



Trophobiosis between a new species of Acropyga (Hymenoptera, Formicidae) and new Neochavesia (Hemiptera, Xenococcidae) from Peru, and establishment of the Acropyga smithii species-group

John S. LaPolla¹, Scott A. Schneider²

I Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD, 21252, USA 2 USDA, Agricultural Research Service, Henry A. Wallace Beltsville Agricultural Research Center, Systematic Entomology Laboratory, 10300 Baltimore Avenue, Beltsville, MD, 20705, USA

Corresponding author: John S. LaPolla (jlapolla@towson.edu)

Academic editor: S. Salata | Received 14 November 2022 | Accepted 10 February 2023 | Published 17 March 2023

https://zoobank.org/D949D6DA-8CEC-4B0D-AFB2-8CE96097039B

Citation: LaPolla JS, Schneider SA (2023) Trophobiosis between a new species of *Acropyga* (Hymenoptera, Formicidae) and new *Neochavesia* (Hemiptera, Xenococcidae) from Peru, and establishment of the *Acropyga smithii* species-group. ZooKeys 1154: 1–16. https://doi.org/10.3897/zookeys.1154.97578

Abstract

We describe a new pair of trophobiotic partners from the ant genus *Acropyga* and the root mealybug genus *Neochavesia*. A recent field study on *Acropyga* ants and associated root mealybugs, conducted in the Peruvian Amazon, led to the discovery of *Acropyga manuense* LaPolla & Schneider, **sp. nov.** and its root mealybug symbiont *Neochavesia podexuta* Schneider & LaPolla, **sp. nov.** The new root mealybug belongs to the family Xenococcidae, whose members are all obligate associates of *Acropyga* ants. Providing joint descriptions of new mutualist partners in the same article is a novel approach for this system, and it offers benefits to the ongoing study of mutualism and patterns of association among these symbiotic ants and scales. Here, we also begin to revise the species-group composition of *Acropyga* by establishing the *smithii* species-group, and we provide updated information to aid in identifying the new ant species and root mealybug species.

Keywords

Ants, Coccoidea, Coccomorpha, mutualism, root mealybug, taxonomy, symbiosis

Introduction

Efforts to understand the obligate symbiotic relationship between *Acropyga* Roger ants (Hymenoptera: Formicidae) and root mealybugs (Hemiptera: Rhizoecidae and Xenococcidae) hinge on an understanding of exactly which species associate with each other. Questions about fidelity and specificity among partnerships cannot be answered without detailed examination of species associations. However, given the cryptic nature of these mutualists (i.e., they are hypogeic, small, and difficult to identify), definitive associations are often difficult to confirm. Additionally, studies of each respective group are typically segregated by taxonomic discipline, and therefore, information about mutualistic partnerships can be disconnected both physically and temporally in the published record.

Schneider et al. (2022) recently detailed a protocol to confirm direct association between root mealybugs and Acropyga ants and reported on several new associations between partnered pairs from the Peruvian Amazon. As part of that field study, we discovered a new species of Acropyga associating with a new xenococcid root mealybug. All species in the family Xenococcidae, which is comprised of the Old World genera Eumyrmococcus Silvestri, 1926 and Xenococcus Silvestri, 1924 and the New World genus Neochavesia Williams & Granara de Willink, 1992, are obligatorily associated with Acropyga. Past experience has shown that when a new species of Acropyga is discovered, it is frequently associated with a new xenococcid as well. This is illustrated by the following (non-exhaustive) list of examples. In the Neotropics, LaPolla (2004) described two new species of Acropyga (A. ayanganna LaPolla and A. stenotes LaPolla) that were each associated with a new species of Neochavesia (N. lapollai Williams and N. linealuma Schneider & LaPolla, respectively). In the Old World, Acropyga kinomurai Terayama & Hashimoto was discovered associating with a new root mealybug species, Xenococcus kinomurai Williams & Terayama (Williams and Terayama 2000); A. nipponensis Terayama with Eumyrmococcus nipponensis Terayama (Terayama 1986); A. pallida (Donisthorpe) with E. adornocapillus (Schneider & LaPolla, 2011); and A. paleartica Menozzi with E. corinthiacus Williams (Williams 1993).

