Article

Trophobiosis between a new species of *Williamsrhizoecus* (Hemiptera: Coccomorpha: Rhizoecidae) and *Acropyga silvestrii* (Hymenoptera: Formicidae) in Tanzania

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Abstract

A new myrmecophilous species of root mealybug, *Williamsrhizoecus udzungwensis* **sp. n.**, is described from individuals found living within a nest of *Acropyga silvestrii* in the Udzungwa Mountains of Tanzania. *Acropyga* ants are highly specialized, obligate associates of scale insects, typically members of the scale family Xenococcidae. *Acropyga* are best known for vertically transmitting trophobiotic partners during their nuptial flights and for housing them within brood chambers. This article presents the first record of trophobiosis between a species of *Williamsrhizoecus* and *Acropyga*, and only the second record of an association between *Acropyga* and rhizoecids in the Old World. This discovery contributes important information about the few species of Rhizoecidae confirmed to engage in these unique symbioses, each putatively the result of a past horizontal transmission event from a xenococcid to a rhizoecid lineage. Included is a discussion on the diagnosis of *Williamsrhizoecus* and an updated key to the species.

Key words: ant, Coccoidea, mutualism, root mealybug, scale insect, trophophoresy

Introduction

Ants from the genus *Acropyga* Roger (Hymenoptera: Formicidae) engage in an obligatory form of mutualism with a small number of scale insect species (Hemiptera: Coccomorpha). Relationships between *Acropyga* and scale insects are notable for their high degree of specialization (LaPolla 2004; Schneider & LaPolla 2011) and long-sustained history, extending back at least 15–20 Ma according to the fossil record (Johnson *et al*. 2001; LaPolla 2005) and potentially as far back as 30 Ma according to divergence dating estimates (Blaimer *et al*. 2016). One striking attribute of these relationships is that *Acropyga* species transmit associated scale insect lineages vertically over generations, a behavior unique to this genus termed trophophoresy (LaPolla *et al*. 2002). Trophophoresy occurs when an alate *Acropyga* queen carries a gravid scale partner between her mandibles during her nuptial flight, with the female scale evidently serving as a "seed individual producing the next generation of nest associates. Observations of this behavior are rare, but have been documented for a phylogenetically diverse set of *Acropyga* species (Blaimer *et al*. 2016; LaPolla 2004; Schneider & LaPolla 2011). The behavior of housing and herding scale insect associates within nest chambers is also worth noting. *Acropyga* protect their trophobionts within brood chambers in the soil, alongside their own eggs and larvae. The ant workers are pastoral; they herd scales between brood chambers and feeding sites, collecting honeydew from them as a food source. Similar nesting behaviors occur in some other obligate ant associations (Delabie 2001; Malsch *et al*. 2001; Schneider *et al*. 2013; Schneider & LaPolla 2011) but are otherwise uncommon or represent examples of social parasitism (Akino *et al*. 1999; Elmes *et al*. 1999).

The primary associates of *Acropyga* are root mealybugs belonging to the family Xenococcidae Tang (Schneider & LaPolla 2011; Williams 1998, 2004). Xenococcids are putatively the oldest group to associate with *Acropyga*

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(Blaimer *et al.* 2016). The two insect groups are co-distributed throughout the tropics and their partnership is mutually obligatory. But *Acropyga* do not associate with xenococcids exclusively; there are rare confirmed examples of *Acropyga* associating with a few species of rhizoecid root mealybugs (Rhizoecidae) (LaPolla *et al.* 2002; Smith *et al.* 2007; Tanaka 2016 and unpublished data) and one species of ensign scale (Ortheziidae) (LaPolla *et al.* 2008). There are additional speculative reports in the literature of *Acropyga* associations with an ensign scale (Ortheziidae), a putoid (Putoidae), several mealybugs (Pseudococcidae), and additional rhizoecids in the Neotropics (Caballero *et al.* 2019; Johnson *et al.* 2001; Williams 1998), but such reports must be viewed with skepticism until a direct ant/scale association is confirmed through observational study. These scale species are common soil inhabitants and may be "neighbors" of *Acropyga* colonies rather than direct associates, or they may be commensal species receiving an indirect benefit from ant defense. On multiple occasions, we have collected free-living scale insect species while excavating colonies of *Acropyga* from the soil (unpublished data). Free-living scales are largely ignored by workers—distinguishing them from true trophobionts, which are gathered, protected, and actively attended by ant workers after the nest is disturbed. The best evidence of direct association is observation of trophophoresy.

