Journal of Natural History

ISSN: 0022-2933 (Print) 1464-5262 (Online) Journal homepage: http://www.tandfonline.com/loi/tnah20

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To cite this article: I. A. Gavrilov-Zimin (2017) A remarkable example of symbiosis between an animal and a fungus in a new species of legless mealybug (Insecta: Pseudococcidae), Journal of Natural History, 51:37-38, 2211-2224, DOI: 10.1080/00222933.2017.1365180

To link to this article: http://dx.doi.org/10.1080/00222933.2017.1365180

JOURNAL OF NATURAL HISTORY, 2017
VOL. 51, NOS. 37–38, 2211–2224
https://doi.org/10.1080/00222933.2017.1365180

A remarkable example of symbiosis between an animal and a fungus in a new species of legless mealybug (Insecta: Pseudococcidae)

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ABSTRACT

A new species and a new monotypic genus of legless mealybug, *Orbuspedum machinator* **gen. et sp. nov.**, are described and illustrated, based on material collected from bamboo twigs in southern Thailand. Larvae and females of the new species each live inside an individual conical domicile constructed from densely packed fungal hyphae of the sooty mould *Capnodium* sp., mixed with small quantities of wax, secreted by the mealybug. The domicile is enlarged as the insect grows; the insect irrigates the hyphae with honeydew through a small orifice at the apex of the domicile. While the insect produces honeydew and feeds the fungus, the fungus grows around the insect, protects it from predators, and buttresses the soft apodal body of the mealybug. Such a remarkable example of mutualistic symbiosis between an animal and a fungus is reported for the first time.

www.zoobank.org/urn:lsid:zoobank.org:pub:C0A84631-C6A0-4177-BC50-A71D6281107F

ARTICLE HISTORY
Received 7 February 2017
Accepted 2 August 2017
Online 13 September 2017

KEYWORDS
Scale insects; sooty moulds; *Capnodium*; honeydew; mutualistic symbiosis

Introduction

The mealybug family Pseudococcidae is one of the largest families of the well-known sup-sucking plant parasites in the taxon Coccinea (also called Coccomorpha, Coccoidea, Coccoptera and Coccina in papers by different authors), or scale insects. More than 2000 species of mealybugs are known in the world fauna to date (Williams and Granara de Willink 1992; Gavrilov-Zimin 2015) and each year new species and genera are discovered in nature and described as new to science (for example, Gavrilov-Zimin 2016a, 2016b). Like all other scale insects, the Pseudococcidae show significant sexual dimorphism. The males usually have well-developed wings and legs like most other insects, and more or less clear subdivision of the body into tagmata: head, thorax and abdomen. The females, in contrast, are neotenic (larva-like), always without wings, with partly or completely fused tagmata and with shortened or even totally reduced legs. The informal morphological group of 'legless mealybugs' comprises at least 26 nominal genera in the world fauna (Gavrilov-Zimin 2015). Most of these genera are known from tropical and subtropical regions of the world, mainly from the Oriental Region and feeding on large

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bamboo-like grasses (Poaceae). In particular, recently (Gavrilov-Zimin 2016b) a new species and monotypic genus of legless mealybug were described from Indonesia (Flores Is.) and another new species from Thailand. During my expeditions in southern Thailand in 2010 and 2013 I have also collected two series of a very peculiar species of legless mealybug that was previously unknown to science. The females and larvae of this species live on twigs of bamboo inside conical domiciles formed by the live hyphae of a fungus *Capnodium* sp. (Capnodiaceae) and the domicile grows together with the insect, which irrigates the hyphae with honeydew. Such mutualistic symbiosis has never been reported before for any other scale insect, nor for any other animals known to the author. Below a taxonomic description of this new mealybug species (placed in a new monotypic genus) together with the description and illustrations of its unusual habitat are provided.

Material and methods

Both adult females and larvae were collected inside fungal domiciles by the author in 2010 and 2013 in southern Thailand, in the vicinity of the Khao Sok National Park (Figure 1), on twigs of an undetermined bamboo (Poaceae). All the material is deposited at the Zoological Institute of the Russian Academy of Sciences (ZIN RAS) (St Petersburg, Russia), excluding one paratype which was sent to Muséum National d'Histoire Naturelle

Figure 1. Vicinity of the Khao Sok National Park, Thailand, the type locality of *Orbuspedum machinator* **gen**. et **sp. nov.**

(MNHN) (Paris, France).The numbers with 'K' refer to a unique collecting number for both ethanol-preserved material and Canada balsam slides.

The method of preparation of the Canada balsam slides can be found in Danzig and Gavrilov-Zimin (2014).