In the above examples, each ant and root mealybug species were described separately from their mutualist partner. Historically, descriptions of new trophobiotic root mealybugs tend to lag behind the descriptions of their mutualist ants, sometimes by many decades. Thus, researchers have had to spend significant effort compiling and reconciling information about species associations scattered across the literature (see Williams 1998; Johnson et al. 2001; LaPolla 2004; Schneider and LaPolla 2011). By combining the methodology outlined in Schneider et al. (2022) with descriptive taxonomy, we aim to present a clearer picture of trophobiotic associations between *Acropyga* and root mealybugs going forward and to keep critical ecological data together in the published record when possible. Here, we describe the trophobiotic association between a new species of *Acropyga* with a new species of *Neochavesia* from Peru.

Materials and methods

Root mealybug specimens

Specimens were preserved in 95–100% ethanol and stored at –80C prior to preparation and subsequently mounted on glass slides in Canada balsam. Morphological terminology for *Neochavesia* conforms to usage from Williams (2004) and Schneider and LaPolla (2011). Measurements were made on a Zeiss Axio Imager.M2 (Carl Zeiss Microscopy, LLC, White Plains, NY, USA) microscope with the aid of an AxioCam and AxioVision software. Slide-mounted specimens were examined under phase contrast and differential interference contrast (**DIC**) microscopy.

Ant specimens

Specimens were preserved in 95–100% ethanol and stored at –80C prior to preparation. Specimens were later point mounted for morphological examination. Ants were measured using a KM33-R micrometer on a Leica MZ16 dissecting microscope to the nearest 0.001 mm. Images were taken with a 10× lens attachment using a Canon EOS 6D Mark II camera with a MP-E 65mm manual focus macro lens on a Macropod Pro 3D and Micro Kit System (Macroscopic Solutions, East Hartford, CT, USA). Images were focus stacked using Zerene Stacker ver. 1.04 software. Morphological terminology used for *Acropyga* description conforms to usage from LaPolla (2004) and LaPolla et al. (2017).

Ant measurements and indices are defined as:

- **EL** (Eye Length): In full-face view, maximum anteroposterior length of the compound eye.
- PFL (Profemur Length): Length of profemur in lateral view.
- **HL** (Head Length): In full-face view, length from a line drawn across the posterior margin of the head to a line drawn across the anterior margin of the clypeus.
- **HW** (Head Width): In full-face view, maximum width of the head between the lateral margins, excluding the compound eyes.
- **ML** (Mesosoma Length): In lateral view, maximum length from the point at which the pronotum meets the cervical shield to the posterior basal angle of the metapleuron.
- PW (Pronotum Width): In dorsal view, maximum width of the pronotum.
- **SL** (Scape Length): In a view perpendicular to the long axis of the scape, maximum length of the scape, excluding the condyle.
- CI (Cephalic Index): (HW/HL) × 100.
- **REL** (Relative Eye Length Index): (EL/HL) \times 100.
- **SI** (Scape Index): (SL/HW) \times 100.

Specimen depositories

Type depositories are abbreviated as follows:

UNMSM Museo de Historia Natural, Universidad Nacional Mayor de San Marcos,

Lima, Peru.

USNM Smithsonian National Museum of Natural History, Coccomorpha collec-

tion at USDA Agricultural Research Service, Beltsville, Maryland, USA.

USNM Smithsonian National Museum of Natural History, Washington, D.C.,

USA. (ant specimens)

Results

Establishment of the Acropyga smithii species-group

Diagnosis of Acropyga smithii species-group

New World species; workers with 8 antennal segments and 4 mandibular teeth (one exception occasionally seen in A. oreithauma where a much smaller tooth is found between the 3^{rd} and basal teeth); males with penial sclerites elongated with distal tips that bend towards gonopods; anterior portion of the ventral margin of the penial sclerites dentate.

Included species:

A. fuhrmanni (Forel, 1914)

A. manuense LaPolla & Schneider, sp. nov.

A. oreithauma LaPolla, Williams & Fan, 2017

A. smithii Forel, 1893

Diagnosis and remarks

LaPolla (2004) created nine informal species-groups within *Acropyga* to replace the old subgenera that had previously been recognized within the genus. Those species-groups were largely based on a phylogenetic analysis of male genitalic characters. The most speciose of the New World groups is the *decedens* species-group, with 12 species included by LaPolla (2004) and a thirteenth species (*A. oreithauma* LaPolla, Williams & Fan, 2017) implied to be included by LaPolla et al. (2017), since they considered it a possible sister species to *A. fuhrmanni* (Forel, 1914).

