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DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGI ANIMAL

DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY

LABORATOIRE DE ZOOLOGIE

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Study of two psyllid species (Psylloidea: Liviidae) associated with ants (Hymenoptera : Formicidae) on two Savannah plant species at Koutaba (West Region Cameroon).

Master's thesis presented in order to obtain a Master's Diploma in Biology of Animal Organisms

Option: ZOOLOGY

By

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DEDICATION

To the LATAR's Family,

Your love, patience, good counsels, efforts, solidarity and availability is my strength

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ABSTRACT

Jumping plant-louse are a common and diverse group of small sap-sucking insects, with a world-wide distribution. They are found on a large range of plant species/varieties (Ouvrard *et al*., 2015). Their main effect on plant is sap deprivation. As they feed, they excrete large amounts of honeydew which is actively collected by ants. In turn ants offer to them a sort of protection. So close relationships between ants and hemipterans are well known in aphids, whiteflies, or scale insects, but so far poorly documented in psyllids. In the present study, two psyllid species, belonging to the family Liviidae, were observed in the presence of several species of ants, feeding on two plant species, *Psorospermum glaberrimum* and *Ozoroa paniculosa* respectively. The medicinal features of the two plant species are well documented; among others are the deworming and anti-plasmodial effects.

The present study aimed at contributing to the psyllid knowledge in Cameroon by providing morphological descriptions which would permit their precise identification, describing their infestation pattern on host-plants, listing their different attendant ant species and finally assessing variations of these psyllids' populations on the plant structures at different phenological stages. Investigations and samplings took place in the Savannah at Koutaba (West Cameroon) (05^0 39'72.7"N; 010^0 47'18.1" E; 1227 m) from February to June 2019.

As results, morphological studies led to the diagnosis of two new species of psyllid, *Paurocephala* sp.n. on *P. glaberrimum*, and *Diaphorina* sp.n. on *Ozoroa paniculosa* . The first species, *Paurocephala* sp.n. is similar to *Paurocephala insolita* Mifsud and Burkhardt 2002 by the whole habitus, especially forewing pattern, but is clearly distinguishable in adult by colouration of thoracic and abdominal segments as well as in immature by the number and distribution of sectasetae on antenna and wing pads and the lack of caudal process. The second species, *Diaphorina* sp.n., is morphologically close to *Diaphorina albomaculata* Capener 1970 but differs to it by the genal processes slightly longer, the more elongate forewing with more expanded white patches, the broader parameres, the more produced male proctiger and the form of female subgenital plate. In the immature of *Diaphorina* sp.n., there are long setae at the rim of the caudal plate while they are lanceolate in *D. alomaculata*.

For the infestation pattern, an unusual behaviour of *Paurocephala* sp.n. was seen as it turned to colonise older leaves of *P. glaberrimum* while *Diaphorina* sp.n., as usual in psyllids,

colonised young leaves. For their attendance by ants, *Camponotus congolensis*, *Camponotus flavomarginatus* and *Tapinoma debouti* were noted as associated ant species of *Paurocephala* sp.n. while *Camponotus congolensis*, *Camponotus flavomarginatus* and *Crematogaster* sp. were the associated ant species for *Diaphorina* sp.n. Ants were always seen building shelters to hide populations of *Paurocephala* sp.n. on the lower surface of leaves of *P. glaberrimum* while these construction were facultative in *Diaphorina* sp.n on leaves of *O. paniculosa*.

RESUME

Les psylles constituent un groupe commun et diversifié de petits insectes suceurs de sève avec une distribution mondiale. Ils sont présents sur une large gamme d'espèces / variétés de plantes (Ouvrard *et al.*, 2015). Leur principal effet sur la plante est l'extraction de la sève. En se nourrissant, ils excrètent de grandes quantités de miellat, substance activement collectée par les fourmis. À leur tour, les fourmis leur procurent de la protection. Ce type d'association étroite entre les fourmis et les hémiptères est bien connu chez les pucerons, les aleurodes ou les cochenilles, mais jusqu'à présent peu documentée chez les psylles. Dans la présente étude, deux espèces de psylle appartenant à la famille des Liviidae ont été observées, en présence de plusieurs espèces de fourmi, sur deux espèces de plantes, *Psorospermum glaberrimum* et *Ozoroa paniculosa* respectivement. Les propriétés médicinales des deux espèces végétales sont bien documentés; entre autres, les effets vermifuges et anti-plasmodiaux.

La présente étude visait à contribuer à la connaissance des psylles au Cameroun en fournissant des descriptions morphologiques qui permettraient leur identification précise, en décrivant leur modèle d'infestation sur les plantes hôtes, en répertoriant les différentes espèces de fourmis qui leurs sont associées et enfin en présentant les variations des populations de ces psylles sur les différentes structures végétales à différents stades phénologiques. Les investigations et les prélèvements ont eu lieu dans la savane de Koutaba (Ouest Cameroun) de février à juin 2019.

Comme résultats, l'étude morphologique a conduit à la description de deux nouvelles espèces de psylle, *Paurocephala* sp.n. sur *P. glaberrimum* et *Diaphorina* sp.n. sur *O. paniculosa*. La première espèce, *Paurocephala* sp.n. est semblable à *Paurocephala insolita* Mifsud et Burkhardt

2002 par son aspect global, en particulier la forme et les motifs de l'aile antérieure, mais s'en distingue clairement au stade adulte par la coloration des segments thoraciques et abdominaux et au dernier stade immature par le nombre et la distribution des soies appelées sectasetae sur les antennes et les bourgeons alaires et par absence d'un processus caudal. La deuxième espèce, *Diaphorina* sp.n., est morphologiquement proche de *Diaphorina albomaculata* Capener 1970 par l'aspect global et l'enfumation de l'aile antérieure, mais s'en distingue par les processus génaux légèrement plus longs, l'aile antérieure plus allongée avec des plages blanches plus étendues, les paramères plus larges, le proctigère mâle plus bombé postérieurement et la forme de plaque

sousgénitale femelle. Au stade immature, *Diaphorina* sp.n. arbore de longues soies au bord postérieur de la plaque caudale alors que ces soies sont lancéolées chez *D. alomaculata*.

Pour ce qui est du modèle d'infestation, un comportement inhabituel a été observée chez *Paurocephala* sp.n.. Les individus de cette espèce, à différents stades de développement colonisaient les feuilles les plus anciennes de *P. glaberrimum*. Cependant, les individus de *Diaphorina* sp.n., comme d'habitude chez les psylles, à différents stades de développement, se trouvaient préférentiellement sur les jeunes feuilles de *O. paniculosa*. Quant à leurs soins par les fourmis, *Camponotus congolensis*, *Camponotus flavomarginatus* et *Tapinoma debouti* ont été vues associées à *Paurocephala* sp.n. tandis que *Camponotus congolensis*, *Camponotus flavomarginatus* et *Crematogaster* sp. étaient les espèces de fourmis associées à *Diaphorina* sp.n. Dans ces associations, les fourmis ont toujours été vues construisant des abris pour cacher les populations de *Paurocephala* sp.n. sur la face inférieure des feuilles de *P. glaberrimum* alors que ces constructions étaient facultatives chez *Diaphorina* sp.n. sur *O. paniculosa*.

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INTRODUCTION

Psyllids (Hemiptera: Sternorrhyncha: Psylloidea), or jumping plant-lice, are a common and diverse group of small sap-sucking insects with a world-wide distribution, found on a large range of plant species (Ouvrard *et al*., 2015). Often, related psyllid species develop on related host-plant taxa, particularly in their immature stages (White, 1970; Hodkinson, 1974; Burckhardt, 1989; Burckhardt *et al*., 2014; Ouvrard, 2014). Like other sap-feeders, psyllids penetrate plant tissues, tapping into transport vessels and siphoning off part of the nutrient-laden liquids. Generally, most psyllids are less harmful to their host plant when present in moderate number. Contrarily, they can really be injurious. For instance, the adverse effects of psyllids on their host can be direct and indirect. Psyllids have lately been shifted to general awareness as they are vectors of serious plant diseases, economically important pests in agriculture and forestry, and potential control organisms of exotic invasive plants. This is the case seen with *Diaphorina citri* Kuwayama, the most serious citrus pest in Asia and America, which transmits the causal agent of huanglongbing (HLB, greening disease), (Bonani *et al*., 2009; Tiwari *et al*., 2011), some Phytoplasma transmitted by the *Cacopsylla* species in Europe and North America which are economically important in apple, pear and stone fruit orchards (Ferre and Denis, 2011), *D. xuani* which causes great damage on seedlings of *Ricinodendron heudelotii* in Cameroon (Aléné *et al*., 2006, 2008) and *D. enderleini* causing damage on *Vernonia amygdalina* (Alene *et al*. 2011). Psyllids mostly attack young plants and shoots of old plants (Hodkinson, 1974; Ossiannilsson, 1992; Souliotis and Broumas, 1998; Alene *et al*., 2008). As they feed, psyllids excrete honeydew (Ossiannilsson, 1992). Honeydew is a sweet, sticky substance whose high concentration is harmful to the psyllids themselves as it reduces their movements and respiration rate. It has also been reported as furthering plant damage (Pedata *et al*., 2012) probably because it is great substrate for the development of sooty mould on the surface of leaves, disturbing the photosynthetic process. Fortunately, this carbohydrate-rich liquid stands as a valuable food resource for various insects such as ants, bees, wasps, flies etc. (Way, 1963; Gullan & Cranston, 2014). Ants actively collect honeydew and live in mutualistic relationships with sap suckers (Way, 1963; Buckley, 1987; Delabie, 2001; Davidson *et al*., 2003). In this intimate relationship, ants receive rich-energy food while providing hemipterans protection from environmental constraints, predators and parasitoids (Heil and McKey, 2003; Novak, 1994; Hodkinson, 1984; Gullan and Martin, 2003; Raman, 2003). This protection can be categorized as "simple" when the ant species surrounds the psyllids or "complex" when the ant species build soil shelters or sand shields round the psyllid colonies as it is the case with *Crematogaster acvipantis* round *Diaphorina enderleini* on *Vernonia amergdalina* (Aléné *et al*., 2011).

Psyllid Population dynamics and their effect on plants are influenced by some environmental factors such as shade (Alene, 2006), temperature, rainfall, etc. (Tamesse, 2014). Plants under shaded areas are less attacked by psyllids since they do not get enough sunlight to manufacture nutrients. Furthermore, high humidity and rainfall decreases the psyllid population size on host plant (Tamesse, 2014). Jumping plant lice usually display mechanisms that protect themselves from loss of humidity or heavy precipitations (Raman 2003, Gullan and Martin, 2003). For example, psyllids, while unable to induce galls at different immature stages produce wax as a strategy to avoid water loss or excessive moisture and for protection against natural enemies (Hodkinson, 1984). However, some species lack functional structures enabling them to produce wax. In this case they need some extrinsic protection which could be provided by ants. This psyllid/ant interaction is weakly documented in our region and even in the world apart from the studies of the hawthorn psyllids and their attendant ants in Germany (Novak, 1994), of *Diaphorina enderleini* and its associated ants on *Vernonia amygdalina* in Cameroon (Aléné *et al*., 2011) and that of *Diaphorina citri* in USA (Navarette *et al*. 2013). With this in mind, two psyllid species feeding on two shrubs in the western highland savanna of Cameroon caught our attention. The first one belonging to the genus *Paurocephala* (Psylloidea: Liviidae) was encountered feeding on *Psorospermum glaberrimum* Hochr (Hypericaceae); the second one belonging to the genus *Diaphorina* (Psylloidea: Liviidae) was observed feeding on *Ozoroa paniculosa* (Sond) R.Fern. & A.Fern. (Anacardiaceae). Their interest lies on two facts: (i) they are closely associated with several ant species in unusual way (ii) they are likely undescribed while their host plants are known to be used as medicinal plants (Lenta *et al*., 2008; Ahmed *et al*., 2014; Gallé, 2015; Nkosinathi, 2017). With the aim of improving knowledge on biodiversity of Cameroon psyllids and supplying more information about psyllid-ant relationships, the present study is going to (i) provide morphological characters for the identification of the studied psyllids, (ii) provide a list of ants involved in the up-cited association and (iii) characterize their relationships with psyllids.

The present dissertation comprises an introduction, three chapters and a conclusion sorted with perspectives. The first chapter is dedicated to the literature review, the second one to the presentation of the study site and methodologies used to achieve objectives assigned to the present work, the third presents the main results and discusses them on the basis of relevant previous works.

CHAPTER I:
LITERATURE REVIEW

I.1. Systematic position of psyllids

The systematic position of psyllids provided here is adapted from several authors, including (Burckhardt and Ouvrard, 2012; Gillott, 2005; Gullan and Cranston, 2014)

- **Kingdom: Animalia**
  - Multicellular living organisms,
  - Heterotroph organisms provided with nervous and muscular systems.
- **Phyllum: Arthropoda**
  - Articulated appendages,
  - Body covered by a hard chitinous exoskeleton,
- **Sub-Phyllum: Hexapoda**
- Body made of three clearly differentiated tagma,
  - Present 3 pairs of articulated legs carried by the thorax
- **Class: Insecta**
  - Body divided into 3; head, thorax and abdomen
  - Thoracic tagma is sub divided into the pro, meso and metathorax
- **Sub Class: Pterygota**
  - Two pair of wings on the meso and meta thorax
  - wings can be secondarily absent
- **Infra class: Neoptera**
  - Wing with one jugal frame or neala
  - Wing folded backward roof-like or plane
- **Super Order: Hemipteroidea or Paraneoptera**
  - Jugal fold with only one nerve ramified at the summit
  - Heterometabolous and exopterygota development
- **Order: Hemiptera**
  - Piercing and sucking mouth parts with long articulated rostrum
  - Heterometaboles, pauromataboles
- **Sub-Order: Sternorrhyncha**
  - Roof-like wings at rest
  - Rostrum present between anterior coxa
- **Super family: Psylloidea**
  - Antenna with 10 articles
  - Broad and rigid metacoxa fixed on the metathorax

- **Family: Liviidae**

- Adult metatibia with an open crown of sclerotised apical spurs (sometimes grouped).
- Larva generally with multiple lanceolate setae or sectasetae. Tarsal arolium bearing unguitractor.

