Miocene ant-mealybug trophobiosis imaged with X-Ray micro-computed tomography

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Abstract

Amber is a remarkable preserving medium for Mesozoic and Cenozoic terrestrial biotas, but even when transparency is good, available viewing angles can be limited. The technique of X-ray micro-computed tomography allows inspection from any desired viewpoint and facilitates detailed anatomical measurements, avoiding parallax errors. Here, I show the use of this technique to study an extremely rare association of a Miocene ant (*Acropyga glaesaria*) trapped in resin while carrying a mealybug (*Electromyrmococcus inclusus*) with which to incubate a new colony. This is only a fourth known example of an *Acropyga* gyne holding a mealybug and the second recorded specimen of *Electromyrmococcus inclusus*. Measurements and other features of both ant and mealybug are consistent with the holotypes.

Keywords: micro-computed tomography, amber, Miocene, symbiosis, trophobiosis, ant, mealybug

Introduction

Inclusions in amber not only preserve individual organisms in fine detail, but can record interactions between organisms, including symbiotic relationships of various kinds (e.g. Johnson et al. 2001, LaPolla 2002, Poinar 2011, 2019).

In recent decades, X-ray micro computed tomography has been used to non-destructively reveal 3D anatomy and relationships in palaeontological specimens (e.g. Grimaldi et al. 2000, Henderickx et al. 2006, Coty et al. 2014, Sutton et al. 2014, Penney et al. 2017). The technique is here applied to a remarkable specimen of Miocene Dominican amber illustrating ant-mealybug trophobiosis (LaPolla 2002), a phenomenon well known in extant ants such as *Acropyga* and *Hypoclinea* in Malaysia (Williams 1978).

The amber of the Dominican Republic is of Miocene age, derived from the resin of an extinct tree *Hymenaea protera* which once grew in northern South America, the island of Hispaniola, and southern Mexico. Specimens are collected from mines in the Cordillera Septentrional mountains of northern Dominican Republic. Poinar (2010) discussed estimates for the age of the amber, which dates from at least the Burdigalian stage of the Miocene, based on associated foraminifera (Iturralde-Vinent & MacPhee 1996, 2019). The age of the amber is estimated to be Early Miocene (20-15 Ma).

The specimen of Dominican amber presented here shows a distinctive female mealybug, *Electromyrmococcus inclusus* Williams & Agosti 2001, held in the mandibles of a winged female ant *Acropyga glaesaria* LaPolla 2005 (2.5 mm total length). The extreme rarity of the specimen is emphasised by Johnson et al. (2001) who noted that "Three pieces of Dominican amber, each containing an *Acropyga* gyne with a pseudococcid, were recovered from approximately 30,000 small pieces of Dominican amber screened by D. Grimaldi." Johnson et al. (2001) illustrated two specimens in which the *Acropyga* gyne holds a mealybug in her mandibles, one in the Harvard collection (AMNH DR-10-228), the other in the collection of the Senckenberg Museum, Frankfurt. In a third specimen (AMNH DR-14-403) the mealybug (*Electromyrmococcus inclusus*) had become dislodged during entrapment in the tree resin. LaPolla (2005) formally described these ants as *Acropyga glaesaria*, arguing that differences in head shape between the Harvard and Senckenberg specimens noted by Johnson et al. (2001) did not warrant the erection of two separate species. A further undescribed specimen has been illustrated in Ross & Sheridan (2013).

Ants and Mealybugs

Johnson et al. (2001) noted that species of the genus *Acropyga* are subterranean ants that rely on mealybugs or aphids to provide nutritional needs in the form of honeydew. Female *Acropyga* (Formicinae) alates carry mealybugs between their mandibles while swarming, using them to inoculate their new nests. They commented that this intimate association between *Acropyga* ants and mealybugs (trophobiosis of Delabie 2001, LaPolla 2002) and the transport of mealybugs by winged females during swarming has existed for at least 15-20 million years (the estimated minimum age of Dominican amber, Grimaldi 1995, Iturralde-Vinent & MacPhee 1996, 2019). It has been suggested that *Acropyga* are completely dependent on their mealybugs for food (LaPolla et al. 2002; LaPolla 2005) and reproductives only emerge from the nest during the mating flight, each carrying a seed bug in their mandibles. *Acropyga* ants are not known in Baltic amber and probably originated in the New World. A number of ant-mealybug associations are known, for example, in Malaysia the subterranean *Acropyga acutiventris* with *Xenococcus annandelei* the aerial *Hypoclinea* sp with *Malaicoccus* (Williams 1978). A recent European occurrence is presented by Scupola et al. (2022).