Taxonomic descriptions

Orbuspedum gen. nov.

Type species *Orbuspedum machinator* **sp. nov.**, here designated.

Description

Adult female. Body spindle-form, with posterior segments of abdomen heavily sclerotized. Antennae each 2-segmented. Legs entirely absent. Anal apparatus simplified, without setae. Vulva of unusual structure, funnel-shaped, directed anteriorly. Anterior and posterior pairs of ostioles absent. Small oval circulus present. Multilocular pores absent. Trilocular pores and simple discoidal pores scattered through entire body surface, except for posterior abdominal segments where they are completely absent. Discoidal pores of irregular structure or duct-like pores absent. Tubular ducts each with very deep collar present on posterior abdominal segments only. Dorsal surface of body covered with small, thin conical setae and minute flagellate setae; ventral surface covered by longer flagellate setae; posterior abdominal segments densely covered by significantly longer flagellate setae on both surfaces. Sacciform invaginations of sclerotized cuticle of different sizes present in transverse rows across most body segments.

Diagnosis

The new genus differs from all other genera of legless mealybugs in having an anteriorly directed vulva. In other characters, *Orbuspedum* **gen. nov**. seems to be similar to the large, widely distributed genus *Antonina* Signoret, but differs from the latter in the total absence of multilocular and irregular disc pores (wax glands); see modern identification keys to genera of legless mealybugs in Hendricks and Kosztarab (1999) and Danzig and Gavrilov-Zimin (2015).

Additionally, the new monotypic genus differs from all other known scale insect genera ecologically, in the peculiar mode of life of females and female larvae inside individual fungal domiciles (see 'Mode of life' section under the species description).

Etymology

The generic name is constructed from appropriate Latin words, and means 'legless'. Gender masculine.

Orbuspedum machinator sp. nov.

(Figures 2–5)