I.2. Taxonomy

For decades, the taxonomy of psyllids has faced a lot of confusion. Crawford (1914) for example erected the mainly tropical genus, *Paurocephala* for *P. psylloptera*, a species that was collected on *Ficus ulmifolia* (Moraceae). He noted a resemblance with the species of the

Pauropsylla Rűbsaamen, which was associated with *Ficus* spp. and thereby implying a close phylogenetic relationship. This produced a lot of substituent confusion until the 1970s. Also,,Mathur (1975) described species of *Paurocephala* from the Indian subcontinent, stating that they are morphologically close to *Pauropsylla*. These two genera were then treated as closely related and placed in the same tribe, Pauropsyllini and further re-examined by Hollis (1984), then placed in the genus *Paurocephala* near *Haplaphalara Uichanco* and *Moraniella Loginova* (Mifsud and Burckhardt, 2002). Also, the taxonomy of *Diaphorina* Lðw, (1880) has faced difficulties over time. This genus replaced *Diaphora* Lðw, 1879 and with time registered 4 synonyms; *Gonanoplicus* Enderlein, 1910; *Pennavena* Capener, 1968; *Eudiaphorina* Loginova, 1975; and *Brachypsylla* Frogatt, 1901. This genus presents till date 77 taxa and 76 species with the first described species *Diaphorina putonii* Lðw, 1879 as type species (Psyl'list database, 02 September 2019). Much difficulty has been occasioned in the past by the poor preservation and loss of type material until Capener (1970) devised a strategy on mounting psyllids and pinning small insects for conservation. For over three decades now, an impressive amount of taxonomic publications has sprouted, doubling the number of described species (Li, 2011). This is seen with Capener (1970); Hollis (1976); Brown and Hodkinson (1988); Hollis and Broomfield (1989), Burckhardt and Basset (2000) just to name a few. The current knowledge of the Psylloidea taxonomy, biology and distribution is far from complete: while for example the Palearctic fauna is comparatively well-known, there are big gaps in knowledge of tropical regions where the global psyllid diversity is largely concentrated, but so far under-studied with many new taxa still awaiting description (e.g. Burckhardt *et al*., 2006; Burckhardt & Queiroz, 2012; Tamesse, 2014). The new and adopted classification of psyllids here consists of eight families: Aphalaridae, Carsidaridae, Calophyidae, Homotomidae, Liviidae, Phacopteronidae, Psyllidae and Triozidae (Burckhardt & Ouvrard, 2012).

I.3. General morphology of psyllids

The general morphology of psyllids presented here is inspired from Ossiannilson (1992), Mifsud and Burckhardt (2002), and Yang *et al*. (2009). Generally, the eggs of psyllids are elliptical, elongated with bold extremes or oval with varying colors depending on the biotic and abiotic factors of the milieu. A basal string-like structure called pedicel that holds the egg to the leaf and whose length and position varies from one taxon to another and is an important characteristic in taxonomy.

Like in other insects, psyllid bodies are divided into three tagma; head, thorax and abdomen. The head is made up of a vertex, divided into two or not by the median suture. Three ocelli are generally present, the paired are situated on the caudo-lateral corners of the vertex and the unpaired on the frons (sclerite varying greatly in size and shape); the genal processes froming the major part of the head. A comparatively large clypeus is present and a short rostrum. Antenna has three to ten segments. The scape, basal segment, and pedicel are usually stouter than the rest of the antenna, the flagellum. The flagellum is further segmented into flagellomeres. The antenna also bears different types of sensory structures varying in number, size and shape from one group to another. The terminal flagellomere usually presents two groups of long setae next to the olfactory organ called rhinaria (Ossiannilsson, 1992). Sensilla are always present on the dorsal surface of the pedicel.

The thorax is generally large and strongly convex dorsally. The size, shape and position of the propleurites are important in taxonomy. Two to three pairs of thoracic spiracles are present (Ossiannilsson, 1992). On the thorax are the wings and legs. The wings are well developed with forewing venation important in taxonomy. Pterostigma absent in Triozidae, is present in most psyllids with usually an incision near its proximal end, the coastal break. In all psyllids, an anal break is observed at the distal end of the claval suture. The anal and coastal breaks communicate through the nodal line. The claval fold is present and important in wing coupling. The surface of the fore wing is more or less densely covered by spinules. The legs on the other hand are segmented, carrying bi-segmented tarsi which end with a pair of claws and two pulvilli. The hind leg is highly modified for jumping. The metatibiae and metatarsi are more or less generally provided with strong setae, spines, spurs or turbercles. On the thorax still, the sensillae are arranged in transverse rows on the outside of the trochanters and in longitudinal rows on the ventral part of the femora.

On the abdomen, only the specialized terminal segments provide characters important in classification. In males, the ninth abdominal sternum is usually more or less helmet-shaped, the convexity directed ventro-caudally and is termed the hypandrium or sub-genital plate. Dorsally, the anal valve or proctiger arises from the frontal margin of the sub-genital plate. The anus is situated at the apex of the proctiger. The two-segmented penis or aedeagus is articulated basally with the proximal end of the proctiger. From the caudal part of the sub-genital plate arise the parameres, very important in separating species. More frontally in the abdomen is the sperm pump. The tergum of the female terminalia consists of the proctiger or dorsal plate on which the anal opening is situated. The anus is surrounded by a ring of wax gland pores, the circumanal pore ring. Ventrally, the sub-genital plate encloses the basal part of the ovipositor which is made up of three pairs of vulvulae; the dorsal, ventral and inner pairs. The ventral and inner pairs are almost or usually entirely concealed by the dorsal pair. The ventral vulvulae are apically serrated, offering useful taxonomic characters.

I.3.1. Morphological and behavioral adaptation of psyllids

I.3.1.1. Eggs

The eggs of psyllids carry a peduncle which can be median, lateral, elliptical or axial. It serves as an attachment structure to the plant allowing the exchange of water, which favours embryonic development (Ossainnilsson, 1992).

I.3.1.2. Nymphs and adults

The ancestral Pterygota insects present chewing type mouthparts made up of a labrum, an anterior extension of the clypeus, a pair of sclerified mandibles, pair of maxillaries each bearing a lateral palp, labium bearing a lateral pair of labial palp and hypopharynx on the top of the labium. Extreme modifications are observed as we move to modern insects like Lepidoptera, Diptera, Hymenoptera and Hemiptera, with the latter presenting segmented flexible labium with a deep groove on its anterior surface (Gillott, 2005). Within this groove protrude two piercing organs; the mandibular and maxillary bristles with chemoreceptors which initiates feeding after prospection (Corio-Costet & Mondy, 2013). The two maxillary bristles are interlocked within the labial groove and form the food and salivary canals. The labium with labial glands which releases enzymes such as amylases for starch hydrolysis, invertase for breakdown of carbohydrates to glucose and fructose, pectinosterases and galacturonidases for the progression of the bristles (Gillott, 2005; Corio-Costet and Mondy, 2013; Gullan and Cranston, 2014). On the thorax, the rapid wing

vibrations are generally associated with sound production which plays a role in courtship (Ossiannilsson, 1992).

On the abdomen is the circumanal ring pore which surrounds the anus. It can be oval or cruciform with one or two rows of pores. The circumanal pore-rings of adults and nymphs produce wax more or less copiously. Additional wax glands may also be present on the integument of nymphs especially on the abdomen as is the case seen in *Euphalerus* spp. feeding on *Lonchocarpus* Spp. (Hollis and Martin, 1997) or in *Diclidophlebia* spp. (Burchhardt *et al*., 2006). This protects the insects from desiccation, from the moisture of their honeydew and from predators which include several species of Miridae, Anthocoridae, Nabidae, Syrphidae, Coccinellidae and Aranidae.

I.4. Biology

All psyllids species undergo sexual reproduction with cross fertilisation except *Cacospylla myrtilli* Wager, 1947 which undergoes facultative parthenogenesis (Ossiannilsson, 1992). In many psyllid species, mating is a simple act which can be resumed in 5 steps; (i) the aggressive male attracted by a passive female approaches her by the side, (ii) with his antenna pointing towards the female, the male turns the female's abdomen to meet he's, (iii) catches the female's valves with his paramere and (iv) inserts the aedeagus. (v) the female start dissociating and the couples eventually separate (Hodkinson, 1974; Aléné, 2006). In most species, this act lasts for about 4 to120 minutes and can be repeated after 10 minutes (Aléné, 2006; Soufo, 2016). Mating occurs many times per day to enable the female lay as many eggs as possible; an example is seen with *D. xuani* which mates two to four times per day (Aléné, 2006).

The life cycle of psyllids comprises of the egg, five immature stages and an adult stage (Hodkinson, 1974; Aléné, 2006; Hodkinson, 2009). Reproductive maturity of both sexes varies, approximately one to three days in males and two to three days in females (Aléné, 2006; Wenninger and Hall, 2007); but in some species the adults are reproductively active few hours after emergence. The age and life-span of a female determines the survival of the species. Oviposition generally begins one day after mating. In any event, young tender shoots or flush are preferred by females as oviposition sites for they better assure high quality food availability, greater light intensity and a complete development of premature stages (Hodkinson, 1974; Cobbinah, 1986; Aléné, 2006;). First and second instars are closely similar. The third instar starts changing in form with visible wing pads. For the fourth instar, the abdomen and head are round, with clearly visible wing pads. The last

instar has the same form as the fourth instar but with developed wing pads and clearly visible sclerites.

Afterward, the dorsal side of the nymph opens, liberating the adult; generally light green to pale in color which deepens with age and maturity. The duration of the developmental cycle and number of annual generations varies from one species to another and also with respect to the biotic and abiotic conditions governing each region (Tamesse and Messi, 2004). Examples include *D. xuani* which in 19-38 days develops from egg to imago with 10-12 generations annually, *Mesohomotoma tessmanni* in 4-8days at 32^0c and 20^0c does so (Van Den Berg, 1990; Aléné, 2006).

I.5. Economic importance of Psyllids

Psyllids attack plants of different families, essentially dicotyledonous plants (Hodkinson, 1974; Hodkinson and White, 1979; Dolling, 1991; Burckhardt *et al*., 2006). Some species are also reported on monocotyledons (Burckhardt, 1987; 2005). Damages caused by psyllids are massive, be it directly or indirectly, and varies from one family to another. Feeding may directly injure leaves, flowers or young fruits, resulting to premature leaf shedding or fruit production decrease, withering of the apical buds and consequently die-back of the stems. Indirect damage of psyllids involves fouling of the leaves or fruits by honeydew, development of sooty mold on honeydew and transmission of the causal agents of plant diseases (Burckhardt 1994). Such effects have been observed on *Ricinodendron heudelotii*, due to *Diclidophlebia xuani* (Aléné *et al*., 2008), or on *Lecaena leucocephala* due to *Heteropsylla cubana* (Aléné *et al*., 2012). Plant would show a loss of vigor or terminals may also be distorted, discolored or even die-back as observed on *Ficus* spp. due to *Homotoma eastopi* (Aléné *et al*., 2014). Some species may cause defoliation, observed on *L. leucocephala* due to *H. cubana* (Aléné et al., 2012). Other are responsible of gall formation which is generally caused by psyllids belonging to the family Triozidae of the genus Trioza (Tamesse *et al*., 2014; Percy *et al*., 2015), *Pauropsylla* spp. on *Ficus* spp. (Hollis and Bromfield, 1989; Percy *et al*., 2015), or Phacopteronidae on Sapindales and on several unrelated Apocinaceae (Malenovský et al., 2007; Malenovský *et al*., 2015; Percy *et al*., 2015).

I.6. Hostplant involved in the study

I.6.1. Ozoroa paniculosa (Sond.) R. Fern. & A. Fern

The systematic position presented here is inspired from the Plant list 2013 accessed 16 September 2019.

Kingdom: Plantae

Clade: Angiosperms

Order: Sapindales

Family: Anacardiaceae Fernandes R. & A. (1966)

Genus: *Ozoroa*

Species: *Ozoroa paniculosa* (Sond.) R. Fern. & A. Fern.

The family Anacardiaceae, which is also known as the cashew family, includes approximately 800 species in 82 genera. They are found primarily in the tropics and subtropics but extending into the temperate zone. The Anacardiaceae includes primarily trees and shrubs, (rarely lianas or subshrubs) with resin canals and clear to milky exudate. Members of the family are cultivated throughout the world for their edible fruits and seeds, medicinal compounds, valuable timber and landscape appeal.