The prior inclusion of *A. fuhrmanni*, *A. oreithauma* and *A. smithii* Forel, 1893 within the *decedens* species-group is challenged by the discovery of a new species, *Acropyga manuense* LaPolla & Schneider sp. nov., along with additional male specimens of *A. fuhrmanni* and *A. smithii* collected from Peru. Evidence drawn from the morphology of males and workers suggests that these four species form a clade within the New World *Acropyga* that is separate from the *decedens* species-group. The penial sclerites of *A. fuhrmanni*, *A. manuense*,

and *A. smithii* share key diagnostic similarities (Figs 1–3). Unfortunately, the male of *A. oreithauma* remains unknown. In all three species the penial sclerites are elongated with distal tips that bend towards the gonopods. These are quite different than the expanded distal tips observed in most of the *decedens* species-group members (the species with such penial sclerites were called the *goeldii* complex by LaPolla (2004) within the *decedens* species-group). The anterior portion of the ventral margin of the penial sclerites also has dentate edges. The workers of *A. fuhrmanni*, *A. manuense*, *A. oreithauma* and *A. smithii* all have 8 antennal segments (occasionally *A. smithii* workers will have as few as 7 segments but this is rarely seen in specimens in collections) and mandibles with 4 mandibular teeth (occasionally a much smaller tooth is found between the 3rd and basal teeth in *A. oreithauma*).

Molecular-based work has suggested that at least *A. fuhrmanni* and *A. smithii* are separated from the *decedens* species-group (Blaimer et al. 2016) and a more recent molecular phylogeny (J. LaPolla, unpub. data) of all described New World *Acropyga* confirms that *A. fuhrmanni*, *A. manuense*, *A. oreithauma* and *A. smithii* comprise a separate clade in alignment with the morphological assessment provided above. Therefore, morphological evidence from both males and workers, combined with molecular results, support the establishment of the *Acropyga smithii* species-group. A revision of the other *Acropyga* species-groups will follow pending publication of molecular phylogenetic results and morphological reassessment.

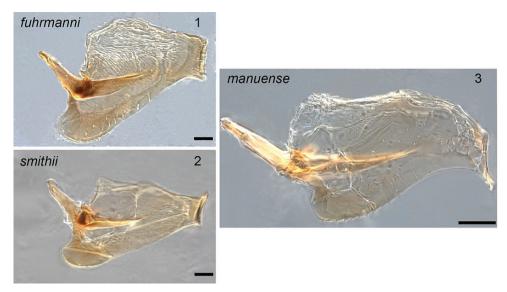
New species descriptions

Hymenoptera Linnaeus, 1758 Formicidae Latreille, 1809

Acropyga manuense LaPolla & Schneider, sp. nov. https://zoobank.org/1C9F0061-5D0F-49B6-A003-3D71BB9AC4E4

Figs 4-6 (worker), 7-8 (queen), 3 and 9-12 (male)

Description. Worker (N = 10). Uniformly yellow; covered in a dense layer of pubescence including lateral portions of pronotum and mesopleuron; scattered erect setae across body. Head slightly longer than wide (CI: 90–99); posterior margin slightly concave medially; posterolateral corners rounded with ca. 6 erect setae found along margin; eyes small with uneven pigmentation (REL: 6–11); 8-segmented, incrassate antennae; scapes short of posterior margin by about ½ to ½ length of pedicel (SI: 71–83); scapes with dense layer of pubescence and scattered erect setae across its length. Clypeus narrow (width in holotype = 0.113 mm) and medially convex. Mandibles with 4 distinct teeth; apical teeth are the longest; teeth 2 and 3 are about equal in size and basal tooth is slightly smaller than teeth 2 and 3; a slight diastema exists between tooth 3 and the basal teeth. In lateral view, mesosoma profile of pronotum steeply rising toward mesonotum (ca. 45° angle if a line is drawn parallel to mesosomal venter). Posterior portion of pronotum and remainder of mesosomal notum with scattered erect setae of varying heights. Highest portion of mesonotum slightly higher than propodeum. Metanotal area dis-



Figures 1–3. Dissected penial sclerites of various species in ectal view. Scale bar: *fuhrmanni* and *smithii* = 0.03 mm; *manuense* = 0.05 mm.

tinct with length in holotype = 0.061 mm. Dorsal face of propodeum flat with length in holotype = 0.122 mm; declivitous face steep (ca. 75° angle). Petiole thick and erect with rounded apex; last $\frac{1}{4}$ of petiole surpasses the most dorsal portion of the propodeal spiracle. Gaster typical of *Acropyga* with thick layer of pubescence and scattered erect setae. Measurements (N = 8). HW: 0.432-0.489; HL: 0.46-0.531; EL: 0.031-0.057; ML: 0.467-0.583; PW: 0.277-0.329; PFL: 0.326-0.413; HL+ML: 0.927-1.09.