Reporting on new associations with *Acropyga* ants must be treated with the utmost care because erroneous records only serve to muddle studies of their trophobiotic relationships. Each relationship with a species from outside of the primary group (i.e. Xenococcidae) is presumably the result of a disruption in the vertical transmission pathway established through trophophoresy, introducing a new horizontally acquired associate lineage. These examples are of special interest because they are instructive as to the evolutionary constraints and frequency of host switching in this system (Schneider & LaPolla 2011); thus, records of associations must be carefully vetted.

Here we report on a new species of root mealybug, *Williamsrhizoecus udzungwensis* **sp. n.** (Hemiptera: Rhizoecidae), the first scale species known to associate with *Acropyga silvestrii* Emery, and the first association recorded between species of *Williamsrhizoecus* and *Acropyga*. We provide updates on the diagnosis of genus *Williamsrhizoecus* and provide a key to the species, based on adult female morphology.

Methods

A single colony of *A. silvestrii* was discovered by JSL while collecting in the Udzungwa Mountains of Tanzania; the colony was found nesting in soil under rocks alongside a stream in rainforest habitat at the foot of the mountain range. Nest tunnels were located within the top 10-15 cm of soil under rocks. Root mealybugs were found residing within the colony's nest chambers. To confirm direct association between *A. silvestrii* and *W. udzungwensis* **sp. n.**, live individuals from both species were kept temporarily in a nest-box for observation. The nest-box was constructed from a Nucons round two-piece clear plastic container (LA Container, Yorba Linda, California, U.S.A.), modified by adding a small hole covered with fine wire mesh affixed to the side for air exchange, and dental cement added to the basin to manage moisture. Following collection, ants and scales were allowed to rest and acclimate for several hours prior to behavioral observations. Simple observations were made in the field without the aid of visual or recording devices.

Following observations, specimens were preserved in 100% ethanol and stored at -20C before being slide-mounted. Root mealybugs were slide-mounted following the protocol of the Systematic Entomology Laboratory (http://www.ars.usda.gov/Main/docs.htm?docid=9832). One specimen (S0430A) was prepared following the same joint DNA-morphology preparation protocol described for armored scale insect specimens in Normark *et al.* (2019). Voucher specimens and the DNA extraction are deposited in the United States National Museum (USNM) scale insect collection, housed at the USDA Agricultural Research Service, Beltsville, Maryland, U.S.A. Morphological terminology follows that used in Kozár and Konczné Benedicty (2007). Measurements were made on a Zeiss Axio Imager.M2 microscope (Carl Zeiss Microscopy, LLC, White Plains, New York, U.S.A.) with the aid of an AxioCam digital camera and AxioVision software. Cleared, slide-mounted specimens were examined using phase contrast and differential interference contrast microscopy.

The holotype data is given exactly as it is on the slide label, with / used to indicate the end of each line of writing on the label.