The genus *Ozoroa*, comprises about 40 species which are often very difficult to distinguish and most of which occur in Southern Africa. They are much-branched shrubs with alternate leaves, small to medium-sized tree up to 15m tall, with milky sap, bark grey and corky. *Ozoroa paniculosa*, commonly known as the bushveld *Ozoroa* or common resin tree is known from Southern Africa where it occurs in Zimbabwe, South Africa, Mozambique, Botswana and Namibia. It has a moderate growth rate, compact shape, fairly hardy with bunches of kidney shaped black, oily seeds which persist on the tree and found on savannah, rocky outcrops. The decoction of the root is considered as an effective remedy against cough and chest pain (Leffers, 2003); the roots considered as a vermifuge in some areas and sold in markets for this purpose (Kerhano & Adam, 1974). The fruit is traditionally used to dye leather and the wood is used for fuel and charcoal production.

I.6.2. *Psorospermum glaberrimum* Hochr

The systematic position of *Psorospermum glaberrimum*, inspired from African Plants Database 16 September 2019.

Kingdom: Plantae

Clade: Angiosperms

Order: Malpighiales,

Family: Hypericaceae

Genus: *Psorospermum* Spach.

Species: *Psorospermum glaberrimum* Hochr.

The Guttiferae Juss, alternately Clusiaceae Lindl. (nom.altern.) including the Hypericaeae Juss and six other (excluding Bonnetiaceae), are trees, herbs, lianas and shrubs usually with milky or colored sap and bearing essential oils or not (Watson and Dallwitz, 2019). The plant list (2010) recognizes nine genera and around 700 species, with members of the family found worldwide except in exclusively cold or dry areas. Their leaves are simple. The genus *Psorospermum* comprises 55 species mostly shrubs or small trees growing in Tropical regions of South America, Africa and Madagascar. The secondary metabolites isolated from this genus include alkaloids anthraquinones, anthrones and bianthrones, vismiones, flavonoids, long chain alcohols, steroids, tannins, terpenes, and xanthones (Francesco *et al*., 2013; Momoh, 2015; Tanemossu *et al*., 2015). The species *Psorosperminm glaberrimum* Hochr is a small to medium-sized shrub distributed in tropical regions. It's ranged from Tropical Africa-Guinea to Ethopia, South to Angola, Zimbabwe and Mozambique, inhabiting wooded savannah and rocky grounds at elevations of 15 to 1950 m (African Flowering plant Database, 16 September 2019). The Bark is used in the treatment of various skin problems like leprosy, parasitic disease craw-craw, scabby, eczema and insect bites (Uphof, 1959; Burkil., 2004; Ruffo *et al*., 2002). Pulped plant or bark in decoction is taken internally for the treatment of dysentery, tuberculosis and whooping cough (Burkil, 2004; Momoh, 2015). Also, crude extract from its stem bark exerts a good anti-plasmodial activity against *P. falciparum* W_2 strain (Lenta *et al*., 2008). The root is used as a mouthwash and gargle to treat tongue diseases and tonsillitis (Ruffo *et al*., 2002). Furthermore, the wood is used to make tool handles and as fuel (Ruffo *et al*., 2002). The pulped roots and bark extracts are mixed with grains and planted to prevent it being dug out by birds.

CHAPTER II:

MATERIALS AND METHODS

II.1. Study site

The present study was carried out in one agro-ecological zone of the Southern Cameroon, the Western Highlands, specifically at the neighborhood of Koutaba. The site was situated at the "Catholic Cistercian Monastery, situated near Tchouffa village (05^0 39'72.7"N; 010^0 47'18.1" E; 1227 m). This zone is made up of the humus ferralitic red soil, suitable for coffee cultivation. The climate is of the Sudano-tropical type with a unimodal pluviometry characterized by 02 seasons; a long rainy season from mid-March to mid-November and a short dry season from ending November to early March (Olivry, 1986). A mean temperature of 25^0C is noted and high precipitations with an average varying from 1313.7 to 1988.6 mm/year, due to an important oceanic influence, with maxima occurring from August to September (Olivry, 1986). The vegetation here is dominated by a shrubby savannah, which usually dries off during the dry season and regularly suffers bush fire, cleared for planting or pasture purposes (Olivry, 1986).

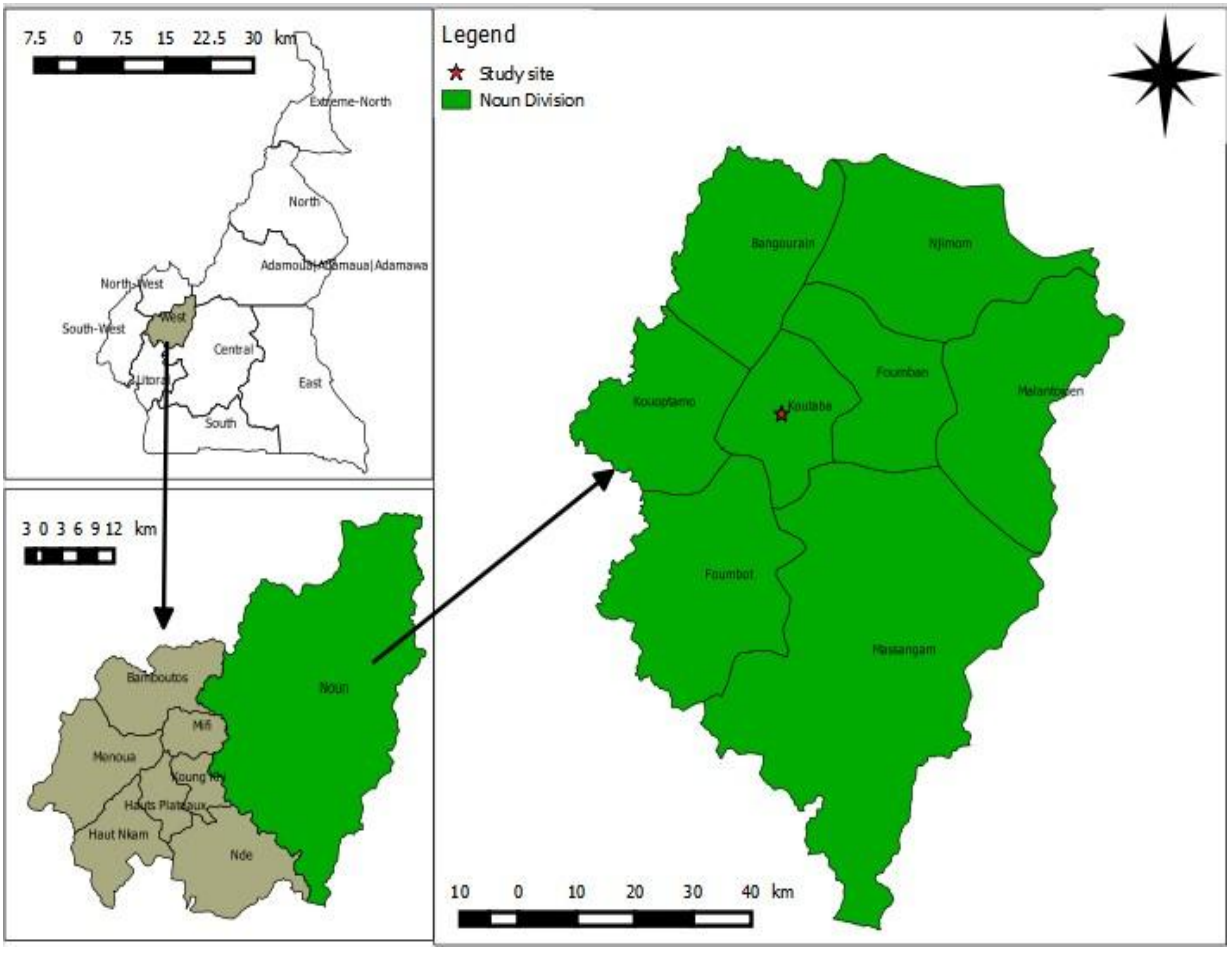


Figure 1: Map of the study site

Figure 2:View of Landscape of the study area.

II.2. Materials

II.2.1. Plant material

The studied host plants are *Psorospermum glaberrimum* Hochr (Hypericaceae) and *Ozoroa paniculosa* (Sond) R Fern& A Fern (1965) (Anacardiaceae) (fig IIa and IIb). They are much branched shrubs with milky sap, colored or not and inhabiting Savannah or wooded rocky areas.

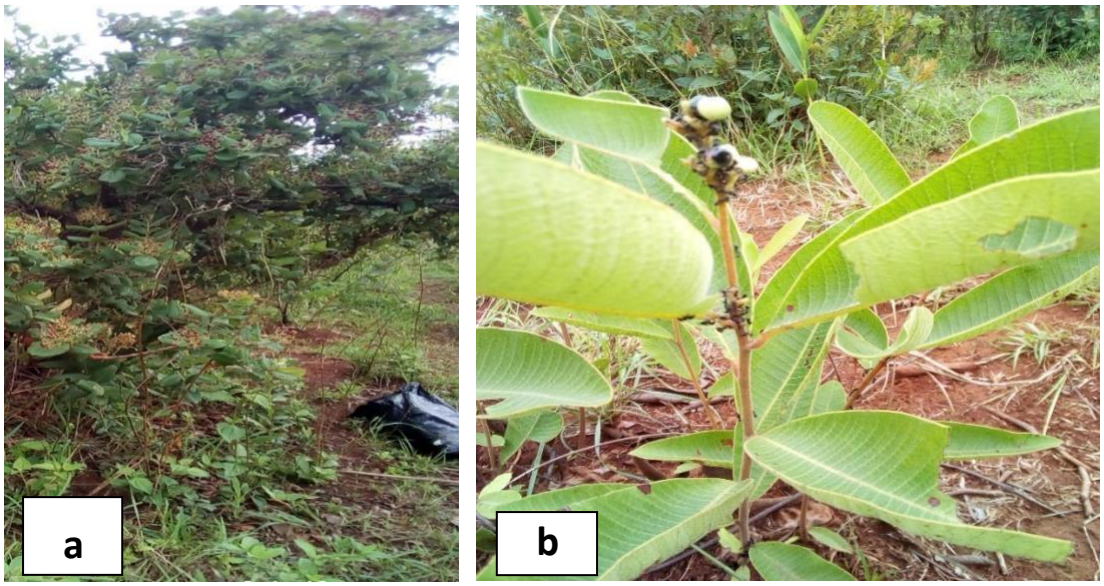


Figure 3:Photographs of the host plants: a. Psorospermum glaberrimum with silvery fruits; b: Ozoroa paniculosa at koutaba with ants and psyllids on stalk and fruits (photo by Latar Nina V., 2019).

II.2.2. Animal material

For the present study, psyllid and ant species were examined or collected from the two host plants presented above.

Ii.3. Methods

II.3.1. Collecting:

The inventory of arthropods found *on P. glaberrimum* and *O. paniculosa* was carried out from February 18 to July 30 2019. Sampling was done on 144 plants of *P. glaberrimum* and 70 plants of *O. paniculosa*. Firstly, the plant was measured using a measuring tape. Age of plant was estimated based on the presence or absence of branches, fruits and leaf texture. On both old and young plants, adults, immatures and eggs of psyllids on one hand and ants on the other were counted and collected per leaf stage, and thereafter preserved in labeled tubes containing 75% alcohol. The different plant organs (leaves, stalks and fruits) infested by psyllid immatures were sectioned using a blade and put in plastic bags, then brought back to the laboratory. Once in the laboratory, the sectioned leaves, stalks and fruits were incubated in closed cylindrical plates and visited every two days till complete degeneration of the plant organs. Thereafter, the emerged adults were gathered and preserved in alcohol as described above.

II.3.2. Measurements:

A bi-ocular lens of Leica brand containing an ocular micrometer graduated from 0-10 unites allowed us to measure the adults and immatures of fifth instar. We measured 60 adults and 17 fifth instars per specie (30♂, 30♀ and 17 instars). On the adults and fifth immatures, we measured organs indicated by Hodkinson and White (1979); Hollis (1976, 1984) and Ossiannilsson (1992) using the following terminology: Body length (BL), body width (BW), head width (HW), vertex length (VL) vertex width (VW), antennal length (AL), flagellum length (F1), metatibia length (Mtl), ultimate rostral segment length (URSL), forewing length (FL), forewing width (WW), male proctiger length (Mpl), female proctiger length (Fpl), female sub-genital plate length (Fsl), length of distal segment of aedeagus (DL), hindwing length (HL), length of the first cubital vein (LCu_1), length of the second cubital vein (LCu_2), length of the first median vein (LM_1), length of the second median vein (LM_2), caudal plate length (Cpl), caudal plate width (Cpw), wingpad length (Fl), tibio-tarsal length (Tbl). The different values in micrometer (µm) were converted to millimeter (mm) using a standard slide graduated from 0.0-1.0mm, observed at different oculars. The different plant measurements were latter discretized into three variables; short (0.10.34), medium (0.35-0.58) and tall (0.59-0.82) since the plant growth was weakly affected by coverage. The different measures were then typed in Excel for further analysis.