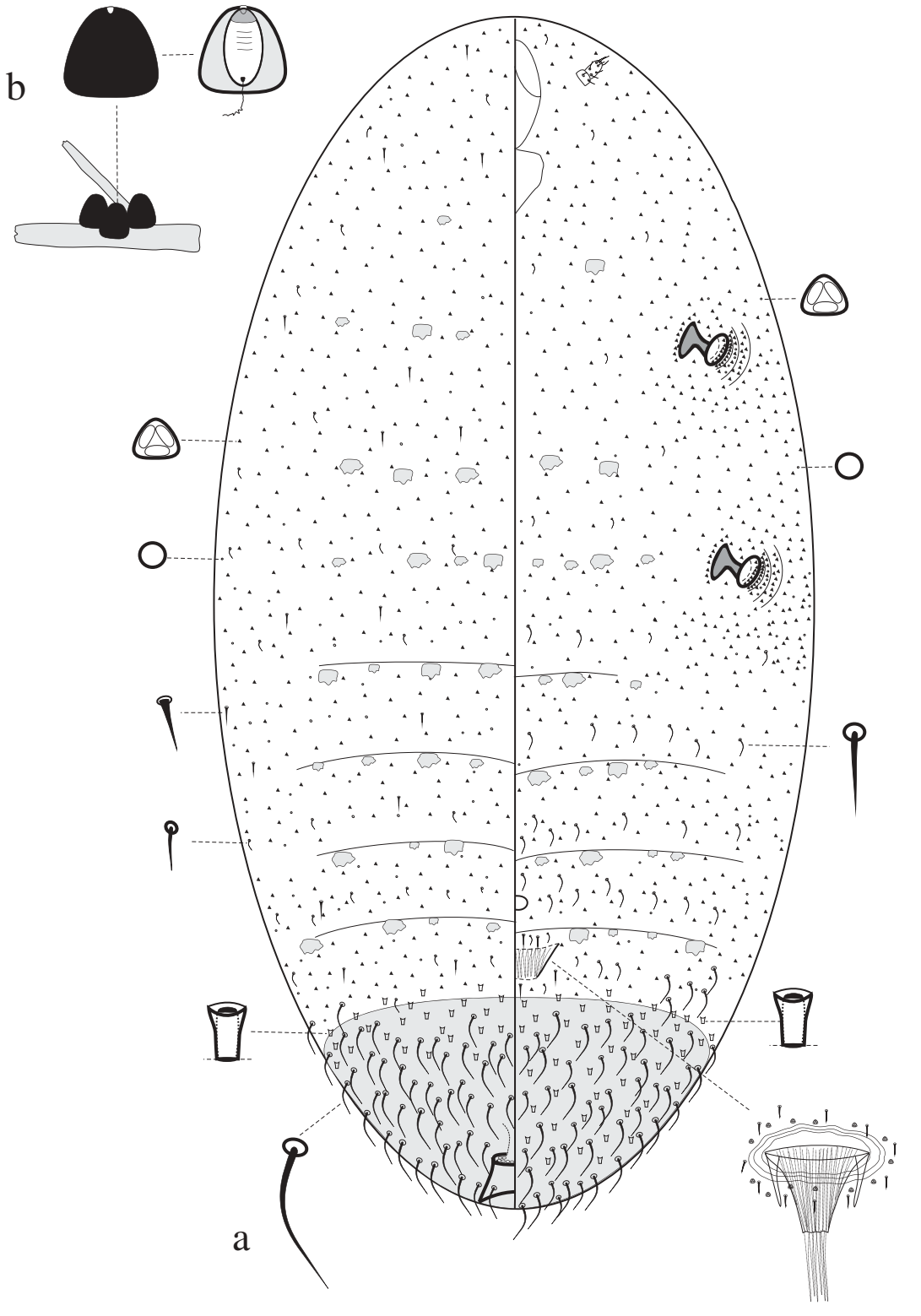

Figure 2. *Orbuspedum machinator* **gen**. et **sp. nov**.; (a) microscopic characters of the holotype; (b) fungal domiciles with a female inside.

Figure 3. *Orbuspedum machinator* **gen**. et **sp. nov**., photograph of the slide-mounted holotype.

Material

Holotype, adult female: K 714, southern Thailand, vicinity of the Khao Sok National Park, on twigs of bamboo, 4 November 2010, coll. Ilya Gavrilov-Zimin. Paratypes: 5 adult females with same collection data as holotype, and 1 female with the same data, but collected 19 November 2013 (K 1151), each mounted singly on separate slide.

Additional, non-type material: K 714, two ultimolarvae, same data as holotype, mounted on separate slides. Series of females in ethanol: K 714 and K 1151, same collection data as holotype, but collected 19 November 2013.

Figure 4. *Orbuspedum machinator* **gen**. et **sp. nov**., photograph of the anteriorly directed vulva and circulus.