Taxonomy

***Williamsrhizoecus* Kozár & Konczné Benedicty, 2007: 354.**
(Type species: *Williamsrhizoecus baskyi*, by monotypy.)

Checklist of species placed in *Williamsrhizoecus*

Williamsrhizoecus baskyi Kozár & Konczné Benedicty, 2007: 355.
Williamsrhizoecus coffeae Caballero & Ramos, 2018: 3.
Williamsrhizoecus epicopus (Williams, 1970): 155.
Williamsrhizoecus udzungwensis **sp. n.**

Generic diagnosis. Body elongate oval. Antennae each with 5 or 6 segments; with 1 blunt sensory seta situated on penultimate antennal segment. Legs well developed. Dorsum and venter with trilocular pores. Multilocular disc pores present or absent. Oral collar tubular ducts absent. Tritubular pores present on dorsum and venter. With flagellate and clavate or falcate setae present on body surface, anal ring, legs, and antennae in varying combinations. Anal ring with oval to elongate pores, some with spicules, and 6–18 flagellate or clavate setae. Dorsal ostioles entirely absent, or only weakly developed if present. Circuli present or absent.

   Comments. Following Kozár & Konczné Benedicty (2007), this genus belongs to the subtribe Rhizoecina based on the presence of tritubular pores; the presence of clavate body setae distinguishes this genus from the others in the subtribe. Note, however, that the study of adult male morphology by Hodgson (2012) found little support for the subdivisions of Rhizoecini (now Rhizoecidae) proposed by Kozár & Konczné Benedicty. This casts some doubt on their decision to separate Ripersiellina from Rhizoecina, which was based on adult females having either bitubular or tritubular pores, respectively. The possession of blunt sensory setae on the penultimate antennal segment is not diagnostic for this genus, as originally described (Kozár & Konczné Benedicty 2007), as this feature is common throughout the family.

   The description of *Williamsrhizoecus* is here updated from the original account (Kozár & Konczné Benedicty 2007) to accommodate recent additions of species, including *W. coffeae* and the new species described here. The genus now includes species with 5 or 6-segmented antennae (originally 5-segmented only), with or without circuli (originally described as present), with or without multilocular disc pores (originally described as absent), with anal ring cells oval to elongate (originally described as elongate), and with 6–18 anal ring setae (originally described as having 6 setae). Caballero & Ramos-Portilla (2018) implied several of these changes when they described *W. coffeae*, but they did not explicitly revise the genus.

   The absence of dorsal ostioles is a rare, notable trait shared by all but one of the species comprising this genus. However, synapomorphies based on the absence of features are more equivocal than those based on presence. Kozár & Konczné Benedicty apparently regarded the presence of clavate setae on the anal ring as more critical to diagnosis, since they chose to recombine *Neorhizoecus epicopus* Williams into *Williamsrhizoecus* based on this trait, despite it being the only member of the genus to possess (weakly developed) dorsal ostioles.

   Williamsrhizoecus is evidently Gondwanan in origin, drawing on the known geographical distribution of the few species that comprise it, which includes Antigua and Barbuda, Colombia, Mexico, Tanzania, and Trinidad and Tobago. The rather disjunct distribution of species may reflect that root mealybugs are generally under-sampled, particularly in the Afrotropical region where only 33 out of 216 total species have been recorded (García Morales *et al.* 2016; last accessed 13.viii.2020). It could also indicate artificiality of the genus, but this question would be best resolved with molecular evidence and morphological data from additional life stages that are unavailable at present.

***Williamsrhizoecus udzungwensis* Schneider & LaPolla sp. n.**
Fig. 1

Material examined. Holotype adult female: TANZANIA: Udzungwa Mountains, attended by / colony of *Acropyga silvestrii* within nest / chambers, under rocks alongside a stream, / -7.8449, 36.8835, 350m; 27 March 2011; / J.S. LaPolla coll. (JSL110327-04A) (USNM).

Paratypes: TANZANIA: 1 adult female, same data as holotype (JSL110327-02A) (USNM); 1 adult female, same data as holotype (JSL110327-02B) (USNM); 1 adult female, same data as holotype (JSL110327-03A) (USNM); 1 adult female, same data as holotype (JSL110327-03B) (USNM) (voucher ID: S0430A); 2 immature instars together on 1 slide, same data as holotype (JSL110327-03C) (USNM); and 1 adult female, same data as holotype (JSL110327-04B) (USNM).