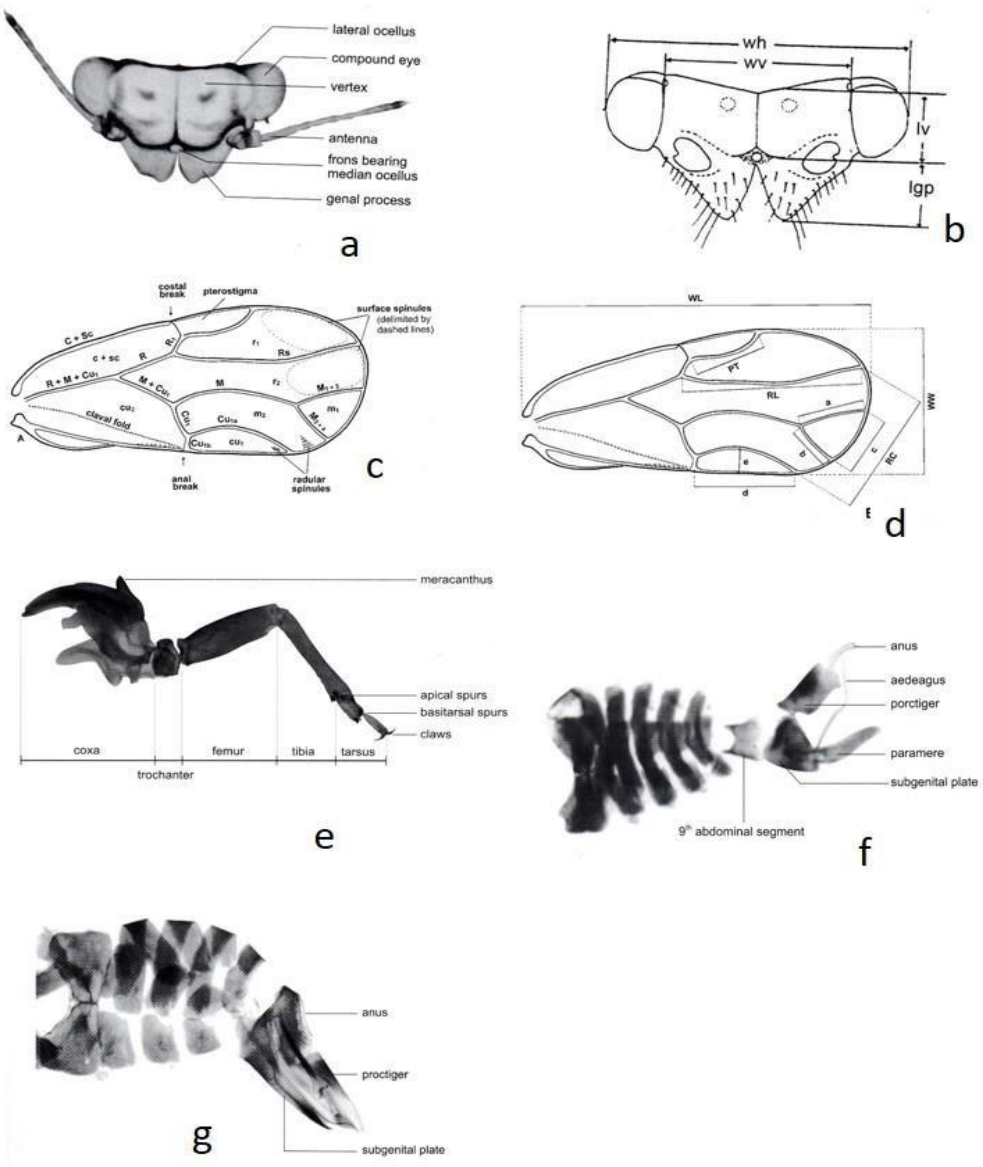


Figure 4: Usual nomenclature and measurements in Psyllids morphology (Pictures borrowed from Mifsud and Burckhardt (2002) and Yang et al. (2009). a: head terminology; b: head measurements; c: forewing terminology; d: forewing measurements; e: metathoracic leg term

II.3.3. Dissection and mounting

The dissection and mounting of the different psyllid parts on slides followed many steps as seen below:

- **Clearing**

The clearing of specimen was done in a solution of dilute potassium hydroxide (KOH) at 10%. The adults and immatures were put in glass containers with dilute KOH, covered and allowed for at least 12 hours, according to the size of the specimen; this was to completely dissolve the inner cavity and to soften the cuticle. The eggs were not treated in KOH. The cleared samples were rinsed with distilled water, and then dehydrated in alcohol at different concentrations (50%, 70% and 95% respectively) for 10 minutes each. Dissections were carried out in the 70% alcohol under a binocular lens (Leica) before complete dehydration in 95% alcohol. The dehydrated samples were passed in LAVANDA essential oil, solvent for Canada balsam, to allow easy manipulation during mounting.

- **Mounting**

Psyllid immatures were delicately spread on a drop of Canada balsam on a slide, and then covered with the slide cover. For the adults, the different dissected organs (head, antenna, metathoracic legs, wings and abdomen) were carefully slide mounted too on a drop of Canada balsam under a binocular lens. The slide mounted specimens were then dried for 30 days in an oven.

- **Drawings**

Drawings were done for both species on transparent papers with the aid of a stereomicroscope (OLYMPUS CX41 brand) with a clear chamber and cleared by a lamp source (SCOTT KL1500 compact). The different drawings were thereafter scanned to obtain an easily manipulable electronic version.

II.4. Statistical Analysis

Statistical Analysis were done using the R software, version 3.6.2. We calculated the abundance and proportion of each species on each host plants, and characterise their relationships. The minimum (min) and maximum (max) values for each measure was derived in Excel and we deployed the Kruskall-Wallis test (X_2) to observe variations in abundance of the psyllid and ant species on one hand, and the Spearman's correlation coefficient (R) to explain the relationships between the psyllid species and their associate ant species on their respective host plants, with the probability value (P) set at 0.005 threshold.

CHAPTER III:
RESULTS AND DISCUSSIONS

III.1. Results

III.1.1. Morphology

III.1.1.1. Study of *Paurocephala* sp. n. (Psylloidea: Liviidae)

III.1.1.1.1. Egg (Fig. 5 a)

Colouration: Whitish when laid and progressively turning milky white to yellow when matured.

Structure: Form elliptical with rounded extremities; the posterior one bearing a lateral short pedicel. Inside, by transparency, with three zones of intense pigmentation, one posteriorly and two small symmetrical anteriorly corresponding to eyes of the embryo.

III. 1.1.1.2. Fifth instar immature

Colouration: Yellowish dorsally and white ventrally (Fig. 5 b) becoming light brown progressively, this colour darkening especially on dorsal sclerites, on base and posterior third of wing pads, on abdominal margins and on intersections of antennal segments.

Structure: Body dorso-ventrally flattened (Fig. 5 c), elongated and divided into two segments: cephalothorax and abdomen. Body length about two times body width, varying from 0.11-0.20 mm long and of 0.03-0.10 mm wide. Head flattened, with two broad dorsal sclerites bearing two symmetrical groups of 3 sectasetae on both side of the body axis, close to the anterior edge; ventral surface with very scarce short setae. Antenna (Fig. 5 d) three segmented, deprived of sectasetae; segments 3 with strangulations and bearing four sub-apical rhinaria; segment 7 with two setae, one distal and one sub-distal. Wing pads (Fig. 5 e) overlapping; upper margin of forewing pad with four sectasetae grouped in 2 + 2; hind wing pad with one sectasetae on distal edge. Legs made up of four segments *viz* coxa, femur, tibia and tarsus; Tarsus ending with two claws, a translucent expanded petiolate arolium, an empodium and two long sub-terminal setae at its extremity (Fig. 5 f). Abdomen elongated, flattened or almost concave on terminal margin with mildly curved extended tubercles carrying sectasetae on the outer margins and simple short setae on the ventral surface. First abdominal tergite deprived of sectasetae; second tergite with a sectaseta on each side; third tergite with a pair of sectasetae on each side. Caudal plate (Fig. 5 g) with three pairs of sectasetae on both sides, a terminal one and three long simple setae on both sides of the slight terminal concavity. Anus terminal, surrounded by a narrow circumanal ring almost rectangular-shaped and made of a single row of pores (Fig. 5 h).

Measurements in mm (17 immatures): BL: 0.11-0.20; BW: 0.03-0.10; AL: 0.03-0.05; Tbl: 0.02-0.04; Fl: 0.02-0.04; Cpl: 0.02-0.05; Cpw: 0.03-0.09.

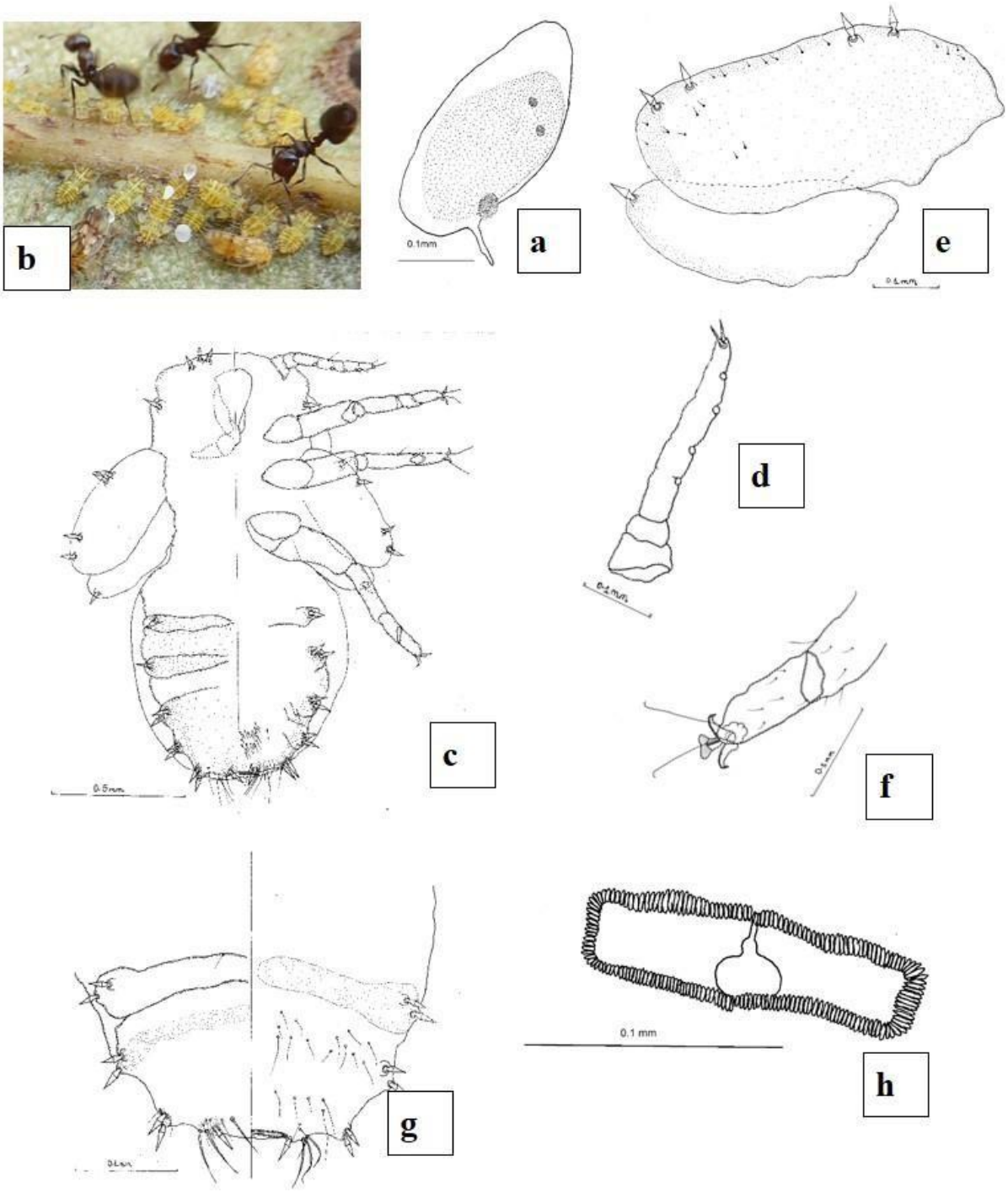


Figure 5: Immatures of *Paurocephala sp.n.* a: egg; b: photo of fifth instar (L_5) with associated ants; c: L_5-habitus; d: L_5-wing pad; f: L_5-metatarsus of hind leg with arolium; g: L_5-caudal plate; h:circumanal ring of pores.

III. 1.1.1.3. Adults

Colouration: Teneral individuals yellow to light brown. Old individuals darkening progressively (Fig. 6 a), thus brown dorsally, and yellowish ventrally. Head light brown, darkening posteriorly; eyes purple; antenna yellow with the two last segments dark brown. Thoracic tergites dark brown, thoracic pleurites and sternites yellow to brownish; forewing hyaline, greyish with spot like brown infuscations on its whitish veins, namely on the base and apex of the pterostigma, on the middle of veins R+M+Cu_1, R and M+Cu_1, on apices of Rs, M_{1+2}, M_{3+4}, Cu_{1a}, Cu_{1b}, and on base and apex of A. Legs with brown coxa; femur, tibia and tarsal segments whitish. Abdominal tergites brown, abdominal sternites light brown, terminalia dark brown.