Queen (N = 4). As in worker with modifications expected for caste and with the following differences: CI: 95–100; SI: 78–87; REL: 26–32. Measurements (N = 3). HW: 0.524–0.59; HL:0.55–0.59; EL: 0.147–0.188; ML: 0.844–1.03; PW: 0.531–0.606; PFL: 0.428–0.454; HL+ML: 1.4–1.62.

Male (*N* = 4). Head yellowish-brown, excluding mandibles and funicular segments which are yellow; remainder of body yellow. Head about as long as wide (CI: 99–108); posterior margin slightly rounded; medially with three prominent ocelli just anterior to posterior margin; posterolateral corners rounded with an indistinct angle and 2–3 erect setae. Compound eyes large, surpassing head margin in full-frontal view (REL: 34–40). 9-segmented incrassate antennae; scapes surpass posterior margin by about length of antennal pedicel (SI: 93–97); scapes covered with dense pubescence and widely scattered short erect setae. Clypeus medially convex (clypeal width in paratype USNMENT01130437 = 0.08 mm long); mandible with three teeth, large apical tooth and two smaller teeth at the basal angle; on some mandibles the two smaller teeth appear to have fused at the basal angle rendering the mandible 2-toothed. Mesosoma covered with dense pubescence and scattered short erect setae on mesoscutum and mesoscutellum. Pronotum small and collar-like with overarching large, rounded mesonotum. Mesoscutellum slightly higher than mesos-



Figures 4–6. *Acropyga manuense* sp. nov., worker USNMENT01130435 (holotype) **4** lateral view **5** full-face view **6** dorsal view.

cutellum in lateral view. Propodeum lower than mesonotum with no separation into dorsal and declivitous faces; propodeum flat (paratype USNMENT01130437 = 0.212 mm long) with a slight slope toward petiole. Petiole thick and erect just surpassing the lowest portion of the propodeum in lateral view. Gaster typical of male *Acropyga* with thick layer of pubescence and scattered erect setae. Gonopods in lateral view tapered to a rounded apex (paratype USNMENT01130437 gonopod length = 0.216 mm long); in dorsal view gonopods medially expanded. Cuspi tubular (paratype USNMENT01130437 = 0.094 mm long); where cuspi meet digiti several peg-like teeth span the surface; several setae extend off of cuspi apex as well; digiti tubular before a distinct right angle bending ventrally where they meet the cuspi; ventral facing portion of digiti taper toward apex becoming

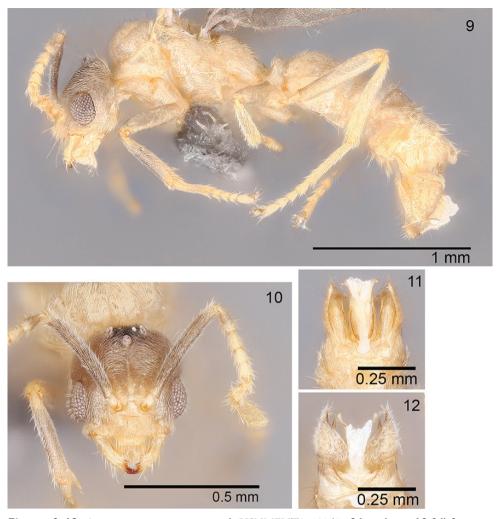




Figures 7, 8. Acropyga manuense sp. nov., queen USNMENT01130436 7 lateral view 8 full-face view.

needle-like (paratype USNMENT01130437 = 0.108 mm long); apex of digiti visible beyond ventral margin of gonopod in lateral view. Penial sclerites elongate (Fig. 3); ventral margin curves from apex through rounded posterior region; along rounded section of penial sclerites margin dentate; apodeme located medially on anterior end of penial sclerites; apex of penial sclerites bend toward gonopods. Measurements (N = 3). HW: 0.389–0.399; HL: 0.36–0.4; EL: 0.128–0.155; ML: 0.663–0.692; PW: 0.465–0.494; PFL: 0.412–0.451; HL+ML: 1.04–1.08.