The *Electromyrmococcus* mealybugs found with the Miocene *Acropyga* females in amber are related to the subterranean (hypogaeic) genera *Eumyrmococcus* Silvestri and *Neochavesia* Williams & Granara de Willink (Pseudococcidae, Rhizoecinae). Given their subterranean lifestyle, and the fact that reproductives only emerge for a short time for their mating flights, *Acropyga* specimens are not expected to be common in amber inclusions and they are, as noted above, indeed extremely rare. *Acropyga* reproductives have, however, been found in at least 6 pieces of Dominican amber (LaPolla 2005, this paper).

While the association of *A. glaesaria* and the *Electromyrmococcus* species is the oldest definitive example of trophobiosis, a possible earlier case is suggested inconclusively by a piece of Eocene Baltic amber containing 13 worker ants associated with several aphids (Wheeler 1915).

Figure 1. A. Photograph of specimen RS.P1033 showing female *Acropyga glaesaria* holding *Electromyrmococcus inclusus*. A number of bubbles in the amber are also visible. Total length of ant is 2.5 mm and length of forewing is approximately 3 mm. Notice the 9-segment antennae of the *Acropyga* and prominent eye. B. Detail view of the *Electromyrmococcus inclusus* presented in ventral view, showing antennae, broad cephalothorax, well developed legs and tapering abdomen with setae extending from two anal lobes. Total length including antennae and setae is about 1.13 mm.

Material and methods

The specimen studied has inventory number RS.P1033 ('StrataSmith Museum' collection, York, UK), measures 10 x 7 x 3 mm and weighs 0.21 g. Photography (Fig. 1) was performed using a Sony 7RIV digital camera mounted on a 100 mm UltraMacro/WeMacro Auto-Rail with Raynox DCR 150 DSLR:Objective Tube Lens and Objective PLAN ACHROMATIC LWD infinity 5X lens. Photographs taken at spaced focal depths were stacked using Helicon Focus Pro software. The X–ray micro-computed tomography image used to examine the specimen was realised in the CT scan facility of the Universiti Malaya, using a Zeiss Xradia Versa 520 machine with a nanofocus transmission tube and movable detector. The voxel size of the reconstructed volume is 4.6 µm. 3D renders, meshes and movies were made using Drishti software (Limaye 2012, Hu et al. 2020). Variations in the material density in the amber piece are visible through changes of greyscale from dark (low density, low attenuation) to light (high density, high attenuation). Transfer functions were designed to reveal preserved cuticle, external surfaces and bubbles (Fig. 2). The ant and mealybug were segmented using Drishti Paint. Weak density contrast made the segmentation process challenging in addition to the partial preservation of cuticle, hence manual interpretation combined with thresholding was used.

Results and discussion

Rendering of the dataset provides a clear image of the ant and mealybug allowing measurements to be made directly, avoiding potential parallax errors. Numerous air bubbles with diameters between 0.05 and 1 mm occur in the sample, one of which impinges on the proximal end of the gaster (Figure 2).

Figure 2. Segmented micro computed tomography image created in Drishti showing *Acropyga glaesaria* ant with *Electromyrmococcus inclusus* mealybug and associated air bubbles. The fine setae developed from the anal lobes of the mealybug are not resolved in the data.

There is curvature to the body of the ant, with gaster bent upwards and head rotated clockwise relative to the alitrunk. Seven measurements were made for comparison with previously reported values from LaPolla (2005) and are found to be consistent with the earlier results, at the lower end of the published ranges (Figure 3).