Structure: Head (Fig. 6 b) short, strongly inclined from the body axis, short, 0.48-0.67 mm wide, with a concave occipital margin; vertex trapezoidal, broad (0.01-0.02mm), divided into two halves by the median suture; its surface sculptured,, with few inconspicuous short setae, two symmetrical pits on each side of the median suture and two lateral ocelli on posterior corners. Frons nearly circular, bearing the median ocellus. Antennal socket oval. Genal cones very short and rounded. Antenna (Fig. 6 c) 0.38-0.57 mm long, made up of 10 segments; scape and pedicel larger than the flagellomeres. First flagellomere longer than the rest; flagellomeres 2, 4, 6 and 7 each with a sub-apical rhinarium, 7^{th} flagellomere without a long basal seta, 8^{th} flagellomere with two strong and long setae, one subapical and one apical (fig 6 d).

Thorax robust, strongly convex dorsally. Pronotum almost as wide as head. Forewing (Fig. 6 e) oblong-oval, broadened on the 2/3 and rounded apically; about 0.16-0.18 mm long and 0.06-0.08 mm wide, with inconspicuous setae from coastal margin to apical margin. Vein Rs slightly curved upward. Radular spinules absent. Hind wing (Fig. 6 f) virtually as wide as forewing, bearing on costal vein 4 setae before the coastal break and 3 groups made up of three, five and one setae respectively after the coastal break. Prothoracic and mesothoracic legs slender. Metathoracic leg robust; metacoxa bearing a thumb-like meracanthus (Fig. 6 g) straight laterally and blunt apically; metatibia long (0.24 to 0.52 mm), ending with an open crown of six apical long strong brown spurs with pointed heads (Fig. 6 h), two on outer side and four on inner side.

Abdomen elongated. Ventral surface of the abdomen carrying short inconspicuous setae. Terminalia different after sexes.

Male terminalia (or male genital segment) (Fig. 6 i) turned upward on the dorsum with respect to the body axis. Male proctiger short, 0.14-0.29 mm long, globular, hairy, with anus surrounded by a transparent short wall and a posterior transparent rounded plate; paramere arched, broad basally, pointed apically with short simple setae. Aedeagus tri-articulated with the basal segment longer than the two others; distal segment about 0.01- 0.02 mm long. Sclerotized end-tube of ductus ejaculatorius long as in fig 6 k.

Female terminalia cuneate (Fig. 6 l), broad basally, tapering apically; female proctiger slightly sloping dorsally bearing a lozenge-shaped dorsal circumanal ring made up of a single row of pores surrounded by short setae, a sub-apical cluster of few long setae and very short and strong setae on posterior end. Female sub-genital plate less acute and shorter than the proctiger, broad and smooth at its base with long setae. Valvulae as in figure 6 l.

Measurements in mm (30♂,30♀): BL: ♂ 1.86-2.71, ♀ 1.90-2.43; BW: ♂ 0.57-0.81, ♀ 0.670.86; HW: ♂ 0.48-0.67, ♀ 0.48-0.62; VW: ♂ 0.01-0.02, ♀ 0.01 ;VL: ♂ 0.33-0.48, ♀ 0.33-0.48; AL: ♂ 0.38-0.52, ♀ 0.43-0.57; URSl: ♂ 0.01, ♀ 0.01; F1: ♂ 0.290.43, ♀ 0.33-0.48; Mtl: ♂ 0.24-0.52, ♀ 0.24-0.52; PL: ♂ 0.14-0.24; FPl: ♀ 0.24-0.52; MPl: ♂ 0.14-0.29; DL: ♂ 0.01-0.02; SL: ♀ 0.19-0.43; FWl: 0.16-0.18; FWw: 0.06-0.08; HL: 0.11-0.15; HW:0.04-0.06 ; LCu_2:0.07-0.09; LCu_1: 0.03-0.04; LR_2: 0.12-0.14; LM_1: 0.02-0.04 ; LM_2:0.05-0.08.

III.1.1.1.4. Discussion

From their study on the genus *Paurocephala*, Mifsud and Burckhardt (2002) recognized 51 described species and 14 non described ones. On the basis of morphological features, they grouped them into six types, no matter phylogenetic relationships between species. As result, all the nine known Afrotropical species of the genus fell into the *gossypii* type. They are; *P. abutili.*, *P. boxi*, *P. hollisi, P. lucida*, *P. medleri*, *P. sinuata, P. urenae* Russell, *P. gossipii* Russell, and *P. insolita* Mifsud and Burckhardt. The *Paurocephala* sp. n. that we describe here, by its morphological characters, is close to *P. insolita* which of the others infests plants of the *Psorospermum* genus. Distinctive characters of these two species are given in the table I.

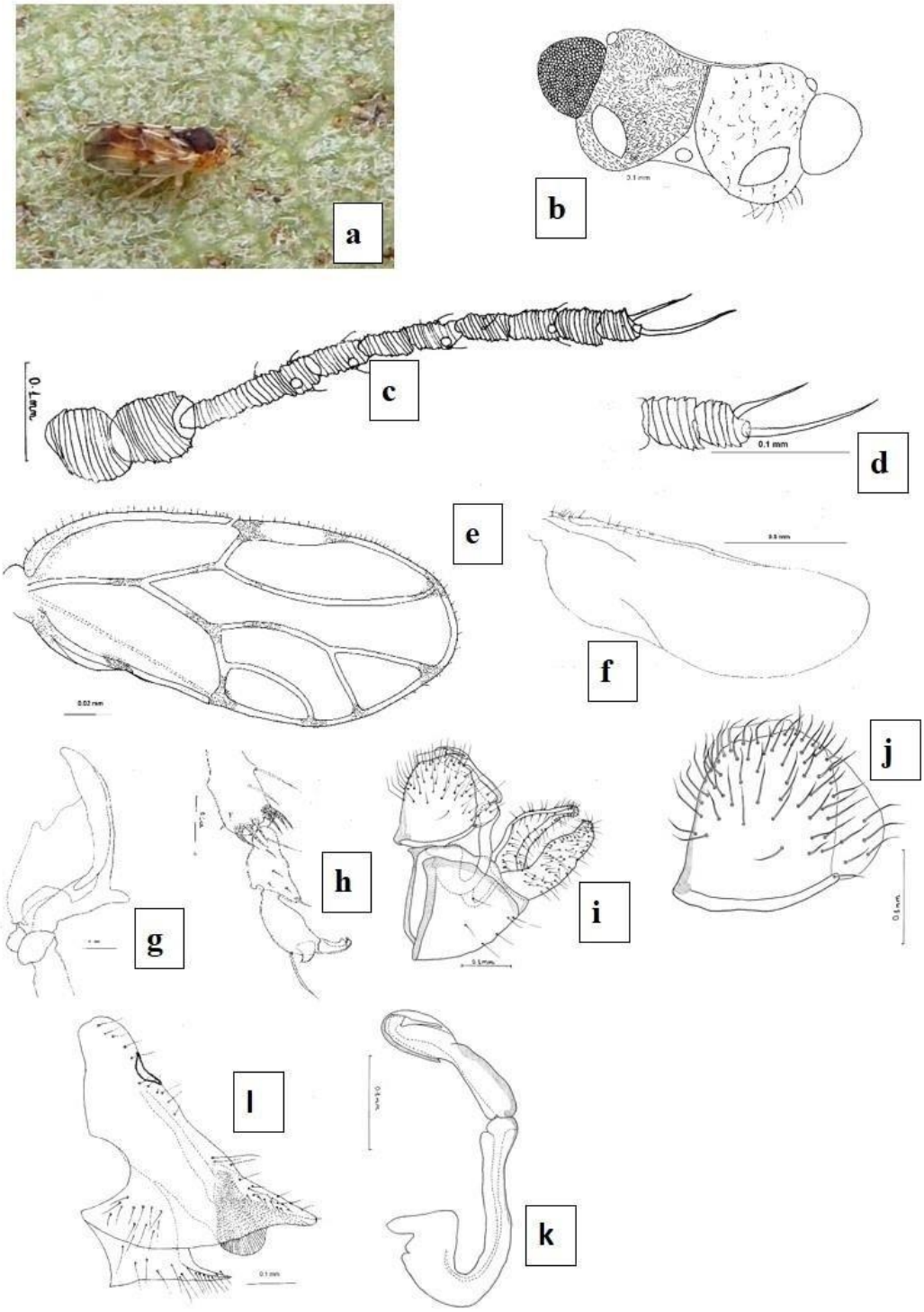


Figure 6: Adults of *Paurocephala sp.n.* a: photograph of a female adult in situ; b: head; c&d: antenna and antennal apex; g: metacoxal; h: apex of metathoracic leg; i: male terminalia; j: male proctiger; k: aedeagus; l: female terminalia

Table 1:Comparison of *Paurocephala sp. n.* and *Paurocephala insolita* Mifsud & Burckhardt, (2002)

Stage	*Paurocephala* sp.n.	*Paurocephala insolita* Mifsud & Burckhardt
Imago	Abdominal tergites brown, sternites light brown, terminalia dark brown.	Abdominal tergites light brown, sternites white, genitalia brown.
	Thoracic tergites dark brown, thoracic pleurites and sternites yellow to brownish	Thorax white to light brown and covered by inconspicuous setae dorsally, laterally and ventrally white.
	Legs apart from coxa, the rest including tarsal segments whitish.	Legs including tarsal segments white to yellow.
	Forewings with inconspicuous setae from coastal margin to apical margin, vein Rs slightly curved upward.	Forewing with inconspicuous setae on veins (vein Rs with 12-16 setae); Rs vein marginally curved in distal third.
	Surface and radular spinules absent.	Surface spinules present in all cells of forewing; radular spinules lacking
Fifth instar	Antenna 3-segmented and deprived of sectasetae	Antenna 3-segmented, segment 1 without a sectasetae, segment 2 with one sectasetae and segment 3 with five sectasetae grouped in 2, 2 and 1.
	Forewing pad with four sectasetae in two groups of 2, 2 and one apical setae on the hind wing pad	Forewing pad with five to seven sectasetae and one to three inconspicuous simple marginal setae.
	Caudal plate without posterior process, with lateral mildly curved tubercle-like extensions bearing sectasetae on the outer margins and simple short setae on the ventral surface.	Caudal plate with large tubercle-like extensions bearing sectasetae and a long posterior process ending in a rounded V-shaped excavation.
	Arolium petiolate	Arolium triangular and petiolate.

III.1.1.2. Study of *Diaphorina* sp. n. (Psylloidea: Liviidae)

III.1.1. 2.1. Egg

Colouration: Colour whitish when laid, gradually turning yellow when matured.

Structure: Form oval with bold ends (Fig. 7 a); the posterior one bearing a short lateral pedicel inside containing three red-pigmented zones; one large posterio-central and two small symmetrical in antero-lateral position representing the embryo's eyes.

III.1.1.2.2. Fifth instar immature

Colouration: Dorsal sclerites dark brown to blackish, with whitish membrane; dorsal caudal plate blackish; ventral sclerites pale brown and membrane whitish to pale beige with purple patches, ventral caudal plate darkening toward posterior end (Fig. 7 b). Eyes pink. Antenna brown basally, pale medially and dark on the two last segments including the precedent segment's tip. Ventral rostrum light brown proximally and dark brown distally. Wing pads dark brown basally and brown apically.

Structure: Body 0.15-0.19 mm long and 0.06-0.10 mm wide (Fig 7 c); well sclerotized, weakly setose. Antenna short made up of three articles (Fig. 7 d), segment 3 longer than the two others, slightly tapering apically, four-strangulated with four rhinaria, each lying ventrally under a strangulation and two terminal strong setae. Dorsal and ventral sclerites scarcely covered with short setae. Cephalothorax with eight large sclerotized plates framed and/or separated by 10 thin elongate ones. Wing pads more than twice as long as wide (Fig 7 c.), well sclerotized and overlapping. Forewing pad with a well-developed humeral lobe, outer margin weakly setose. Leg tetra-articulated (Fig 7 e.), provided with small, short regular setae; tarsus end with a long seta and two claws framing a transparent fan-shaped central arolium. Abdomen short, almost as long as wide, showing anteriorly on both side of the body axis four well sclerotized large sclerites, the three first followed by tinnier ones, and posteriorly a caudal plate of about two times wider than long with a posterior rim concave medially, comprising four long marginal thick setae on both side of the body axis. Ventral plates as in fig 7 f, with three long thick setae and a thin one on both side of the body axis. Anus nearly terminal surrounded by a narrow kidney-shaped circumanal ring made up of a single row of pores.

Measurments in mm (17 immatures): BL: 0.15-0.19; BW: 0.06-0.10; AL: 0.03-0.04; Mtl: 0.04; FL: 0.02-0.05; CPl: 0.04-0.45; CPw: 0.07-0.09.