Material examined. *Holotype*: Peru • worker; Madre de Dios, Las Cruces, Manu Paradise Lodge, nest behind lodge, in soil around small rotting branches; 13.055°S, 71.544°W; 31.v.2019; J.S. LaPolla and S.A. Schneider leg.; USNMENT01130435 (UNMSM). *Paratypes*: Peru • same data as holotype; 9 paratype workers: USN-



Figures 9–12. *Acropyga manuense* sp. nov., male USNMENT01130437 **9** lateral view **10** full-face view **11** ventral view of genitalia **12** dorsal view of genitalia.

MENT01130438 (USNM), USNMENT01130439 (USNM), USNMENT01130450 (USNM), USNMENT01130451 (USNM), USNMENT01130452 (USNM), USNMENT01130453 (USNM), USNMENT01130454 (USNM), USNMENT01130465 (USNM), USNMENT01130483 (USNM); paratype queens: USNMENT01130436 (USNM), USNMENT01130467 (USNM), USNMENT01130468 (USNM), USNMENT01130480 (USNM); paratype males: USNMENT01130437 (USNM), USNMENT01130466 (USNM), USNMENT01130481 (USNM), USNMENT01130482 (USNM).

Etymology. The epithet is a noun in apposition, referring to its type location near Manu National Park, Peru.

Remarks. The workers of *A. manuense* are similar in overall appearance to both *A. fuhrmanni* and *A. smithii* with all three possessing 8 antennal segments (although *A. smithii* workers can occasionally be found with as few as 7 segments) and 4 mandibular teeth. One of the most obvious ways *A. manuense* workers differ from both *A. fuhrmanni* and *A. smithii* workers is in mesosomal pubescence. In *A. manuense* workers possess thick pubescence that extends down the lateral portions of the pronotum and onto the mesopleuron. In *A. fuhrmanni* and *A. smithii* pubescence is very sparse to lacking on the lateral portions of the pronotum and the mesopleuron.

Several measurements will also allow for separation of the three species. The smallest of the three species is *A. smithii* with a head width and length less than 0.4 mm, whereas both *A. fuhrmanni* and *A. manuense* have head widths and lengths greater than 0.4 mm. The scape length in *A. smithii* is less than 0.27 mm, while it is greater than 0.3 mm in both *A. fuhrmanni* and *A. manuense*. The small size of *A. smithii* makes it fairly easy to distinguish from both *A. fuhrmanni* and *A. manuense*. Workers of *A. fuhrmanni* and *A. manuense* possesses different eye sizes with a relative eye length index between 12–15 in *A. fuhrmanni* and between 6–11 in *A. manuense* (Fig. 13). The cephalic index is also instructive with *A. fuhrmanni* generally having a value over 100 with *A. manuense* less than 100.

The most striking differences between *A. fuhrmanni*, *A. smithii* and *A. manuense* are found in males. The mandibles of the three species' males differ in the number and size of teeth, with *A. manuense* possessing three distinct equally sized teeth. Both *A. fuhrmanni* and *A. smithii* only have two teeth: a prominent apical tooth and a much smaller tooth at the basal angle. There are several genitalic differences between the three species such as each possessing uniquely shaped penial sclerites (Figs 1–3). The digitus is very distinctive within *A. manuense*, being ventrally directed, elongated and coming to a prominent point (Figs 9, 11). The elongated, pointed structure of the digitus is unique among all *Acropyga* species.

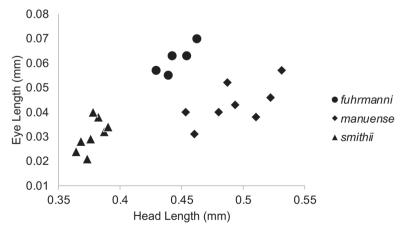


Figure 13. Bivariate plot of eye length vs. head length among measured *A. fuhrmanni*, *A. manuense* and *A. smithii* workers.

In the key to New World workers provided by LaPolla (2004), specimens of *A. manuense* will key to the lug for *A. fuhrmanni*. That lug would become a new couplet as follows:

- 1 Lateral portions of pronotum and mesopleuron with spare to no pubescence; ocular index between 12–15; cephalic index above 100........Acropyga fuhrmanni (Forel)

Hemiptera Linnaeus, 1758 Xenococcidae Tang, 1992

Neochavesia podexuta Schneider & LaPolla, sp. nov. https://zoobank.org/EA866C98-2812-44DC-8B50-0A59D80755B5 Fig. 14