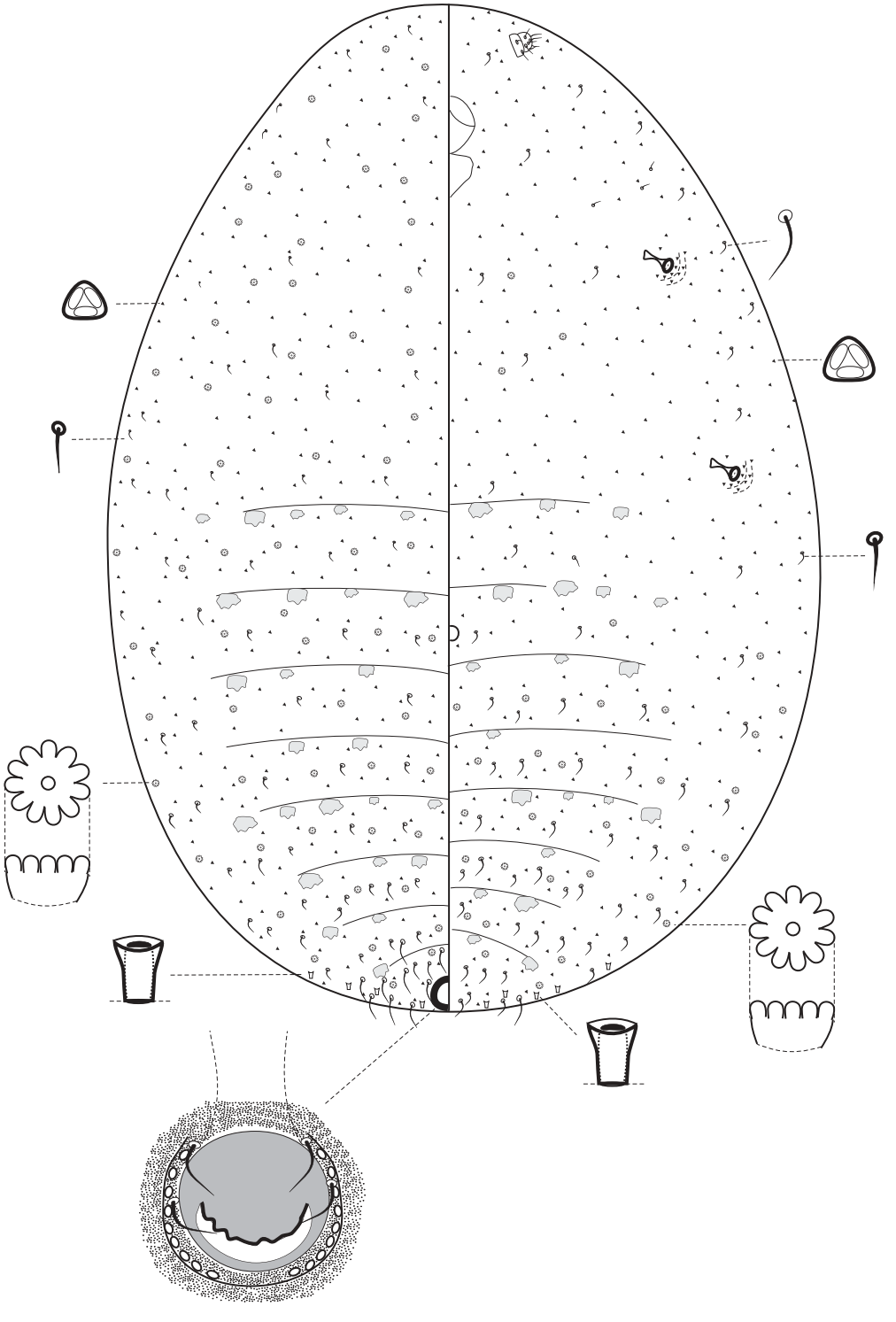

Figure 5. *Orbuspedum machinator* **gen**. et **sp. nov**., microscopic characters of the ultimolarva.

Description

Adult female. Body spindle-form, up to 5 mm long, with posterior segments of abdomen heavily sclerotized. Antennae very short, each 2-segmented, about 50 µm long. Legs entirely absent. Anal apparatus simplified, without setae; anal ring located inside of short anal tube; opening of anal tube at apex of abdomen. Vulva of unusual structure, funnel-shaped, opening directed anteriorly (Figure 4). Both pairs of ostioles absent. Small oval circulus, about 70 µm wide, present. Multilocular pores absent. Trilocular pores (each about 5 µm in diameter) and simple discoidal pores (each about 4 µm in diameter) scattered over entire body surface except posterior abdominal segments, where they are totally absent. Discoidal pores of irregular structure or duct-like pores absent. Tubular ducts, each about 7 µm long, with very deep collar, present on posterior abdominal segments only. Dorsal surface of body covered by small thin conical setae and minute flagellate setae; ventral surface covered by longer flagellate setae; posterior abdominal segments densely covered by significantly longer flagellate setae on both surfaces. Sacciform invaginations of sclerotized cuticle of different sizes present in transverse rows across most segments of body.

Female ultimolarva (probably third immature instar). Body broadly oval, enlarged posteriorly, about 1.5 mm long. Antennae each 2-segmented, very short, about 40 µm long. Legs absent. Anal apparatus located on body surface (without anal tube) at apex of abdomen; structure of anal apparatus rather unusual, with anal opening covered by sclerotized operculum; anal ring with one row of very small poorly visible pores and with four minute setae (see detail in Figure 5). Both pairs of ostioles absent. Small round circulus about 20 µm wide present. Multilocular pores, each about 8 µm in diameter, of asteroid form, with one central loculus and 11 peripheral loculi, scattered over entire dorsal surface of body and venter of abdominal segments; occasional pores also present in medial zone of thoracic sternites. Trilocular pores, each about 3 µm in diameter, sparsely scattered over entire body surface. Tubular ducts, each about 5 µm long, same structure as in adult female, few, present in marginal zone of two posterior abdominal segments. Minute flagellate setae sparsely scattered over entire body surface; longer setae present around anal opening. Sacciform invaginations of sclerotized cuticle of different sizes present in transverse rows on abdominal segments.

Males and larvae of other stages unknown.

Etymology

The species name is a masculine Latin word which means ‘constructor’. So, the binomen is translated as ‘legless constructor’, referring to the fungal domicile in which it is found.

Mode of life

The collected adult females and larvae inhabit individual conical domiciles on thin twigs of an undetermined species of bamboo (Poaceae). The domiciles are intensely black, each up to 7 mm wide and 8 mm high. In some cases the individual domiciles merge at their bases (Figure 6). Each domicile has a small orifice at the apex, which is used by the encapsulated insect for sprinkling the honeydew. The domicile consists of densely packed hyphae of sooty mould *Capnodium* sp. (Capnodiaceae) (Figure 7), which are mixed with small portions of wax produced by the mealybug.