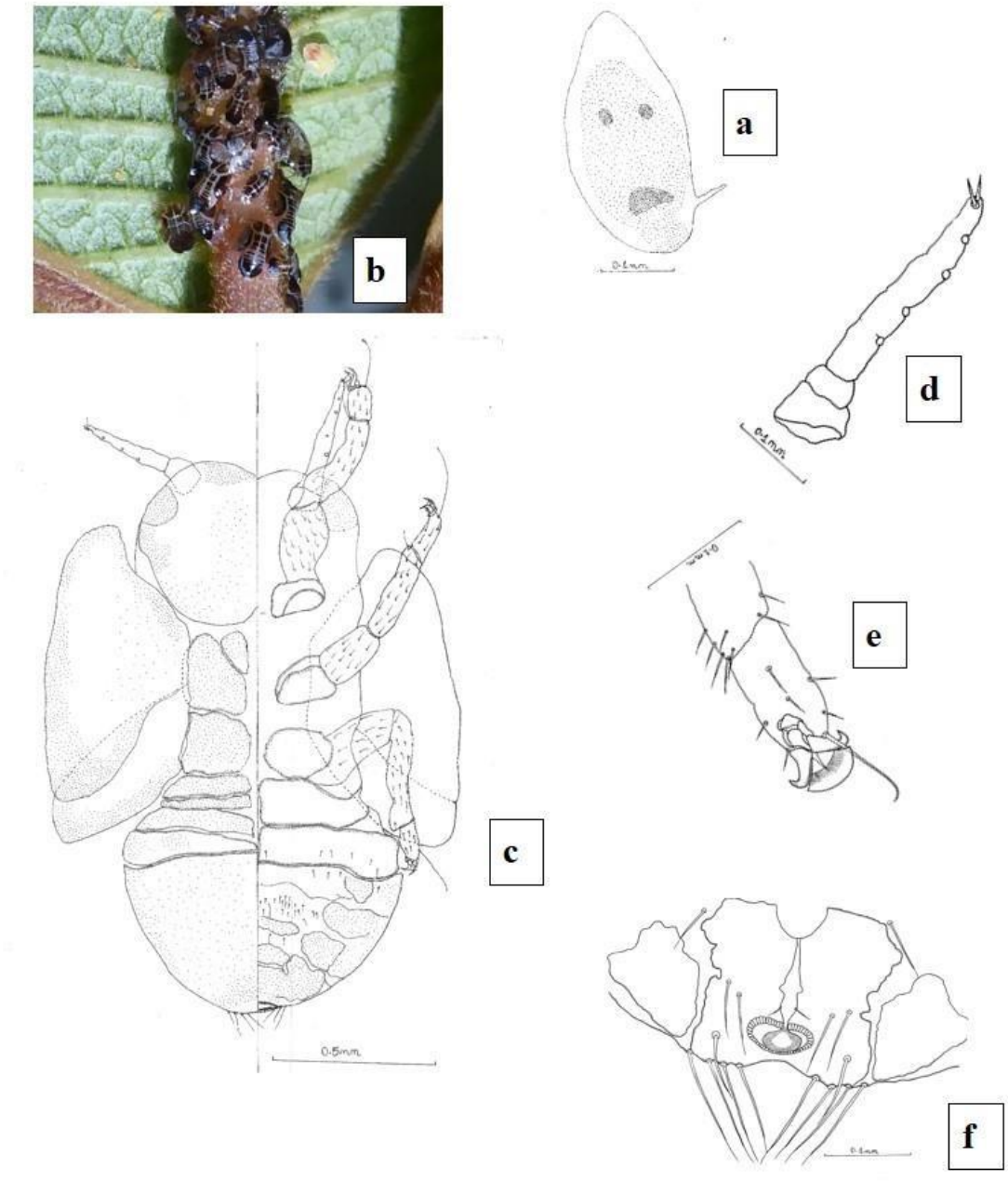


Figure 7:Immatures of *Diaphorina sp.n*. a: egg; b: photograph of a colony of fifth instar immatures (L_5) in situ; c: L_5 habitus; d: L_5-antenna; e: L_5 metatarsus with arolium; f: end of caudal plate with circumanal ring of pores

III.1.1.2.3. Adults

Colouration: Body sclerites dark brown to black dorsally, light brown-purple ventrally, membranes beige (Fig. 8 a). Head blackish with red lateral eyes. Antenna light brown basally, dark brown to black apically on the two last segments and on the tip of the precedent segment. Thorax almost black dorsally, brown ventrally. Forewing darkly infuscate with coastal vein light brown and several white patches *viz* an elongate one on most of subcostal cell (c+sc), another one also elongate on apical half of second marginal cell (m_2), two small ones respectively close to tipmargins of first radial cell (r_1) and first marginal cell (m_1), a very small one close to tip-margin of first cubital cell (cu_1), several whitish spots in the second cubital cell (cu_2) above claval fold and in basal third of r_1. Hind wing pale with brown edges and inconspicuous venation. Legs with dark brown coxae and femurs, pale tibias and tarsi. Genital segments dark brown to black.

Structure: Body three times longer than large. Female more robust than male. Head (Fig. 8 b) on the same plane as body axis, about twice as wide as long; vertex rectangular, broad, strikingly divided into halves by a median suture; occipital margin weakly concave, anterior margins rounded above antennal socks to bases of genal cones, presence of pronounced double depressions (fovea) on each vertex half near posterior margin; vertex surface coarsely sculptured; vertex and genal cones surface covered with scarce simple short setae; frons spherical bearing a prominent median ocellus; lateral ocelli close to inner margin of compound eyes. Genal process conical, broad, and elongate. Antennal socks oval, broad at ocular side and tapering at genal side; antenna (Fig. 8 c) nearly as long as head width, made up of ten segments; scape and pedicel short and thick, flagellum slender basally, thickened apically, antennal segments 4, 6, 8 and 9 carrying each a sub apical rhinaria; segment 10 ending with two apical setae, one short and strong, the other long.

Thorax robust, dorsally convex with bended ribbon-shaped pronotum. Forewing (Fig. 8 d) evenly broadening apically, more than 2.4 times longer than wide; $R+M+Cu_1$ slightly longer than $M+Cu_1$, R about three times longer than $M+Cu_1$, the latter being more than 1.5 times longer than R_1; pterostigma moderately broad and long. Hind wing (Fig. 8 e) with inconspicuous venation, costal vein (C+Sc) carrying fifteen setae in four groups (4+6+4+1), the first group proximal to costal break and three others distal from costal break. Legs penta-segmented. Prothoracic and metathoracic legs less robust carrying yellowish spurs with sharp edges at the apical regions of the tibia. Metathoracic leg robust; coxa with a thumb-shaped meracanthus (Fig. 8 f); metatibia long without knee-spines, covered with simple sectasetae and bearing a crown of six dark apical spurs

disposed in two inward and four outward ; metabasitarsus with 2 apical spurs on both sides; tarsus ending with a long apical seta and 2 claws (Fig. 8 g).

Abdomen elongate. Male terminalia (Fig. 8 h) turned upward with respect to the body axis. Male proctiger elongate, almost straight dorsally, protruded medially toward posterior side, tubular apically, and ending with a transparent structure surrounding the anus, its surface densely covered by long setae; sub-genital plate rounded ventrally, sparsely covered with moderately long setae. Paramere tubular, weakly arched inward, rounded apically, ending with a short pointed tooth. Aedeagus tri-articulated densely covered with long setae oriented inward and downward.

Female terminalia conical (Fig. 8 i). Female proctiger slightly longer than ventral plate, broad anteriorly, evenly tapering towards apex, with an acutely rounded tip; surface covered with moderately long setae especially around a kidney-shaped circumanal ring of pores, setae on apical third short and strong. Female sub-genital plate broad, short, strongly bent ventrally with a pointed apex; surface covered by sparse short setae.

Measurements in mm (30♂, 30♀): BL: ♂ 1.95-2.62, ♀ 1.86-2.90; BW: ♂ 0.57-0.81, ♀ 0.520.95; HW: ♂ 048-0.67, ♀ 0.48-0.67; VW: ♂ 0.03-0.04, ♀ 0.04; VL; ♂ 0.29-0.48, ♀ 0.29-0.48 ; AL: ♂ 0.38-0.52, ♀ 0.38-0.52; URS: ♂ 0.01, ♀ 0.01; F1: ♂ 0.29-0.43, ♀ 0.29-0.43; Mtl: ♂ 0.430.62, ♀ 0.43-0.62; PL: ♂ 0.19-0.33;FP: ♀ 0.19-0.48; MP: ♂ 0.19-0.29; DL: ♂ 0.01-0.02; SGPl: 0.24-0.62; FWl: 0.19-0.22; FWw: 0.08-0.10; HL: 0.13-0.18; HW: 0.03-0.08; LCu_2: 0.10-0.15; LCu_1: 0.02-0.06 ; LR_2: 0.15-0.18 ; LM_1: 0.03-0.05; LM_2: 0.10-0.13.

III.1.1. 2.4. Partial discussion on *Diaphorina* sp.n. morphology

The genus *Diaphorina* Löw, 1880) comprises 76 species (Ouvrad, 2019), more than 40 of which are from the afro-tropical region (Capener, 1970; Burckhardt, 1985; Hollis, 1987; Mifsud and Burckhardt, 1998). It is represented by many species in Southern Africa. Of the eleven species described by Capener (1970), *Diaphorina* sp.n. that we describe here, by its morphological characters, is particularly close to two species *viz D. albomaculata and D. bicolor* (Table II). The species can also be reproached to *D. enderleini* (Mifsud and Burckhardt, 1998; Aléné *et al*., 2011) by its vestigial circumanal pore ring.

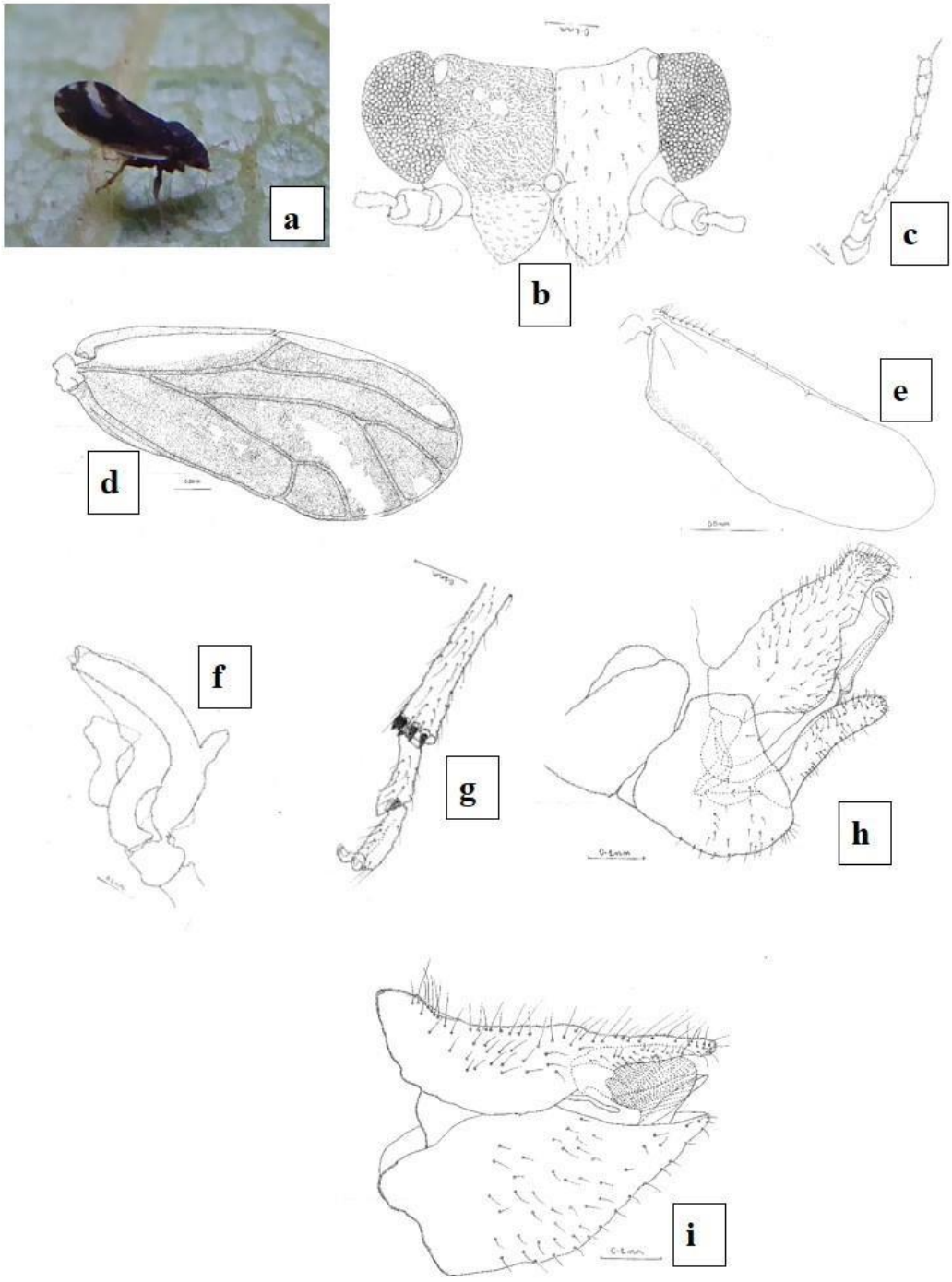


Figure 8: Adults of *Diaphorina sp.n.* a: photograph of an adult in situ; b: head; c: antenna; d: forewing; e: hind wing; f: metacoxal with meracanthus; g: apex of metathoracic leg showing tibial and basitarsal spur

Table 2: Comparison of *Diaphorina bicolor* Capener, *Diaphorina albomaculata* Capener and *Diaphorina sp.n*.

