Insectes Sociaux
https://doi.org/10.1007/s00040-020-00754-9

Insectes Sociaux

SHORT COMMUNICATION

A subterranean ant *Acanthostichus* Mayr, 1887 is revealed in Costa Rica

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Received: 25 October 2019 / Revised: 16 January 2020 / Accepted: 28 January 2020

Abstract
In this study, pinned insect specimens derived from Malaise traps in Costa Rica were identified using a collaborative process, resulting in a significant addition to our understanding of the range of a rare Neotropical ant genus. The specimens were imaged and the COI barcode region of mitochondrial DNA was sequenced. The images and sequence data were shared in an open access database. Public exposure from such a digitally based approach to biodiversity analysis allowed us to collaboratively uncover the first known Costa Rican occurrence of the subterranean ant genus *Acanthostichus*, filling in a heretofore confusing hole in its geographic range.

Keywords Formicidae · Barcode · Area de Conservación Guanacaste (ACG)

For insect species that are known only from the type collection, the data associated with these type specimens represent the entirety of what we know about the ecology of the species. Currently, 20% of all species are known only from the types (Deng et al. 2019). Thus, much of the value enclosed in natural history collections, museums and herbaria come from the iterative analysis of insects on pins (Meineke et al. 2019). In this study, a new genus for Costa Rica was uncovered when pinned insect specimens from an ongoing inventory were imaged and sequenced and then the data were shared in a publicly available database. This digitally driven "crowd-sourcing" approach to biodiversity analysis allowed us to discover the first Costa Rican occurrence of a subterranean genus of ants and to fill in a previously confusing hole in its geographic range.

Species in the ant genus *Acanthostichus* Mayr 1887 are subterranean and widely distributed, from the southern United States to northern Argentina (Borowiec 2016; MacKay 1996) (Fig. 1). However, despite this broad distribution, it is uncommon to collect *Acanthostichus*, and our understanding of their distribution contains significant gaps. For example, despite many years of research and sampling by multiple investigators, the genus has never been documented in Costa Rica (http://antmaps.org/index.html?mode=diversity&genus=Acanthostichus).

Using a Townes-style Malaise trap, which passively collects bulk samples of mostly flying insects, we collected two ant specimens (winged queens) in dry forest in the Area Administrativa in Sector Santa Rosa of Area de Conservacion Guanacaste (ACG), Costa Rica on 28 June, 2009. In August, 2010, the specimens were point-mounted and imaged (Figs. 2, 3). A single leg was removed from each animal's right side for DNA extraction, amplification and sequencing of the DNA barcoding region of the cytochrome *c* oxidase (COI) gene. The images, sequences and metadata were then made publicly available on the Barcode of Life Data System website (BOLD—Ratnasingham and Hebert 2007) where they became the sole members of the Barcode Index Number or BIN, BOLD:AAM7086 (Ratnasingham and Hebert 2013—https://doi.org/10.5883/bold:aam7086). The specimens were eventually included in a published test of elevation and diversity (Smith et al. 2014), but all data were public beforehand. The two ants were provisionally identified as *Cerapachys* Smith, F., 1857, a genus with a complicated taxonomic history involving several genera of Dorylinae (Borowiec 2016). After a comparison of all the

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Published online: 20 February 2020

Fig. 1 Distribution of the genus *Acanthostichus* across North, South and Central America. Nations with any state or provincial record of *Acanthostichus* are coloured red. Costa Rica is coloured orange. Distributional data from Boroweic (2016) and Antmaps (Janicki et al. 2016). An up to date version of the antmap distribution, with specific state/provincial estimates of species diversity, can be seen here: https://antmaps.org/?mode=diversity&genus=Acanthostichus