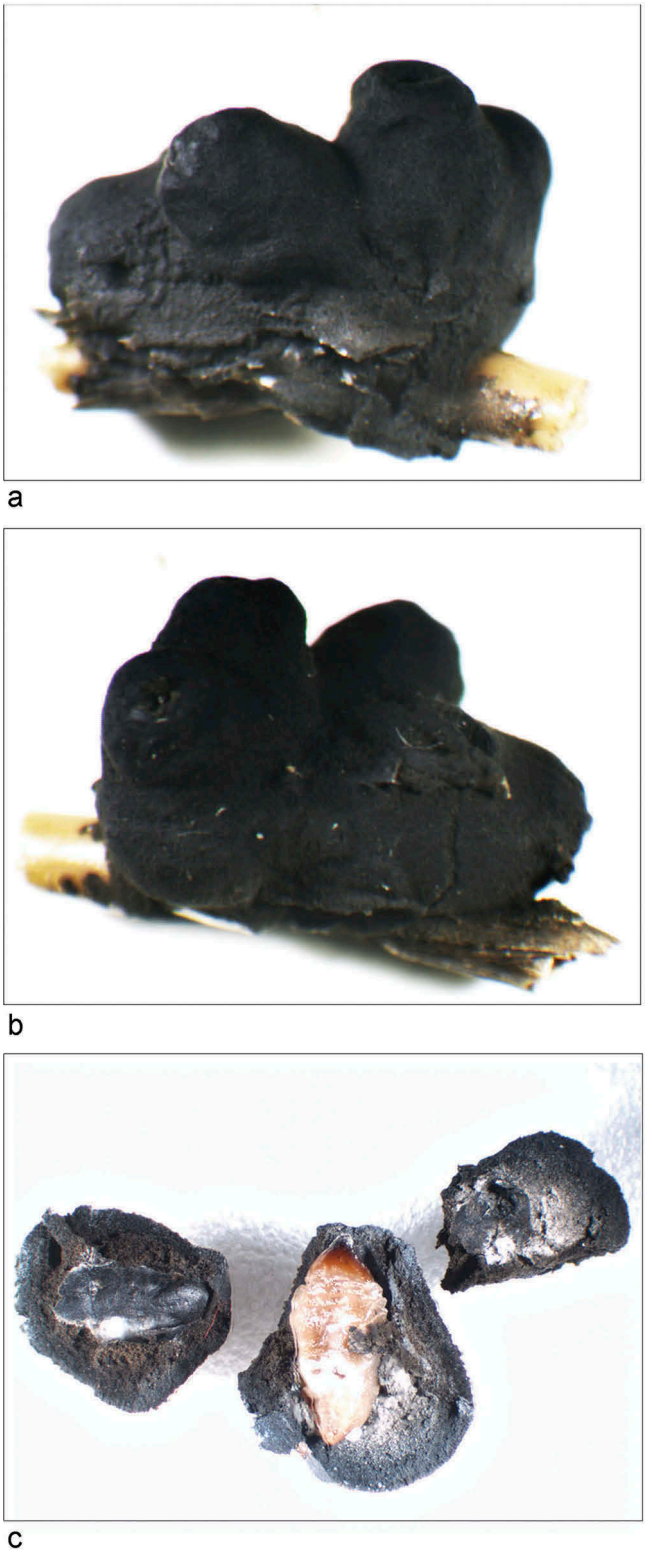

Figure 6. Fungal domiciles of *Orbuspedum machinator* **gen**. et **sp. nov**.; (a, b) several merged domiciles on a twig of bamboo; (c) a domicile divided into three parts.