Stage	*Diaphorina bicolor* Capener	*Diaphorina albomaculata* Capener	*Diaphorina* sp.n.
Imago	Head dark brown to black, cylindrical genal processes constricted at base.	Head brown with short sub-conical genal processes	Head blackish with long and broad sub-conical genal processes
	Forewing bicoloured, with a broad white margin along subcostal and anal margins, the latter suffused at base, a small white lunate spot at apex anterior to Rs, another between Rs and M_{1+2} and a third between M_{1+2} and 3+4, the rest of wing dark brown with veins black	Forewings smoky brown to black, strikingly relieved by a broad milky white area occupying most of subcostal cell and including C + Sc**,** a similar white area along anal fold, and generally with a large white area between M+ $M_{1+\ 2}$ and Cu_1 inwards from margin of wing. In some specimens of both sexes the middle area is entirely black; R, R_1 and pterostigma blackish, other veins same shade as membrane	Forewing darkly infuscate, evenly broadening apically with coastal vein light brown and several white patches area on the subcostal cell (c+sc), second marginal cell (m_2), first radial cell (r_1), first marginal cell (m_1), first cubital cell (cu_1), several whitish spots in the second cubital cell (cu_2) above claval fold and in basal third of r_1.
	Forewings about 2.2 times longer than wide, pterostigma thin.	Forewing about twice as long as wide, pterostigma broad.	Forewing 2.4 times longer than wide, pterostigma broad.
	Hind wings with veins well developed and about 10	Hind wings milky white.	Hind wing pale brown with inconspicuous venation.

	strong bristles at base along anterior margin		
	Hind leg with tibia bearing seven small black apical spines.	Hind Leg with tibia bearing eight apical spines	Hind leg with tibia bearing 6 dark apical spurs.
		Ventral female sub-genital plate weakly bent ventrally with scattered micro-hairs	Ventral female sub-genital plate strongly bent ventrally
	Male proctiger not protruded posteriorly	Male proctiger weakly protruded posteriorly	Male proctiger markedly protruded posteriorly
	Paramere shorter than proctiger and rounded apically	Paramere tubular	Paramere broader in apical two third
Immatures		Brownish above, paler below	Dark brown to blackish above, pale brown below
		With 8-10 lanceolate sectasetae at tip of abdomen	with 8-10 long simple setae at tip of abdomen.
		Circumanal ring very close to apical margin and heart-shaped	Circumanal ring very close to apical margin and kidney-shaped.
		Wing pads margined with extremely minute setae	Wing pads almost bare marginally

III.1.2. Ecology of the Psyllids

III.1.2.1. Ecology of *Paurocephala* sp.n.

III.1.2.1.1. Entomofauna living on *Psorospermum glaberrimum*

The psyllid species *Paurocephala* sp.n. was encountered at different developmental stageswith three ant species on *Psorospermum glaberrimum* (fig. 13 a and b). The ants were

Camponotus congolensis Emery, 1899, *Camponotus flavomarginatus* Mayr, 1862 and *Tapinoma debouti* ….

Of all these ant species, *C. flavomarginatus* turned to be more abundant with 561 individuals followed by *C. congolensis* (431 individuals) and then *T. debouti* (10 individuals) (Fig. 9).

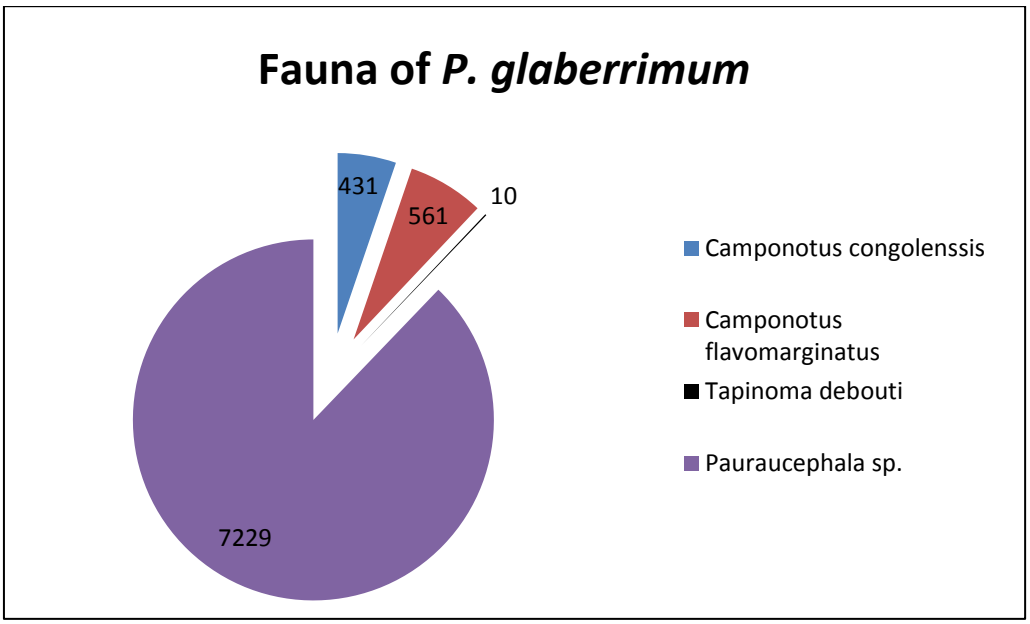


Figure 9:Insects' fauna of *Psorospermum glaberrimum*

Individuals of *Paurocephala* sp.n. were observed feeding on leaves. They were seen on the lower surface of the leaves, lined on the principal vein or at the borders of the leaf. Their feeding action which is sap sucking caused leaf depigmentation and degeneration.

III.1.2.1.2. Distribution of insects' location on *Psorospermum glaberrimum*

The assessment of the psyllid infestation and its attendant ants on *P. glaberrimum* showed significant variations of populations on the different leaf stages (Table III). Indeed, *Paurocephala* sp.n., *C. congolensis*, *C. flavomarginatus* and *T. debouti* were more abundant on the old leaves (46.85%, 43.62%, 74.33%, and 50% respectively) than on the young leaves (21.07%, 22.04%, 8.56%, and 0.00% respectively) with high significant differences ($P< 10^{-3}$). The distribution of the psyllid population (fig. 10) showed that very young leaves were weakly colonised, young leaves were moderately infested, whereas old leaves were heavily occupied.

These results suggest that the more leaves were old, the more they were attractive for *Paurocephala* sp.n., which is an unusual behaviour in psyllid.

Table 3:Variation of *Paurocephala sp.n.* and its associated ants on *Psorospermum glaberrimum* (O = old leaves, Y = young leaves, VY = very young leaves)

Species	*P. glaberrimum*	Leaf stages			Kruskall test(X2)
	O	Y	VY	Total	
Paurocephala sp.	3387(46.85%)	2319(32.08%)	1523(21.07%)	7229	X2=0.42; P>0.05
Camponotus congolensis	188(43.62%)	148(34.34%)	95(22.04%)	431	X2=0.47; P>0.05
Camponotus flavomarginatus	417(74.33%)	96(17.11%)	488.56%)	561	X2=17.71; P<0.0001
Tapinoma debouti	5(50.00%)	5(50.00%)	0(0.00%)	10	X2=8.17; P<0.0001

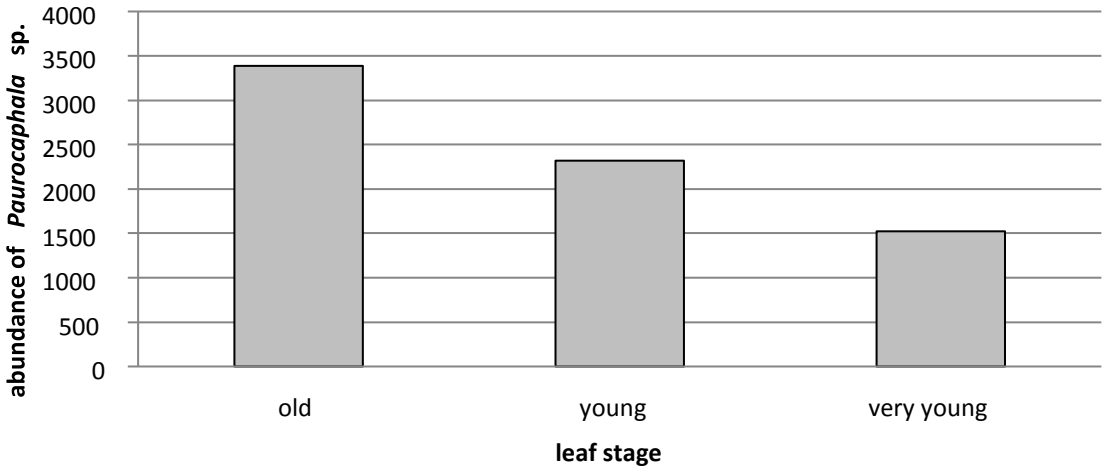


Figure 10: Distribution of *Paurocephala sp.n.* on *Psorospermum glaberrimum* leaves.

III. .1.2.1.3. Relationships between *Paurocephala* sp.n. and its associated ant species

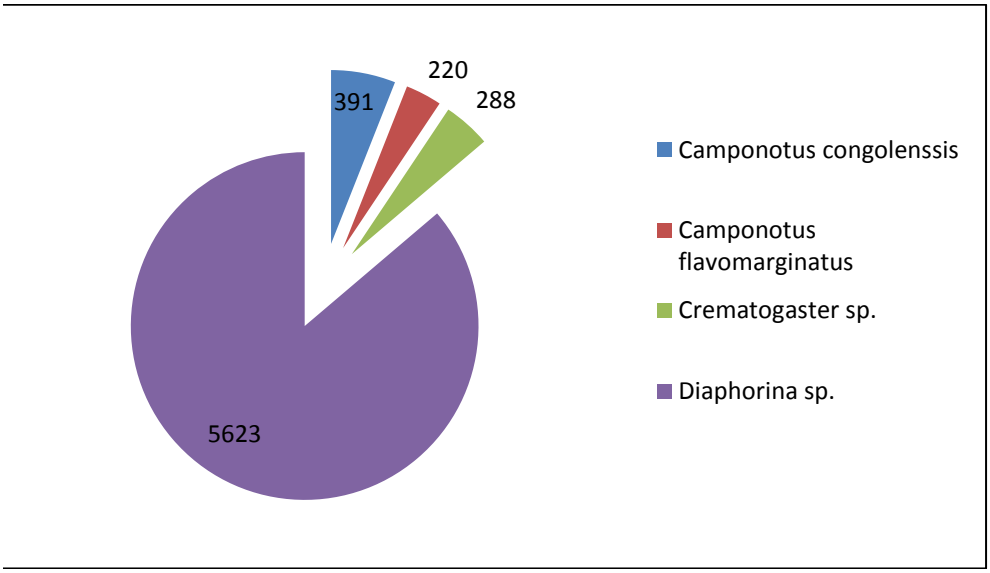
The spearman's correlation test performed between *C. congolensis* and *Paurocephala* sp.n. showed a positive but not significant correlation. (R=0.33; P = 0.002). This implies that the population of *paurocephala* sp.n. increased according to that of *C. congolensis*. By contrast, *C. flavomarginatus* and *Tapinoma debouti* showed no significant correlation with *Paurocephala* sp.n. (R=0.02, P value= 0.87 and R=0.13, P value= 0.25), implying that their abundance had not any

influence on that of *Paurocephala* sp.n. Furthermore, ants associated with *Paurocephala* sp.n. always built shelters around the psyllid colonies to protect them against their rivals or other environmental factors such as natural enemies or weathers (fig 13 c and d).

III.1.2.1. Ecology of *Diaphorina* sp.n.

III.2.2.1. Entomofauna living on *Ozoroa paniculosa*

The psyllid species *Diaphorina* sp.n. was encountered at different stages on *O. paniculosa* with three associated ant species (fig. 13 e), *viz C. congolensis, C. flavomarginatus* and *Crematogaster* sp. Of these ant species, *C. congolensis* was the most abundant with 391 individuals, followed by *Crematogaster* sp. (288 individuals) and lastly *C. flavomarginatus* (220 individuals).



III.2.2.2. Distribution of insects' location on *Ozoroa paniculosa*

The assessment of the psyllid infestation and its attendant ants on *O. paniculosa* (Table IV) showed no significant variations of populations' proportions on the different leaf stages.

Indeed, percentages of *Diaphorina* sp.n. on young and very young leaves were approximately the same (37.24% on very young and 38.86% on young leaves). Individuals of *Diaphorina* sp.n. were observed feeding on young plants of *O. paniculosa*, especially on young leaves and stems (plant stalk). The plant stalks were occupied till the point of attachment of the seeds. The immatures were seen on the lower surface of leaves, aligned on both sides of the principal vein and on the plant stalk. Of its associated ants, *C. congolensis* was predominantly found with immature colonies of *Diaphorina* on the very young leaves (47.57%) (fig. 13 e) while *C. flavomarginatus* was mostly abundant on young leaves. These ants would often build shelters around the *Diaphorina* sp.n. colonies (fig. 13 f) but not always.

The distribution of the psyllid population (fig. 12) showed that, no matter no significant differences, the very young leaves were mostly colonised, the young leaves were moderately infested, whereas the old ones were weakly occupied. These results suggest that young leaves, as usual in psyllid habits, were more attractive for *Diaphorina* sp.n. than the old ones.

Table 4:Variation of *Diaphorina sp.n.* and its associated ant species *Ozoroa paniculosa* (O = old leaves, Y = young leaves, VY = very young leaves).