Fig. 2 One of the two ants that constitute the first example of the ant genus *Acanthostichus* known from Costa Rica. Lateral image of the sample ACGAE480-10 collected on June 26, 2009. Focus-staked image taken with a Leica Z16 APO. A high-resolution version of this view and others can be annotated here: http://www.gigapan.com/gigapans/216979. Full collection information can be viewed here: http://www.boldsystems.org/index.php/Public_RecordView?processid=ACGAE480-10

publicly available sequences labelled as *Cerapachys* from BOLD, MGB emailed MAS on 17 April, 2018 as to the correct generic identity of the Santa Rosa samples (based on an exploration of diverse *Cerapachys* sequences on BOLD and subsequent examination of morphological features including a prominent subpetiolar process). We thereby confirmed the identification and the occurrence of *Acanthostichus* in Costa Rica. The barcode sequence was > 20% divergent from other *Acanthostichus* samples in the database, and thus this record likely represents a new species for the barcode library. What remains unclear is whether it is a new species to science or an extreme southerly range extension for *A. texanus*. Determining this will require further integrative taxonomy that includes examination of morphological characters, comparison with type specimens, and additional genetic comparisons based on multiple and independent DNA markers.

Ants are common animals in many ecosystems and have received years of effort by the many taxonomists dedicated to the task of describing ant diversity (currently estimated at 13.4 K species globally), yet many species remain unencountered, or at least, undescribed. Total ant diversity is expected to eventually be between 20 and 30 K species (Steiner 2018). Continuing to unravel the diversity of ants and other small arthropods will require open data and access to primary collections, particularly from diversity hotspots

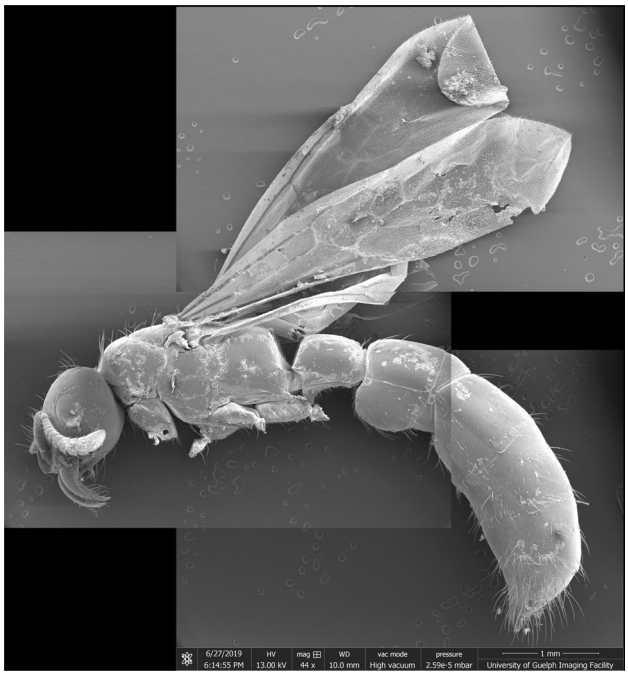

Fig. 3 A composite scanning electron micrograph (SEM) of the second of two samples (ACGAE481-10) collected in the Area Administrativa in Santa Rosa, ACG, Costa Rica. The SEM was captured using a FEI Quanta FEG 250 equipped with a tungsten filament after sample preparation using a stereomicroscope, then adhered on an aluminium stub using double-sided carbon tape, placed stub in a Cressington Auto/SE sputter coater equipped with a gold target and a thin gold film (~20 nm) was sprayed on the sample. A high-resolution version of this image can be explored and annotated here: http://www.gigapan.com/gigapans/216546. Full collection information is here: http://www.boldsystems.org/index.php/Public_RecordView?processid=ACGAE481-10

such as the Neotropics. The global community of myrmecologists has made early successful investments in this open and collaborative style of work (e.g. AntWeb (www.antweb.org), AntCat (www.antcat.org), AntWiki (www.antwiki.org), Global Ant Database (http://globalants.org/—Parr et al. 2017), Ant Maps (http://antmaps.org—Janicki et al. 2016), antbase (http://antbase.org) as well as aggregative databases such as the Global Biodiversity Information Facility (GBIF) and the Barcode of Life Data System (BOLD) (respectively www.gbif.org, with > 1.8 million ant collection records, and www.barcodinglife.org, with more than 94 K public ant specimen records as of July 2019, http://www.boldsystems.org/index.php/API_Public/combined?taxon=Formicidae&format=tsv).