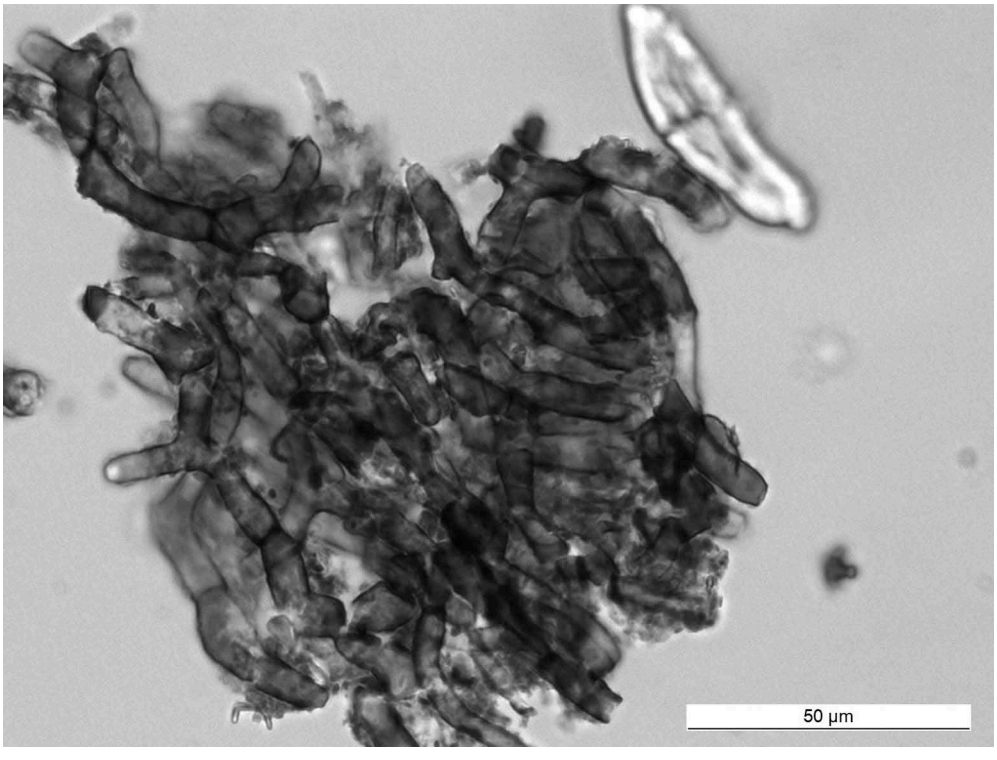

Figure 7. Hyphae of sooty mould under the microscope.

Other larval stages of the mealybugs, and males, have not been found. All the studied females contained ovarioles at an early stage of development. Spermatheca and sperm bundles inside the oviducts were not detected. From the vulva, an unpaired oviduct is anteriorly directed proximally, then bifurcates into two thicker paired oviducts distally.

Discussion

As in many other sap-sucking insects, mealybugs produce honeydew – a sugar-rich liquid that is the final product of digestion. Drops of honeydew may be consumed by ants of different species (more rarely by some other Hymenoptera) in the course of their well-known symbiosis with scale-insects, aphids and some other honeydew-producers, or may be simply sprinkled onto a surface of the host plant. Honeydew deposits on the surface of the plant are usually colonized by sooty moulds, an ecological group of fungi from the families Antennulariellaceae, Capnodiaceae, Chaetothyriaceae, Coccodiniaceae, Euantennariaceae, Metacapnodiaceae, Trichomeriaceae, Schifferulaceae and some occasional genera from other families (Chomnunti et al. 2014). Natural colonies of sooty moulds usually have a mixed taxonomic composition and include hyphae of different species, that significantly impedes identification. The sooty moulds growing on honeydew use its sugars, proteins, minerals and some other organic compounds (Auclair

1963). A comprehensive review of the general morphology, ecology and taxonomy of sooty moulds was published recently by Chomnunti et al. (2014). As well as this review, there are older published works on this subject, indicating that sooty moulds are usually saprobes causing damage to plants and host insects, covering the foliage and insect cuticle with black mycelia that prevent normal photosynthesis and respiration. However, until now no examples of mutualistic symbiosis between insects and sooty moulds have been reported, so far as the author knows. Moreover, the construction of the individual domicile using growing fungal hyphae seems to be unique in the animal kingdom. It is well known that many invertebrates can use fungal colonies (not sooty moulds) as shelters. Such examples are also known in some mealybugs and giant scales (Watson et al. 1960; Watson and Cox 1990; Williams 2004), which were sometimes collected on roots or trunks of host plants under the mycelium of 'higher fungi' Basidiomycota (*Boletus dimocarpicola* M. Zang et Sittigul, *Septobasidium pinicola* Snell and different *Diacanthodes* spp.), but in all these cases the animal and the fungi are able to live separately from each other, i.e. any obligate symbiosis and special co-adaptations are absent. In contrast, the newly discovered *Orbuspedum machinator* **sp. nov**. and its 'constructional' fungi are strongly co-adapted and closely depend on each other. The insect produces honeydew that feeds the fungi, and the fungi that grow around the insect provide it protection from predators, and buttress the soft apodal body of the mealybug. Most scale insects are sessile through almost all their lives and protect themselves by various passive methods: production of abundant wax around the body, dense sclerotization of the cuticle, inhabiting sheltered locations such as cracks in the bark, leaf sheaths, other small cavities in the plant's surface, etc. The body of *O. machinator* **sp. nov**. is free of wax, and soft apart from the sclerotized posterior abdominal segments, which are used to block the orifice at the apex of the fungal domicile. Meanwhile the surface of the domicile is solid and dense; its walls protect the insect.