Description of adult female (N = 6). Mounted on microscope slide, body approximately oval and membranous throughout, 0.88–0.95 mm long, 0.64–0.73 mm wide, widest near abdominal segment II. Abdomen gently tapering toward posterior end; abdominal segment VIII approximately 250 μm wide at base. Anal lobes poorly developed, indicated by slight protrusions of the body margin, each lobe with a cluster of several dorsal clavate setae, each seta 10–18 μm long. Anal ring dorsal, located slightly anterior of body apex, with two concentric rows of ovoid cells, some lateral cells each bearing a spicule. Anal ring setae arranged in 9 pairs, numbering 18 in total, each seta approximately 19–24 μm long. Antennae 6-segmented, situated close to each other on ventral submargin of head. Stout flagellate setae present on each antennal segment, 12–21 μm long; with 1 falcate sensory seta present on segment V and 3 falcate sensory setae present on segment VI. One sensory pore and two seta-like sensillae present on antennal segment II. Average antennal segment lengths in μm: I – 32, II – 19, III – 16, IV – 12, V – 13, VI – 38; overall length 130 μm. Eyes absent. Dorsal ostioles absent.

Labium 3-segmented, 76 μm long and 48 μm wide, with few short flagellate setae present on each segment. Cephalic plate absent. Legs well developed; hind leg average length measurements in μm: trochanter + femur 108; tibia + tarsus 129. Ratio of lengths of metatrochanter + femur to tibia + tarsus, 0.84; ratio of lengths of metatibia to tarsus, 1.39. Hind tarsus widest at base and tapering to claw, about 24 μm long. Claws with simple digitules, shorter than the claw, and without denticles. Several flagellate setae present on each leg segment, about 15 setae present on each hind tibia. Spiracles normal for the family, each approximately 23 μm in diameter at widest point. Circulus absent.

All body setae on dorsum and venter clavate to falcate, 8.5–10 μm long, either linear or curved, widest at tip or widest just proximal to tip and tapering distally. Setae arranged in irregular rows separated by bald intersegmental regions, otherwise fairly evenly distributed throughout. Trilocular pores numerous across dorsum and venter, interspersed with setae. Tritubular pores also numerous on both dorsum and venter, though less common than trilocular pores; arranged in singular transverse rows toward the midline or posterior edge of each segment, those on dorsum of abdomen mostly located medially to submarginally, those on venter of abdomen mostly located marginally to submarginally, except for segment VII, where they extend from midline to margin. Multilocular disc pores absent.

Comments. *Williamsrhizoecus udzungwensis* **sp. n.** is similar to *W. coffeae* in that they both possess 6 antennal segments, lack ventral abdominal circuli, and have clavate body setae. However, in *W. udzungwensis* **sp. n.** (Fig. 1) the body setae are short and thickened throughout the length of the seta, sometimes curved and appearing falcate (sickle-shaped), whereas in *W. coffeae* the body setae have a longer flagellate stem that terminates in a dilated tip. The same trait distinguishes *W. udzungwensis* **sp. n.** from *W. baskyi*, which is also from Tanzania. Additionally, whereas *W. udzungwensis* **sp. n.** lacks circuli and has six antennal segments, *W. baskyi* has two circuli and only five antennal segments.

There are similarities between *W. udzungwensis* **sp. n.** and the only other rhizoecid confirmed to associate with *Acropyga* in the Old World (Tanaka 2016), *Ishigakicoccus shimadai* Tanaka. Besides the obvious ecological connection, both species lack dorsal ostioles and both have body setae that could be described as clavate. The body setae of *I. shimadai* are mostly flagellate and hooked at the apex, but rarely they are knobbed at the apex (Tanaka 2016), suggesting a potential affinity to *Williamsrhizoecus*. Tanaka considered *Ishigakicoccus* as being similar to *Capitisetella* and *Pseudorhizoecus* because they all lack dorsal ostioles, but did not consider *Williamsrhizoecus* in his discussion, which differs from the other genera by having tritubular pores. Tanaka used the presence of two types of wax pores in *Ishigakicoccus*, small pores with 6 loculi surrounding a central chamber and large 3–5 locular pores without a central chamber, as justification for establishing a new genus (Tanaka 2016). These structures are distinct from the multilocular disc pores found in *Williamsrhizoecus*, which are present only in *W. coffeae*.