Many arthropod specimens and species currently found on BOLD were caught by passive intercept traps, such as Malaise traps, and often initially lack a formal scientific name. They are eventually associated with a taxonomic rank based on the iterative curation of barcode sequences and specimen morphology made possible by making those data public prior to publication or formal taxonomic identification; their BIN code thus serves as a proxy interim species name (though we recognize that, just as with morphological identification, several species may be included in the same BIN (e.g., Janzen et al. 2017). For example, there are multiple cases where species of European Hymenoptera have been uncovered as adventive in Canada through the comparison of sequenced and named European samples with BOLD records of insects Malaise-trapped in Canada (e.g. Moffat and Smith 2014; Smith et al. 2018). Interestingly, the area where the two *Acanthostichus* specimens were collected in 2009 has been intensively and continuously Malaise-trapped in the intervening decade with no further captures, and the two specimens described in this note remain the sole members of the BIN BOLD:AAM7086. While Malaise trapping will help call attention to members of this subterranean genus, other more specialised collection techniques are required to better understand *Acanthostichus* natural history.

While *Acanthostichus* contains 23 known extant species (Borowiec 2016), much remains unknown regarding the distribution and natural history of species in this taxon. They are in the Dorylinae, the subfamily that contains army ants and driver ants, but they do not exhibit typical "army-ant" behaviours and are thought to be subterranean termite hunters (Borowiec 2016). Information about the dispersal strategies adopted by *Acanthostichus* is scant. For many species (19 of 24), we do not yet know what the queens look like. In some cases, the queens are wingless (or ergatoid) (*A. brevicornis, A. quadratus* and *A. laticornis*) while two other species (*A. texanus* and *A. emmae*) have winged queens, like the Costa Rican samples documented here (Borowiec 2016; MacKay 1996). Indeed, the natural history of subterranean ants is an emergent area of ant biology and specialised traps and surveys (Wilkie et al. 2007) continue to help us document new species biology. Our results might help direct the placement of specialised subterranean traps to further document the workers of this species.

Rapid posting of biodiversity data to public databases allows the convergence of distributed expertise. In this instance we filled a large gap in the distribution of a subterranean ant genus. Subterranean ants contain taxa about which we know the least (Pacheco and Vasconcelos 2012)—so DNA and images on winged individuals is a useful strategy to increasing our understanding of species ranges and natural history. In this case, to put a scientific name on something that can now be recognized at the species level by its barcode or BIN required field workers operating Malaise traps, imaging of specimens, DNA barcoding, and a final recognition by taxonomic specialists. While Malaise traps can be efficient tools to capture ant diversity (e.g. Longino and Colwell 1997), ant researchers are frequently unable to use the preponderance of Malaise-collected ants (winged males and queens) due to the disconnect that exists in many

taxa between worker taxonomy and reproductive (particularly male) identification (Yoshimura and Fisher 2007), a problem easily overcome with DNA barcodes (e.g. Smith et al. 2015). If publishing primary collection information, specimen photographs as well as sequence data becomes routine; such data can then be used to inform conservation and our ecological understanding of species distributions.

Acknowledgements Funding and infrastructure was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants and Canada Foundation for Innovation (CFI) grants to MAS. Ant research by MGB and JTL was supported by National Science Foundation grant DEB-1354739 (Project ADMAC) and DEB-1932405 (Ants of the World). We would like to thank Danny McIsaac and Lauren Janke for taking the Leica image and Mohamed Elmakamandy Elmankabady for taking the SEM. The ongoing work in ACG is supported by the Wege Foundation of Grand Rapids, Michigan, the International Conservation Fund of Canada (Nova Scotia), the private donors to the Guanacaste Dry Forest Conservation Fund, and the 34 ACG parataxonomists who collect, rear, and database ACG insects, the government of Costa Rica, and the University of Pennsylvania. USDA is an equal opportunity provider and employer.

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