The life cycle of scale insects includes three or four postembryonic instars in the female and four or five stages in the male (Danzig 1986). All studied legless mealybug species have four female instars: three larval instars and the adult female (Yang and Kosztarab 1967). Unfortunately only two instars of *O. machinator* **sp. nov**., the ultimolarva and the imago, were found in the studied population, so it is not known how many earlier instars (1 or 2?) are present in the female life cycle and how the construction of its peculiar domicile is started. In legless mealybugs legs are present in the primolarva only (the so-called crawlers), which travel to new feeding sites on the host-plant and settle to feed and produce honeydew; this is probably the first stage of the fungal domicile formation (Figure 8). While the body of the primolarva lies parallel to the surface of the host plant, it is likely that honeydew is accumulated just outside the anal opening and sooty moulds start intensively growing around this area, gradually elevating the posterior end of the insect's body into a perpendicular position. All the subsequent instars are already positioned perpendicular to the plant surface, so they uniformly sprinkle honeydew around their bodies, forming an approximately conical mass of fungal hyphae with the orifice at the apex. A somewhat similar situation is known in the mealybug *Glycycnyza turangicola* Danzig, which lives on the twigs of *Populus diversifolia* Schrenk (Salicaceae) in a desert climate in Central Asia (Uzbekistan). Larvae and females of this

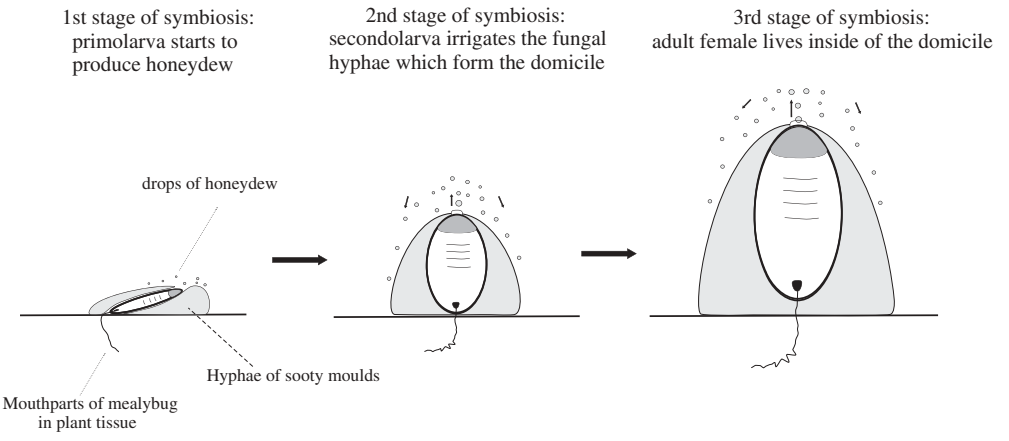

Figure 8. Hypothetical scheme of the formation of a fungal domicile by *Orbuspedum machinator* **gen**. et **sp. nov.**

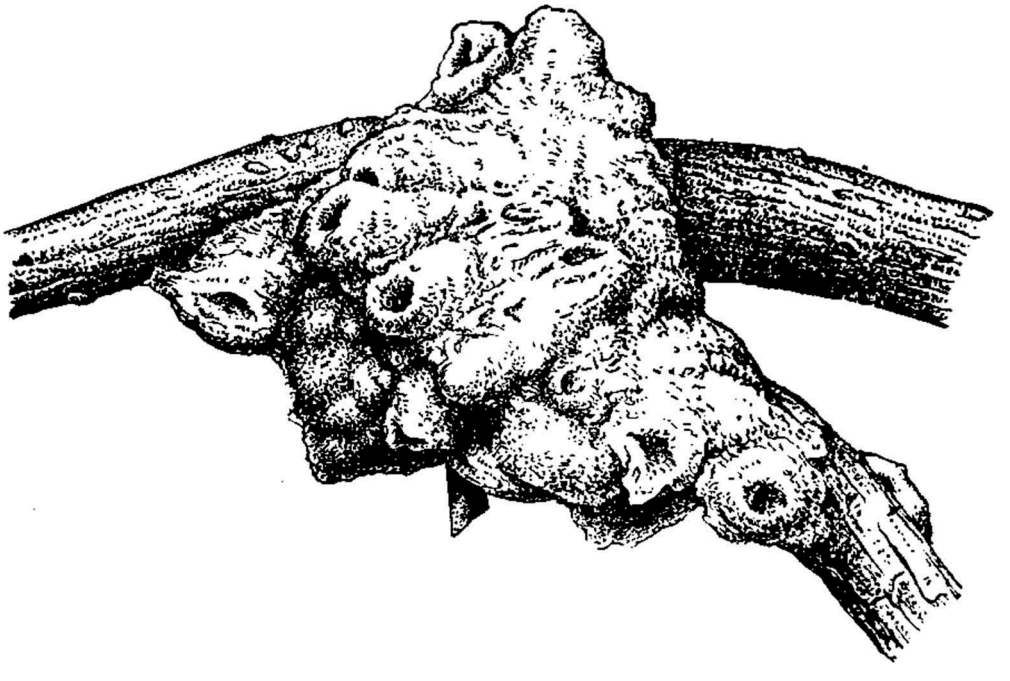

Figure 9. Colony of *Glycycnyza turangicola* Danzig, 1974 on a twig of *Populus diversifolia* (after Danzig 1974).

species produce honeydew which rapidly dries to form solid conical shelters that become merged in colonies (Figure 9) (Danzig 1974). Similar colonies are also known in some other scale insects from other families, but in all those cases there are no known associations with fungi.