Description. Adult female (N = 7). In life, body bright white to cream colored and free of wax. Mounted on microscope slide, body elongate-pyriform, 1.35-1.58 mm long, 0.55-0.73 mm wide at widest point; head and thorax dilated with widest point at metathorax and abdominal segment I. Abdomen constricted after segment III; segments IV–VII gently tapering in width posteriorly with another constriction between segments VII and VIII. Dorsal posterior half of abdominal segment VIII sclerotized; anterior half membranous and free of setae, forming a distinctive bald patch; width of segment 145 µm wide. Anal lobes well developed and separated from abdominal segment VIII on venter and margins of dorsum by an intersegmental line. Anal lobes diverging with a roughly U-shaped notch between them, each rounded at posterior end. Dorsum of each anal lobe with numerous long flagellate setae, longest about 135 µm, situated at posterior end, with those at anterior end about 35 µm; ventral surface with similar setae 32-90 µm long. Anal ring roughly triangular, without cells or setae, 65 μm wide; anterior end lying along the intersegmental division that separates the anal lobes from abdominal segment VIII, posterior edge removed from the apical notch between lobes by about 1X length of anal ring. Long antennae widely spaced on dorsal head margin; basal segment set into a notch on the head and articulating; each with four segments, 652-663 µm in overall length; average lengths of segments (base to apex) 56 μm, 297 μm, 95 μm, 215 μm; the apical segment appearing partially divided, indicating an obsolete fifth segment; few flagellate setae on basal antennal segment, numerous such setae on all other segments, 30–55 µm long. Legs well developed; average length of metatrochanter + femur 212 μm long; metatibia + tarsus 150 μm long; tarsus swollen basally and abruptly tapering; with metaclaw 70-80 µm long, longer than tarsus. Ratio of length of metatibia to tarsus, 1.60; leg segments with multiple stout flagellate setae. Labium 3-segmented, 147 μm long, longer than clypeolabral shield, 70 μm wide; basal segment with three pairs of setae; eight pairs of setae on terminal seg-

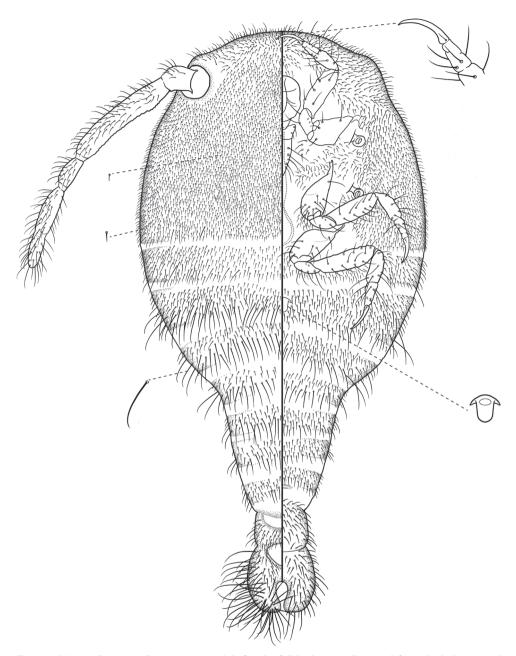


Figure 14. *Neochavesia podexuta* sp. nov. Adult female, full body view, illustrated from the holotype and paratypes. Illustration by Taina Litwak (USDA, ARS, Systematic Entomology Laboratory) with edits by SAS.

ment. One round circulus present, situated towards center of abdominal segment II, $16~\mu m$ in diameter, conical and projecting from derm, cup-shaped internally. Spiracles normal, $35~\mu m$ in diameter at widest point.

Dorsal surface of head and thorax crowded with slender flagellate setae, about 17 μ m long, few approaching 45 μ m at posterior end of metathorax, most with small setal collars, longer setae with slightly wider collars; abdominal segments with similar setae but less densely crowded after segment I; on each segment, setae at anterior end shorter and finer than posterior setae, ranging from 19–75 μ m on segment I, upwards of 95 μ m on segments II–VI, shorter setae 25–40 μ m on VII–VIII, longest setae on anal lobes, from 32–135 μ m. Venter with similar setation; less densely crowded on head and with greater variation in setal lengths, 15–40 μ m; thoracic margin to submargin similar to dorsum; thoracic submedian similar to ventral head; abdominal segments I–IV similar to dorsum, segments V–VIII with shorter setae, anal lobes with numerous long setae but generally shorter than those found on dorsum. Pores and ducts absent.