Acropyga workers were observed actively carrying individuals of *W. udzungwensis* **sp. n.** around within the nest-box and gathering them together into small chambers that the workers had excavated from loose soil. The behavior of arranging root mealybugs into protected clusters is a critical observation of direct association. No mating swarms of *A. silvestrii* were observed, so trophophoresy of *W. udzungwensis* **sp. n.** (transportation of gravid females by *A. silvestrii* queens) cannot be confirmed at present.
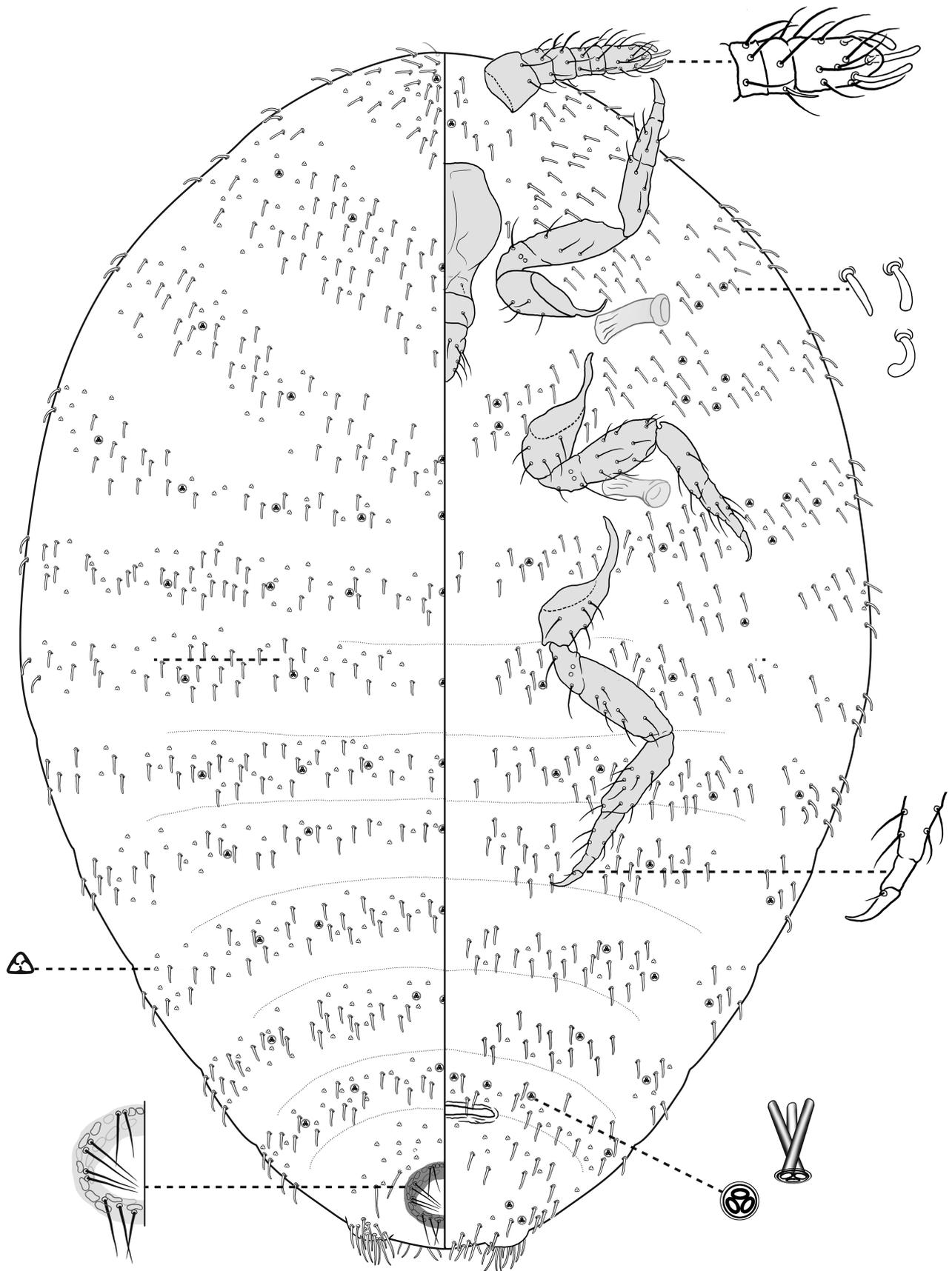
FIGURE 1. *Williamsrhizoecus udzungwensis* **sp. n.** Adult female, full body view, illustrated from the holotype and paratypes. Illustration by Taina Litwak (USDA, ARS, Systematic Entomology Laboratory) and SAS.