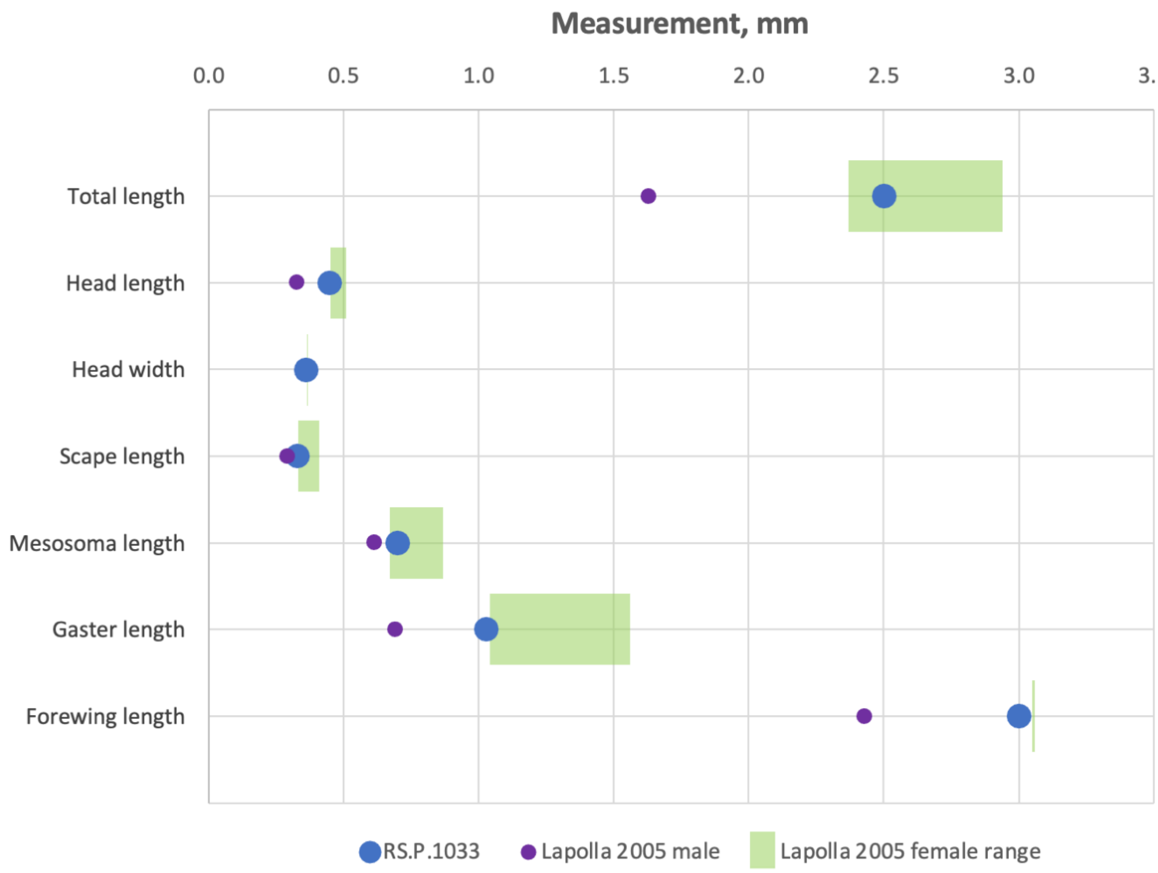

Figure 3. Comparison of measurements (mm) between RS.P.1033 described here and the range in values recorded by LaPolla (2005) from the three known specimens of *Acropyga glaesaria* females at that time (in green bars). Measurements from the small male specimen are also shown.

The mealybug is one of three species described in the extinct genus *Electromyrmococcus* in the Rhizoecinae subfamily of Pseudococcidae (Williams & Agosti appendix in Johnson et al. 2001). This is a second specimen of *E. inclusus*. The holotype (AMNH DR-14–403) is an adult female with broad cephalothorax and tapering abdomen terminating in anal lobes each of which carries four setae. A notable constriction between the abdomen and the thorax was thought to be a result of compression between the mandibles of the carrying ant, although in that case, ant and mealybug were separated when they became trapped in resin.

The specimen of *E. inclusus* illustrated here conforms with the description of the holotype (AMNH DR-14–403), showing distinctive cephalothorax and groups of four setae developed from well-defined anal lobes. Williams & Agosti (2001) reported total length (not defined) as 0.76 mm. Front of cephalothorax to end anal lobe in RS.P1033 is 0.64 mm and total length including antennae and setae is about 1.13 mm. Cephalothorax width of 0.36 mm in RS.P.1033 is smaller than the 0.46 mm reported for the holotype. As in the holotype, a strong constriction is present where the mealybug was clasped by the ant mandibles; this measures 0.12 mm in RS.P1033, half of the 0.24 mm maximum width of abdomen. Setae developed from anal lobes have the same approximate length in both holotype (0.265 mm) and RS.P.1033 (0.27 mm). Recorded antenna lengths are 0.31 (RS.P1033) and 0.365 in the holotype. It is not known what errors may be associated with optical measurements (for example associated with parallax) in amber specimens, but three decimal places may be overly precise.

Conclusions

An excessively rare specimen of an associated winged female *Acropyga glaesaria* ant and *Electromyrmococcus inclusus* mealybug has been studied with X-ray micro computed tomography, allowing an accurate set of measurements to be made for comparison with previously documented examples. Measurements are consistent with those reported for the three previously reported female *Acropyga* specimens, towards the lower end of the range documented by LaPolla (2005). The specimen also provides only a second example of *Electromyrmococcus inclusus* which is highly comparable with the holotype and also displays the constriction associated with carrying by the ant gyne seen in that specimen.