Material examined. *Holotype*: Peru • 1 adult female; Madre de Dios, Las Cruces, Manu Paradise Lodge, from *Acropyga manuense* nest behind lodge, in soil around small rotting branches; 13.055°S, 71.544°W; 31.v.2019; J.S. LaPolla and S.A. Schneider leg.; UNMSM (nest ID PER01-02; prep S0401E). *Paratypes*: Peru • 6 adult females; same data as holotype; USNM (nest ID PER01-01; preps S0400A,B,C,D,E,F) • 1 immature female; same data as holotype; USNM (nest ID PER01-02; prep S0401A).

Informal synonyms. This species was previously referred to as "*Neochavesia* undescribed" in Schneider et al. (2022).

Etymology. The epithet is a noun in apposition, referring to the distinctive bald patch located just anterior to the anal opening. The Latin 'podex', meaning fundament/ anus, was combined with 'exutus', meaning bared or stripped.

Remarks. *Neochavesia podexuta* bears a distinctive bald patch on the dorsal anterior surface of abdominal segment VIII. On slide-mounted specimens, it often appears as though this segment, including the anal lobes, has become detached from the rest of the body although it is still intact. In life, species of *Neochavesia* hold their abdomen in a curled position over their dorsum, resembling the tail of a scorpion; this bald membranous patch is located where the cuticle would curve inward. Adult females of *Neochavesia podexuta* are similar to *N. cephalonodus*, *N. eversi*, *N. iwokramae*, and *N. lapollai* in possessing antennae that articulate at the basal segment with a sclerotized prominence (forming a socket) on the head. They also lack a pair of setae on the middle labial segment, a characteristic shared among these species as well. The new species is most akin to *N. eversi*; the former can be distinguished from the latter by the bald patch on VIII described above and by their longer antennae. In *N. podexuta*, the body is about 2.0–2.5 times longer than the antennae with the second segment almost 300 μm long; whereas in *N. eversi*, the body is roughly 3.5 times the length of the antennae with the second segment only about 150 μm long.

Direct association between *A. manuense* and *N. podexuta* was confirmed by collecting ants and root mealybugs into a nest-box and observing interactions (Schneider et al. 2022). Ants gathered root mealybugs into protective clusters within the nest-box and were observed actively tending them. These observations were made over a 48-hour period in field-based laboratory conditions.

Key to adult females of Neochavesia Williams & Granara de Willink, 1992

Adapted from Schneider and LaPolla (2011) and Williams (2004).

1	Anal lobes fused with abdominal segment VIII, without intersegmental line; antennae situated toward ventral head margin
-	Anal lobes separated from abdominal segment VIII by intersegmental line; antennae situated on lateral margin or dorsally on head
2	Trilocular pores present on head and thorax
_	Trilocular pores absent
3	Claws over 1/2 length of tarsi; one circulus present on third abdominal segment; posterior abdominal segments with rows of rigid thorn-like setae
	Neochavesia linealuma Schneider & LaPolla
_	Claws between 1/4 and 1/3 length of tarsi; two circuli present on abdominal segments II and III; all setae on abdominal segments flagellate
	Neochavesia weberi (Beardsley)
4	Antennae normally five segmented
_	Antennae normally four segmented
5	Second antennal segment $203-293~\mu m$ long; anal ring transversely elliptical and situated between anal lobes and abdominal segment VIII; many long stout setae
	on head margin
_	Second antennal segment $81-185~\mu m$ long; anal ring round to triangular and situated near proximal base or in middle of anal lobes between anterior edge and base of
	U-shaped notch between anal lobes; head without long stout setae
6	Second antennal segment 81–95 µm long, antennae articulating with promi-
	nences on head; anal ring situated in middle of anal lobes between anterior
	edge and base of U-shaped notch between anal lobes
_	Second antennal segment 168–185 µm long, antennae not articulating with prominence on head; anal ring situated near proximal base of anal lobes
7	Claws shorter than tarsi; second antennal segment 365–415 µm long; promi-
	nences enlarged giving head a swollen appearance
_	length, prominences on head relatively small
8	Body roughly 2.0–2.5 times longer than antennae, with second antennal seg-
O	ment almost 300 µm in length; dorsal anterior surface of abdominal segment
	VIII membranous and free of setae, appearing bald
_	Body roughly 3.5 times longer than antennae, with second antennal segment
	about 150 μm in length; dorsal anterior surface of abdominal segment VIII
	sclerotized and bearing setae

Acknowledgements

Support for this research was provided by the National Science Foundation (award number 1754242 to JSL and SAS). We thank Dr. Roxana Arauco Aliaga for accommodation at Cocha Cashu Biological Station and for assistance with permitting (SERFOR #003620). The research was supported also in part by the U.S. Department of Agriculture, Agricultural Research Service. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture; USDA is an equal opportunity provider and employer.