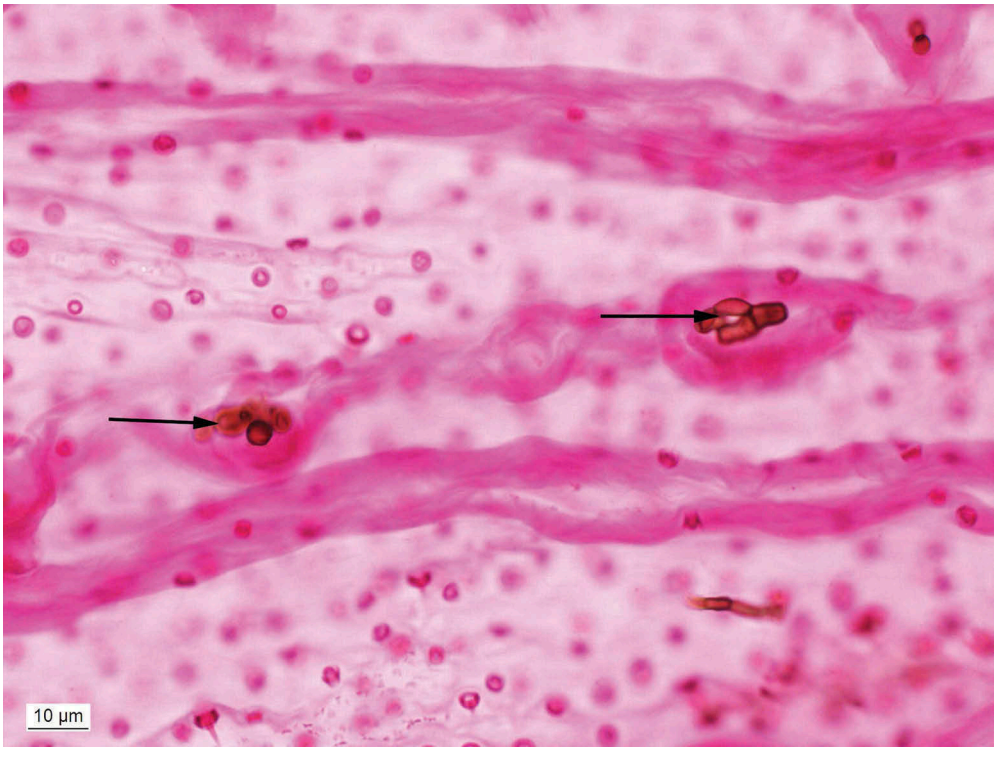

Figure 10. Pieces of fungal hyphae inside of the cuticular sacciform invaginations in *Orbuspedum machinator* **gen**. et **sp. nov.**

It is unclear if the symbiosis between *O. machinator* **sp. nov**. and *Capnodium* sp. is initiated anew between each crawler and fungi that are constantly present on bamboo twigs, or if the crawler acquires small pieces or spores of the mother's fungal domicile and transports these to a new place. The last hypothesis seems to be highly possible, since some of the studied females and ultimolarvae had pieces of the fungal hyphae inside their cuticular sacciform invaginations (Figure 10).

Acknowledgements

The author thanks Dr Dmitry A. Gapon for consultations on Latin grammar and Dr Eugenia Labina for linguistic corrections. I am also very grateful to Dr Putarak Chomnunti from Mae Fah Luang University (Thailand) for the identification of the fungus. The work was performed in the context of the state research project no. 01201351189 in the Zoological Institute of the Russian Academy of Sciences. The expedition expenses were covered by grants of the fund of the President of the Russian Federation: MK-6075.2010.4 and MK-300.2013.4 and by a personal travel grant from the Percy Sladen Memorial Fund (UK).

Disclosure statement

No potential conflict of interest was reported by the author.

Funding

The expeditional work was supported by the fund of the President of the Russian Federation: MK-6075.2010.4, MK-300.2013.4 and by Percy Sladen Memorial Fund (UK).

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