lasts 2–3 days (Kolesik 2015). The symptoms were observed after two weeks from the emergence of adults. The first instar is generally responsible for the initiation of gall induction (Rohfritsch 1992). The presence of the black spot in the middle of the gall and the first age larva directly above this spot makes us believe that the larva penetrates from this place.

The univoltine gall-midge species can be classified into four types, type IA, type IIA, type IB, and type IIB, based on the overwintering sites and larval developmental stadia. According to Yukawa (1987, cf. Tokuda 2012), the larvae of type IIB do not mature until the following spring. The duration of the first larval stadium is the longest among the four types. *Braueriella phillyreae* adopt the type IIB strategy. The duration of the pupal stage from the first pupal to the first emergence adult was a month; Yukawa & Miyamoto (1979), Sunose (1983), and Tokuda & Yukawa (2005) mentioned that the duration of the pupal stage varies with species. It usually ranges from ten days to one month. The observations of the current study on the effect of weather conditions on the emergence of gall midges; are in accordance with Yukawa & Rohfritsch (2005), who indicated that various climate conditions such as temperature, rain, and cloudiness had been reported to affect the daily activities of gall midges. At the end of the larval third instar, an opening is prepared, closed only with a delicate cuticle before entering the stage of the pupa. The emergence from the gall is by this opening from the upper surface of the leaf galls. At the time of emergence of adult midges, the pupa wriggles around, rising to the epidermal covering, pierces the dry epidermal lid by the antennal horns, sticks out on the surface of the gall. Then the pupal skin ruptures open for the emergence of the adult midge (Mani 1964). Gagné (1994) mentioned the species that pupate in the galls usually possess well-developed pupal antennal horns.

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