References

- Blaimer BB, LaPolla JS, Branstetter MG, Lloyd MW, Brady SG (2016) Phylogenomics, biogeography and diversification of obligate mealybug-tending ants in the genus *Acropyga*. Molecular Phylogenetics and Evolution 102: 20–29. https://doi.org/10.1016/j. ympev.2016.05.030
- Forel A (1893) Formicides de l'Antille St. Vincent, récoltées par Mons. H. H. Smith. The Transactions of the Entomological Society of London 1893: 333–418.
- Forel A (1914) Quelques fourmis de Colombie. In: Fuhrmann O, Mayor E (1914) Voyage d'exploration scientifique en Colombie. Mémoires de la Société Neuchâteloise des Sciences Naturelles 5(2): 1–1090. [9–14]
- Johnson C, Agosti D, Delabie JH, Dumpert K, Williams D, Tschirnhaus MV, Maschwitz U (2001) Acropyga and Azteca ants (Hymenoptera: Formicidae) with scale insects (Sternorrhyncha: Coccoidea): 20 million years of intimate symbiosis. American Museum Novitates 3335: 1–18. https://doi.org/10.1206/0003-0082(2001)335<0001:AAAAHF>2.0.CO;2
- LaPolla JS (2004) *Acropyga* (Hymenoptera: Formicidae) of the world. Contributions of the American Entomological Institute 33(3): 1–130.
- LaPolla JS, Williams JL, Fan E (2017) *Acropyga oreithauma*, a new species from Panama. Transactions of the American Entomological Society 143: 1–5. https://doi.org/10.3157/061.143.0102
- Schneider SA, LaPolla JS (2011) Systematics of the mealybug tribe Xenococcini (Hemiptera: Coccoidea: Pseudococcidae), with a discussion of trophobiotic associations with *Acropyga* Roger ants. Systematic Entomology 36(1): 57–82. https://doi.org/10.1111/j.1365-3113.2010.00546.x
- Schneider SA, Sodano J, LaPolla JS (2022) Distinguishing symbiotic partners of *Acropyga* ants from free-living soil inhabitants. Neotropical Entomology 51(4): 641–647. https://doi.org/10.1007/s13744-022-00948-9
- Silvestri F (1924) A new myrmecophilous genus of Coccidae (Hemiptera) from India. Records of the Indian Museum 26: 311–315. https://doi.org/10.26515/rzsi/v26/i4/1924/162665
- Silvestri F (1926) Descrizione di un novo genere di Coccidae (Hemiptera) mirmecofilo della Cina. Bollettino del Laboratorio di Zoologia Generale e Agraria della R. Scuola Superiore d'Agricolture in Portici 18: 271–275.

- Terayama M (1986) A new species of the anomalous ant-attended mealybug *Eumyrmococcus* (Homoptera, Pseudococcidae) from Japan. Kontyû 54: 509–512.
- Williams DJ (1993) A new species of mealybug from Greece, the first from Europe belonging to the ant-attended genus *Eumyrmococcus* Silvestri (Hemiptera: Coccoidea: Pseudococcidae). Entomologist's Gazette 44: 216–220.
- Williams DJ (1998) Mealybugs of the genera *Eumyrmococcus* Silvestri and *Xenococcus* Silvestri associated with the ant genus *Acropyga* Roger and a review of the subfamily Rhizoecinae (Hemiptera, Coccoidea, Pseudococcidae). Bulletin of the Natural History Museum London (Entomology) 67: 1–64.
- Williams DJ (2004) A synopsis of the subterranean mealybug genus *Neochavesia* Williams and Granara de Willink (Hemiptera: Pseudococcidae: Rhizoecinae). Journal of Natural History 38(22): 2883–2899. https://doi.org/10.1080/00222930310001657856
- Williams DJ, Granara de Willink MC (1992) Mealybugs of Central and South America. CAB International, Wallingford, 635 pp.
- Williams DJ, Terayama M (2000) A new species of the mealybug genus *Eumyrmococcus* Silvestri (Hemiptera: Pseudococcidae: Rhizoecinae) associated with the ant *Acropyga* (*Rhizomyrma*) *kinomurai* Terayama et Hashimoto (Hymenoptera: Formicidae) in the Ryukyu Islands, Japan. Entomological Science 3: 373–376.