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Acrobat ants go global – Origin, evolution and systematics of the genus *Crematogaster* (Hymenoptera: Formicidae)

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ABSTRACT

This study unravels the evolution and biogeographic history of the globally distributed ant genus *Crematogaster* on the basis of a molecular phylogeny, reconstructed from five nuclear protein-coding genes and a total of 3384 bp of sequence data. A particular emphasis is placed on the evolutionary history of these ants in the Malagasy region. Bayesian and likelihood analyses performed on a dataset of 124 *Crematogaster* ingroup taxa lend strong support for three deeply diverging phylogenetic lineages within the genus: the *Orthocrema* clade, the Global *Crematogaster* clade and the Australo-Asian *Crematogaster* clade. The 15 previous subgenera within *Crematogaster* are mostly not monophyletic. Divergence dating analyses and ancestral range reconstructions suggest that *Crematogaster* evolved in South-East Asia in the mid-Eocene (40–45 ma). The three major lineages also originated in this region in the late Oligocene/early Miocene (~24–30 ma). A first dispersal out of S-E Asia by an *Orthocrema* lineage is supported for 22–30 ma to the Afrotropical region. Successive dispersal events out of S-E Asia began in the early, and continued throughout the late Miocene. The global distribution of *Crematogaster* was achieved by subsequent colonizations of all major biogeographic regions by the *Orthocrema* and the Global *Crematogaster* clade. Molecular dating estimates and ancestral range evolution are discussed in the light of palaeogeographic changes in the S-E Asian region and an evolving ocean circulation system throughout the Eocene, Oligocene and Miocene. Eight dispersal events to/from Madagascar by *Crematogaster* are supported, with most events occurring in the late Miocene to Pliocene (5.0–9.5 ma). These results suggest that *Crematogaster* ants possess exceptional dispersal and colonization abilities, and emphasize the need for detailed investigations of traits that have contributed to the global evolutionary success of these ants.

1. Introduction

Ants are arguably one of the most abundant and ecologically dominant groups of arthropods in the world. They are able to occupy all major habitat types and ecosystems (Wilson and Hölldobler, 2005). Most ant genera, however, have succeeded only in colonizing one or a few biogeographical regions and are fairly restricted in their habitat preferences. Global distribution is rare among ant genera, but the notable exceptions to this rule are often very diverse and species-rich groups that have been ecologically highly successful. Such an example is the focal group of this study, *Crematogaster*. The genus currently comprises 467 nominal species (excluding subspecies; cf. Bolton, 2011) distributed widely in tropical and temperate latitudes, although with a much elevated diversity in subtropical and tropical regions. These ants occur mostly in forest, woodland or savannah habitats, where they inhabit both the ground as well as the canopy level. Most tropical species nest arboreally, in dead branches, under bark or in independent carton nest structures. Ground nesting probably occurs more frequently in temperate areas, and then often under stones – but some species in the tropics also have adapted to leaf litter and soil habitats (Hosoishi et al., 2010). Arboreal species of *Crematogaster* in particular can be dominant elements of the ant fauna, with polydomous and strongly territorial colonies (Blaimer, 2010; Dejean et al., 2010).

As is the case in many other widespread genera, the species-level taxonomy of *Crematogaster* ants is difficult (Brown, 1973; Ward, 2007). Many synonyms and undescribed species likely still exist, although much progress has been made recently (e.g. Blaimer, 2010, 2012a, 2012b; Hosoishi and Ogata, 2009; Longino, 2003). On the genus level, *Crematogaster* is easily recognizable by the unique dorsal attachment of the postpetiole (3rd abdominal segment) to the rest of the metasoma (i.e. the gaster). This feature constitutes the strongest morphological synapomorphy of the genus (Bolton, 2003) and also confers the ability to raise the gaster high over the rest of the body in a defensive posture. Reminding of a balancing act, this behavior gave these ants their common name: acrobat ants. Confronted with an ever-mounting diversity of species through new descriptions, taxonomists have very early on attempted to erect an internal subgeneric classification system for the genus based on morphology. Most of the currently recognized 15 subgenera were established by Forel and Santschi (for details see Blaimer, in press). These subgeneric descriptions mostly did not provide concise and

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clear diagnostic character states for identification, and their validity as natural groups in a phylogenetic sense is doubtful.

An equal conundrum is the relationship of *Crematogaster* within the largest subfamily of ants, the Myrmicinae. Bolton (2003) assigned the Asian endemic genus *Recurvidris* to the same tribe, Crematogastrini, on basis of some morphological similarities. Molecular phylogenetic studies have not been able to confirm this, nor any other close relationships with high support (Brady et al., 2006; Moreau et al., 2006; P.S. Ward pers. comm.). Brady et al. (2006) estimated a timeframe for the evolution of the Myrmicinae of ca. 80–90 ma. Within this subfamily, *Crematogaster* is placed in a well supported clade together with other genera between which relationships, however, remain unresolved (P.S. Ward, pers. comm.).