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References

Coty, D., Aria, C., Garrouste, R., Wils, P., Legendre, F. & Nel, A. 2014. The first ant-termite syninclusion in amber with CT-scan analysis of taphonomy. *PLOS ONE*, 9(8), e104410. https://doi.org/10.1371/journal.pone.0104410

Delabie, H.J.C. 2001. Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): an overview. *Neotropical Entomology,* 30, 501–506. DOI:10.1590/S1519-566X2001000400001

Grimaldi, D., Nguyen, T. & Ketcham, R. 2000. Ultra-high-resolution X-ray computed tomography (UHR CT) and the study of fossils in amber. In: Grimaldi, D. (ed.), *Studies on fossils in amber, with particular reference to the Cretaceous of New Jersey.* Backhuys Publishers, Leiden. 77–92.

Henderickx, H., Cnudde, V., Masschaele, B., Dierick, M., Vlassenbroeck, J. & Van Hoorebeke, L. 2006. Description of a new fossil *Pseudogarypus* (Pseudoscorpiones: Pseudogarypidae) with the use of X-ray micro CT to penetrate opaque amber. *Zootaxa*, 1305, 41–50.

Hu, Y., Limaye, A. & Jing, L. 2020. Three-dimensional segmentation of computed tomography data using Drishti Paint: new tools and developments. *Royal Society Open Science*, 7, 201033. https://doi.org/10.1098/rsos.201033

Johnson, C., Agosti, D, Delabie, J.H., Dumpert, K., Williams, D.J., Van Tschirnhaus, M. & 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. doi:10.1206/0003-0082(2001)335<0001:AAAAHF>2.0.CO;2.

Iturralde-Vinent, M.A. & MacPhee, R. D. E. 1996. Age and paleogeographical origin of Dominican amber. *Science*, 273, 1850–1852. https://doi.org/10.1126/science.273.5283.1850

Iturralde-Vinent, M.A. & MacPhee, R. D. E. 2019. Remarks on the age of Dominican amber. *Palaeoentomology*, 2, 236–240. DOI: 10.11646/PALAEOENTOMOLOGY.2.3.7

LaPolla, J.S., Cover, S.P. & Mueller, U.G. 2002. Natural history of the mealybug-tending ant *Acropyga epedana*, with descriptions of the male and queen castes. *Transactions of the American Entomological Society,* 128, 367–376.

LaPolla, J. S. 2005. Ancient trophophoresy: a fossil Acropyga (Hymenoptera: Formicidae) from Dominican amber. *Transactions of the American Entomological Society*, 131, 21–28. https://www.jstor.org/stable/25078875

Limaye, A. 2012. Drishti, a volume exploration and presentation tool. *Developments in X-Ray Tomography VIII,* 85060X.

Penney, D., Dierick, M., Cnudde, V., Masschaele, B. & Vlassenbroeck, J. 2007. First fossil Micropholcommatidae (Araneae), imaged in Eocene Paris amber using X-ray computed tomography. *Zootaxa,* 1623, 47–53. https://doi.org/10.11646/zootaxa.1623.1.3

Poinar, G.O. 2010. Palaeoecological perspectives in Dominican amber. *Annales-Société Entomologique de France*, 46, 23–52. 10.1080/00379271.2010.10697637

Ross, A. & Sheridan, A. 2013. *Amazing Amber*. NMS Enterprises Limited - Publishing. 64 pp.

Scupola, A., Durante, A., Gianuzzi, F. & Pellizzari, G. 2022. The coccid-tending ant genus *Acropyga* Roger and its obligate associated myrmecophilous scale insect genus *Eumyrmococcus* new to Italy (Hymenoptera: Formicidae; Hemiptera: Xenococcidae). *Fragmenta entomologica*, 54, 89–94. https://doi.org/10.13133/2284-4880/722

Smejkal, G.B., Poinar, G.O., Righetti, P.G., Chu, F. 2011. Revisiting Jurassic Park: the isolation of proteins from amber encapsulated organisms millions of years old. In: Ivanov, A., Lazarev, A. (eds) *Sample Preparation in Biological Mass Spectrometry*, Springer, Dordrecht, 925-938. https://doi.org/10.1007/978-94-007-0828-0_45

Soriano, C., Archer, M., Azar, D., Creaser, P., Delclos, X. 2010. Synchrotron X-ray imaging of inclusions in amber. *Comptes Rendus Palevol*, 9, 361–368. https://doi.org/10.1016/j.crpv.2010.07.014

Wheeler, W. M. 1915. The ants of the Baltic amber. *Schriften der Physikalisch-Okonomischen Gesellschaft zu Konigsberg*, 55, 1–142.

Williams, D.J. 1978. The anomalous ant-attended mealybugs (Homoptera, Pseudococcidae) of south-east Asia. *Bulletin of the British Museum (Natural History), Entomology Series*, 37, 1–72.