Prior to this study, the only known root mealybug association with an African *Acropyga* species was between *A. arnoldi* Santschi and the xenococcid, *Eumyrmococcus scorpioides* (De Lotto) (Prins 1982; LaPolla & Spearman 2007). *Eumyrmococcus williamsi* Kozár & Konczné Benedicty also occurs in Tanzania, in the Uluguru Mountains, but the *Acropyga* species associated with this xenococcid was not recorded at the time of its collection (Kozár & Konczné Benedicty 2007). *Eumyrmococcus williamsi* was later found to associate with a new species of *Acropyga* (JSL manuscript in preparation). Interestingly, the neighboring Udzungwa and Uluguru Mountain ranges each harbor a distinct *Acropyga* species partnered with a root mealybug.

Ecological associates. *Acropyga silvestrii* Emery (Hymenoptera: Formicidae); *W. udzungwensis* **sp. n.** was feeding on roots, host plant not recorded.

Etymology. The specific epithet is an adjective formed from Udzungwa, the mountain range where it was discovered, together with the Latin suffix *-ensis*, meaning of or from a place.

Key to species of *Williamsrhizoecus* based on the adult female
Based on keys in Kozár and Konczné Benedicty (2007) and Caballero & Ramos-Portilla (2018).

1.   Circulus present; antenna 5 segmented 2
-     Circulus absent; antenna 6 segmented 3
2.   Body setae clavate; anal ring setae flagellate; with 2 circuli . . . .***baskyi***
-     Body setae flagellate; anal ring setae clavate; with 1 circulus . . . .***epicopus***
3.   Multilocular disc pores present; body setae clavate, elongate, dilated only at tip; anal ring setae numbering 6–17 . . . . . **coffeae**
-     Multilocular disc pores absent; body setae clavate to falcate, short, stout throughout length; anal ring setae numbering 18 . . .
. . . .***udzungwensis*** **sp. n.**

Discussion

The classification of root mealybugs has changed rapidly in recent years, as thoroughly reviewed by Hodgson (2020), and some explanation is warranted to avoid confusion regarding the distinction between xenococcid and rhizoecid root (or hypogaeic) mealybugs. Within the past two decades the root mealybugs have been recognized as tribes within the broadly defined family of mealybugs (Pseudococcidae: Rhizoecini and Xenococcini; Hardy *et al.* 2008) or a single subfamily of mealybugs (Pseudococcidae: Rhizoecinae; Downie & Gullan 2004); subsequently, root mealybugs were recognized as a distinct family comprising two subfamilies (Rhizoecidae: Rhizoecinae and Xenococcinae; Hodgson 2012), and they are now regarded as two separate families (Rhizoecidae and Xenococcidae; Danzig & Gavrilov-Zimin 2014; Hodgson 2020). The available molecular evidence indicates xenococcids are the sister group of rhizoecids (Downie & Gullan 2004; Hardy *et al.* 2008). Further discussions on their affinities and classification can be found in Hodgson (2012) and Gavrilov-Zimin (2018). The most important ecological distinction between the two families is that the xenococcids are all obligate associates of *Acropyga* ants and have been so for tens of millions of years (Blaimer *et al.* 2016; Johnson *et al.* 2001); whereas only a few rhizoecids associate with *Acropyga* and the ages and specificities of these relationships are unknown. Most of the 216 described rhizoecid species are free-living (Kozár & Konczné Benedicty 2007) or only facultatively associated with ants.