	O. paniculosa leaf stages				kruskall.test
Species	O	Y	VY	total	(X2)
Diaphorina sp.n.	1344 (23.90%)	2185 (38.86%)	1523 (21.07%)	7229	X2=1.78; P=0.41
Camponotus congolenssis	78 (19.95%)	127 (32.48%)	95 (22.04%)	431	X2=0.36; P=0.84
Camponotus flavomarginatus	72 (32.73%)	92 (41.82%)	48 (8.56%)	561	X2=0.65; P=0.72
Crematogaster sp.	80 (27.78%)	103 (35.76%)	0 (0.00%)	288	X2=0.27; P=0.87

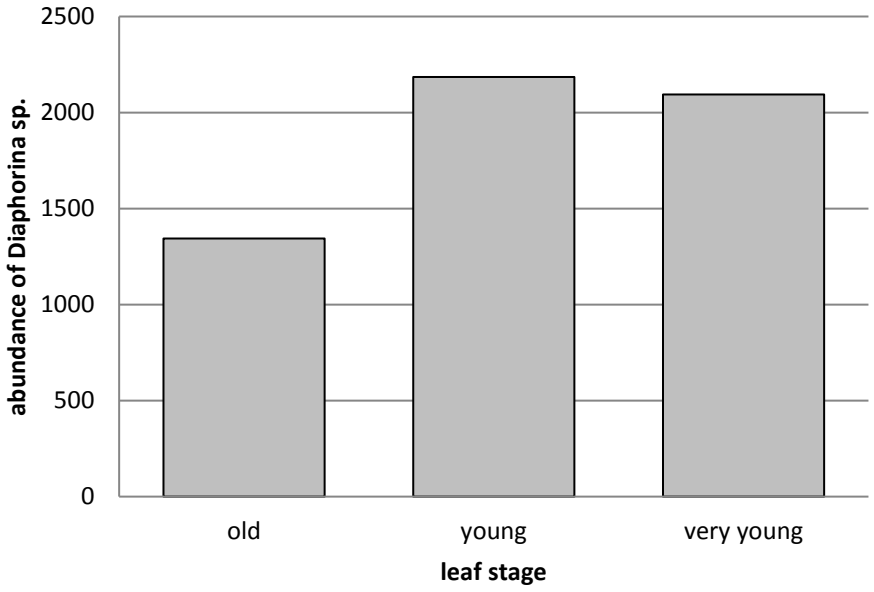


Figure 12: Distribution of *Diaphorina sp.n.* on *O. paniculosa*

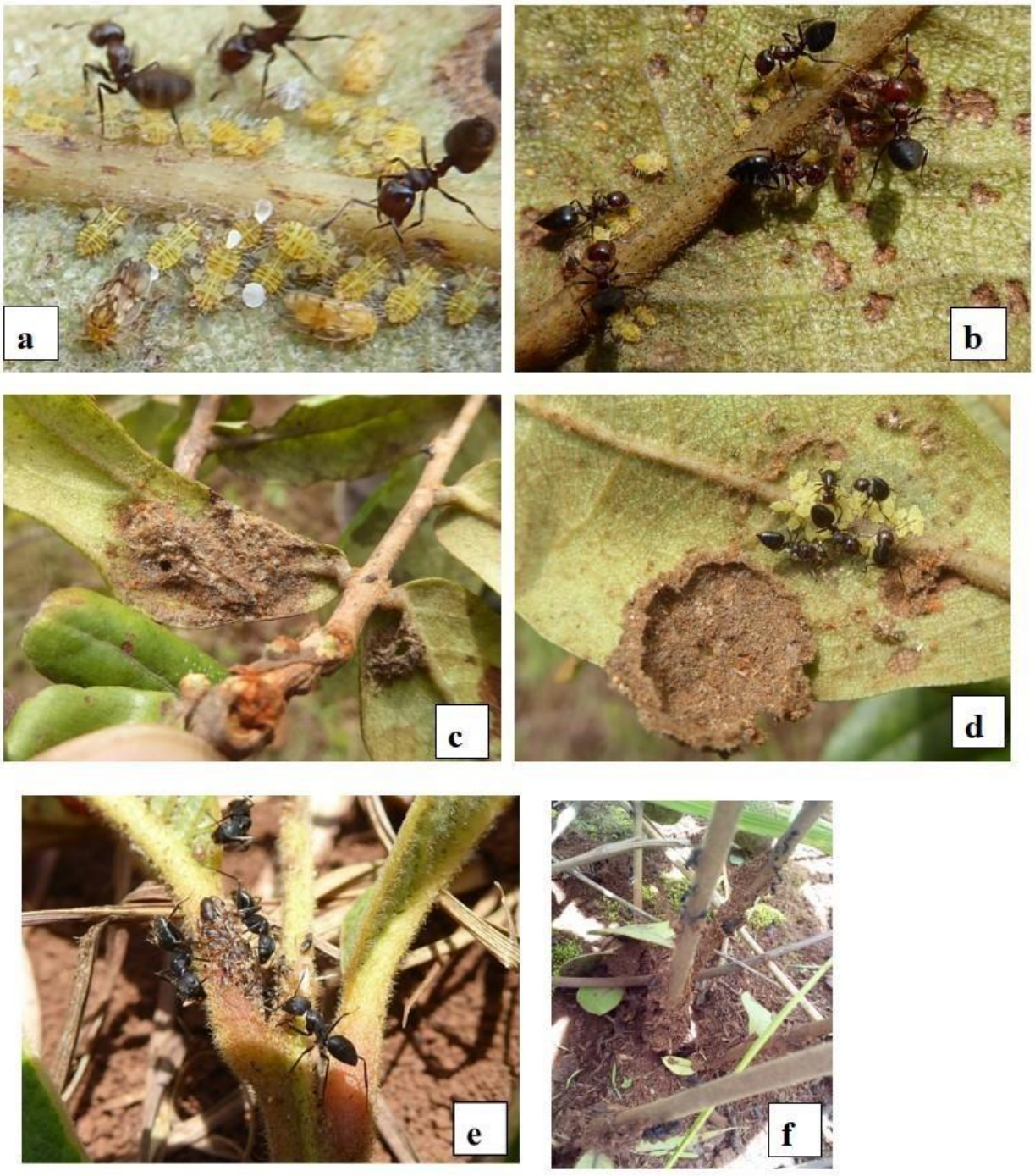


Figure 13: Photographs of studied and some of their associated ants. a: a colony of *Paurocephala sp.n*. with *Tapinoma debouti*; *Paurocephala sp.n.* with *Crematogaster sp*.; c: *Paurocephala sp.n.* under shelter built by *Crematogaster sp*.; d: Shelter removed showing young immatures of Paurocephala sp.n. surrounded by *Crematogaster sp*. workers; e: immatures of *Diaphorina sp.n*. with *Camponotus congolensis*; f: shelter built by *Camponotus congolensis* at the base of *Ozoroa sp*. to protect colonies of *Diaphorina sp.n*

III.2.2.3. Relationships between *Diaphorina* sp.n. and its associated ant species

The spearman's test performed between ants and *Diaphorina* sp.n. showed positive and significant correlations with *C. congolensis* (R=0.49, P<0.001) and *Crematogaster* sp (R=0.50, P<0.001). This implies that the population of *Diaphorina* sp.n. was closely dependant on those of these two ant species. However, the ants' nests were not always built around the psyllid, suggesting that these constructions were facultative for this psyllid species.

III.2 General Discussion

Concerning the distribution of psyllid population on the different leaf stages, *Paurocephala* sp.n. population size varied significantly on the different leaf stages of *P. glaberrimum* and occurred more abundantly on old leaves than on young ones. However, *Diaphorina sp.n.* did not vary significantly on *O. paniculosa* but its densities were somehow higher on young leaves than on old ones. The significant variation observed in *Paurocephala* sp.n. was far different from that observed generally in psyllids (Ossiannilsson 1992; Aléné *et al*. 2008; Cario-Costet and Mondy 2013; and Ouvrard 2014), and moreover, the psyllid species studied by these authors had their populations more abundant on young vegetative organs than on old ones. Indeed, psyllids would mostly attack young plants and shoots of old plants, laying their eggs on young tender tissues to ensure the availability of resources (food and habitat) as the young emerges. *Diaphorina* sp.n. is the one which fit with this usual habit in psyllids.

As for relationships between the two psyllid species and their associated ants, the positive and significant correlations observed between *C. congolensis* and *Crematogaster* sp. and *Diaphorina* sp.n. suggested that as the population of *C. congolensis* and *Crematogaster* sp. increased, that of *Diaphorina* sp.n. also increased accordingly, meaning that the psyllid fitness was closely dependant on the presence of the two ant species. No significant correlation was noticed between *C. flavomarginatus* and *Diaphorina* sp.n., implying that this ant species did not contribute in the fiftness of the psyllid. For *Paurocephala* sp.n., a positive and significant correlation was noticed between its population and that of *C. congolensis*, suggestion a close relationship between the psyllid and the ant species. For the two other ant species, *C. flavomarginatus* and *T. debouti*, their populations showed no significant correlation with that of *Paurocephala* sp.n., implying that their fluctuations had no influence on that of *Paurocephala* sp.n. It results from this analysis that relationship between the two psyllid species and *C. flavomarginatus* in not close, probably because

this ant species usually feed on other food resources in addition to honeydew produced by hemipterans, as noticed by Aléné *et al*. (2019).

The two described psyllid species have in common, in immature stages, a very weakly developed and non-functional circumanal ring which is almost vestigial in *Paurocephala* sp.n. (very terminal). This character is shared by *Diaphorina enderleini* whose circumanal ring is also vestigial as described by Aléné *et al*. (2011). As result, all these psyllid species do not produce wax which would surround and protect immatures from environmental constraints and their natural enemies. *Diaphorina* sp.n. and *Paurocephala* sp.n. on the other hand put forth fantastic strategies as they do not produce wax. These strategies were their associations with some ant species. Hence *C. congolensis, C. flavomarginatus* and *Crematogaster* sp. were seen around *Diaphorina* sp.n. colonies on *O. paniculosa* while *C. congolensis* and *C. flavomarginatus* were seen around *Paurocephala* sp.n. colonies on *P. glaberrimum*. These associations could be simple as the ant individuals simply surrounds the psyllid colony (Fig. 13 a, b and e) or become complex when the ant build sand shelters round the psyllid colonies. This complex level of originality is put forth by *C. congolensis*, and *C. flavomarginatus* round *Diaphorina* sp.n on *O. paniculosa* and and by *Camponotus flavomarginatus* round *Paurocephala* sp.n on *P. glaberrimum*. So kind of relationships were registered by Aléné *et al*. (2011) where *Phiedole megacephala* workers built sand shelters to surround *D. enerleini* on *Vernonia amygdalina*. These shelters built by ant are probably also a means for the ants to protect their food resource (honeydew) and young stage (eggs and nymphs) from weathers and natural enemies in hemipterans as noticed by several authors (Stadler and Dixon, 2005; Oliver et al., 2007; Aléné *et al*., 2011, Aléné *et al*., 2019).

CONCLUSION AND PERSPECTIVES

Finally, the present work termed the study of two psyllid species belonging to the family Liviidae, both associated with ants (Hymenoptera: Formicidae) on two Savannah plant species at Koutaba (West Cameroon).

Morphological studies led to the diagnosis of two new species of psyllid, *Paurocephala* sp.n. found on *Psorospermum glaberrimum*, and *Diaphorina* sp.n. found on *Ozoroa paniculosa*.

The first species, *Paurocephala* sp.n., is similar to *Paurocephala insolita* Mifsud and Burkhardt 2002 by the whole habitus in adult stage, but is recognisable by abdominal tergites brown, sternites light brown, terminalia dark brown; thoraxic tergites dark brown, thoracic pleurites and sternites yellow to brownish; forewings with inconspicuous setae from coastal margin to apical margin, vein Rs slightly curved upward. In fifth immature instar, diagnostic characters are: antenna three segmented, deprived of sectasetae, wing pads overlapping; upper margin of forewing pad with four sectasetae grouped in 2 + 2, hind wing pad with one sectasetae on apical edge, abdomen elongate, lacking a caudal process, almost concave on terminal margin with mildly curved extended tubercles carrying sectasetae on the outer margins and simple short setae on the ventral surface, anus terminal, surrounded by a vestigial circumanal ring almost rectangular-shaped and made of a single row of pores.

The second species, *Diaphorina* sp.n., is morphologically close to *Diaphorina albomaculata* Capener 1970 but its diagnostic characters in adult are: the genal processes slightly longer, the more elongate forewing with more expanded white patches, the broader parameres, the more produced male proctiger and the form of female sub-genital plate. In the immature instar of *Diaphorina* sp.n., there are long setae at the rim of the caudal plate while they are lanceolate in *D. alomaculata.*

The distribution of psyllid populations on different leaf stages was as usual in hemipterans in general and in psyllids in particular for *Diaphorina* sp.n. as this species, at different stages, infested at approximately the same rate, young and very young leaves, without significant differences in densities. Conversely, in *Paurocephala* sp.n., the distribution of population on different leaf stages varied significantly between young and old leaves; the old leaves being more infested than the young. This is uncommon in sap sucking insects in general.

For the relationships between psyllids and ants, it results that populations of *Camponotus congolensis* and *Crematogaster* sp. were positively and significantly correlated to those of *Diaphorina* sp.n. with a complex association existing between them. Thus, these ants often built

shelters around *Diaphorina* colonies. For *Paurocephala* sp.n., its populations were positively correlated to those of *Camponotus congolensis* suggesting mutualistic relationships between the psyllid and this ant species, which in addition always built shelters around *Paurocephala* sp.n. colonies.

As perspectives, furthers studies on biology and population dynamics of the psyllids species in association with their attendant ants are to be considered as well as factors determining the distribution of the studied psyllids on different leaf stages of their host plants.

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