Concerning the trophobionts of *Acropyga* that fall outside of Xenococcidae, association has been unequivocally confirmed for only four described species: *Acropygorthezia williamsi* LaPolla & Miller, from the family Ortheziidae (LaPolla *et al.* 2008); and *Ripersiella colombiensis* (Hambleton) (Smith *et al.* 2007), *Ishigakicoccus shimadai* (Tanaka 2016), and *Williamsrhizoecus udzungwensis* **sp. n.** from the family Rhizoecidae. For each of these records, direct association was confirmed either through carefully documented observation or collection of *Acropyga* mating swarms. We have collected at least eight additional undescribed species of rhizoecid confirmed to associate with *Acropyga* species in the Neotropics (unpublished data). Additional speculative reports from the literature include (by family): *Insignorthezia insignis* (Browne) (Ortheziidae); *Dysmicoccus brevipes* (Cockerell), *D. caribensis* Granara de Willink, *D. radicis* (Green), *D. texensis* (Tinsley), *Phenacoccus sisalanus* Granara de Willink, *Pseudococcus elisae* Borchsenius, *P. jackbeardsleyi* Gimpel & Miller, *P. landoi* (Balachowsky) (Pseudococcidae); *Puto barberi* (Cockerell) (Putoidae); *Capitisetella migrans* (Green), *Geococcus coffeae* Green, *Pseudorhizoecus proximus* Green, *Ripersiella andensis* (Hambleton), *Rhizoecus americanus* (Hambleton), *R. arabicus* Hambleton, *R. caladii* Green, *R. coffeae* Laing, *R. colombiensis* Ramos & Caballero, *R. mayanus* (Hambleton), *R. moruliferus* Green, and

R. spinipes (Hambleton) (Rhizoecidae) (Bünzli 1935; Caballero *et al*. 2019; Delabie *et al*. 1991; Johnson *et al*. 2001; Williams 1998). The details on how these associations were verified varies by publication. Bünzli (1935) noted observing trophophoresy involving one or more of the rhizoecid species he listed as associating with *Rhizomyrma* (= *Acropyga*), but did not specify which species were carried by the ant queens. Delabie *et al*. (1991) made careful observations but identified associated scales to genus level only, specifically *Geococcus* and *Rhizoecus*. *Geococcus coffeae* Green has been repeatedly reported as an associate of *Acropyga* (see Johnson *et al*. 2001) and Williams (1998) suggested it is readily attended by *Acropyga paramaribensis* Borgmeier. According to Johnson *et al*. (2001), *Pseudorhizoecus proximus* has only been found near or within *Acropyga* colonies, supporting reports that it is a direct associate, but not ruling out the possibility it is a commensal.

Treating these records as speculative associations reflects that the literature lacks details on how associations between ants and scales were observed and confirmed, and is not intended to disparage the work of any authors or collectors. From experience, the collection of *Acropyga* colonies and observation of species interactions requires great care and investment of time to be certain about direct association between ants and scales, often requiring hours of effort for a single colony. It is understandable that collectors who are not explicitly interested in studying the trophobiotic associations of *Acropyga* would be unlikely to invest such time and care in collecting. Detailed descriptions of the methods of collection and observations help in confirming direct mutualistic association between species, and should be published whenever possible. We highly recommend the use of nest-boxes for a period of observation to draw unequivocal links between directly associated species in the literature.

Acknowledgements

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