In this study, I examine the global phylogeny and biogeography of the genus *Crematogaster*, with a special focus on the Malagasy fauna. In Madagascar, *Crematogaster* is moderately diverse with 32 known (described and undescribed) species, which fall into several morphological species-groups (and five of the nominal subgenera), whose taxonomy is in the process of revision (Blaimer, 2010, 2012a). Acrobat ants in Madagascar are predominantly arboreal and one of the most conspicuous ant groups in all forest habitats. Species distribution patterns are characterized partly by widespread species found across large parts of the island, and cases of local endemism especially in mountainous regions (Blaimer, unpubl.). All Malagasy *Crematogaster* are endemic to the island, although four species also occur in the greater Malagasy region (including Comoros, Mayotte, Seychelles and the Mascarenes).

Since its last contact with India ca. 80–87 ma during Gondwanan break-up (Storey et al., 1995; Upchurch, 2008), the continental island Madagascar has remained in complete isolation from other landmasses. It is separated from the African mainland by the Mozambique channel, which is at least 430 km wide at its narrowest width. A few small oceanic islands break up this distance, with the most notable in size being the Comoros Islands. Unraveling the geographic origins of Madagascar's hyperdiverse and highly endemic biota has fueled numerous molecular phylogenetic studies. It is nowadays a widely accepted view that most of this unique species diversity has been generated by transoceanic dispersal and subsequent radiations ('neoendemisms'), rather than paleoendemisms with a Gondwanan origin (see review of Yoder and Nowak, 2006). Considering that the subfamily Myrmicinae originated only around the time of Madagascar's separation from India, *Crematogaster* therefore must have also reached the island via transoceanic dispersal. The questions remaining to be investigated are when and from where acrobat ants have colonized Madagascar, and, considering the diversity of morphological species-groups, how many dispersal events have taken place.

In this study, I reconstruct a framework phylogeny for *Crematogaster* ants to improve current understanding of relationships within the genus and to elucidate their global evolutionary and biogeographic history. I hereby first seek to reveal the phylogenetic structure within the genus and investigate whether subgenera represent monophyletic groupings. My second objective is to infer the center of origin for acrobat ants and sketch a time-calibrated picture of their subsequent spread across the world. Thirdly, I comprehensively investigate the biogeography of *Crematogaster* in the Malagasy region to understand their faunal affinities and the timeline of colonization of Madagascar by acrobat ants.

2. Materials and methods

2.1. Taxon sampling

Taxa were selected for this study with the goals of representing the phylogenetic diversity of the whole genus worldwide and the entire Malagasy *Crematogaster* species diversity. I was guided by previous subgeneric assignments and geographic distribution as indicators to select species for molecular sampling, and I attempted to sample subgenera in proportion to their size and distribution. Table 1 provides an overview of the current size and distribution of the subgenera, and indicates the number and distribution of sampled taxa. These numbers were taken from Bolton (2011), while also including some unpublished data on new species and subgeneric transfers (pers. observ.; S. Hosoishi, pers. comm.; H. Feldhaar, pers. comm.). Further included in the study are eight members of other ant genera (*Metapone*, *Vollenhovia*, *Tetramorium*, *Recurvidris*, *Leptothorax*, *Temnothorax*, *Aphaenogaster*, *Stenamma*) within the subfamily Myrmicinae, ranging from moderately to distantly related to *Crematogaster*.

2.2. Species identification and morphological observations

Crematogaster ants are challenging to identify to species level. Most specimens were identified using either reference collections or images, existing identification keys or original species descriptions in the literature. Taxa bearing the label "cf" before the species name were usually identified using literature only. This denotation indicates that identification may not be fully accurate, but that the specimen is expected to have a close morphological affinity to the applied name. Malagasy taxa labeled with code names represent undescribed species, while in cases of taxa from other regions this could mean either "undescribed" or "no identification possible". These codes are not intended to for use in formal nomenclatural purposes.

Color images of voucher specimens were created with a JVC KY-F75U digital camera, a Leica MZ16A stereomicroscope, Syncroscopy Auto-Montage (v5.0) software and Zerene Stacker (v1.02) software. These are publicly available on AntWeb (www.antweb.org). For Malagasy taxa the molecular voucher specimens have not been imaged, but representative images for respective species are available on AntWeb. Species distributions were plotted with ArcMap (v9.3) within the software ArcGIS, based on coordinates (latitude and longitude) as given in the Supplementary Table 1.

2.3. Molecular data collection

DNA was extracted from 124 ingroup specimens using a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, USA), following the manufacturer's protocol but eluting the extract in sterilized water rather than the supplied buffer and at half the suggested volume. I used primarily a non-destructive method (cuticle pierced prior to extraction), enabling me to retain and re-mount voucher specimens after extractions. In cases where multiple individuals from colony series were available, a destructive technique (entire ant pulverized) was preferred. Five nuclear protein-coding genes were selected for amplification: long wavelength rhodopsin (LW Rh, 856 bp exon /199 bp intron), arginine kinase (ArgK, 390 bp exon), carbamoylphosphate synthase (CAD, 536 bp exon/193 bp intron), wingless (Wg, 409 bp exon) and DNA topoisomerase 1 (Top1, 802 bp exon). Four of these genes are widely used for phylogenetic inference in ants and primers are available (Blaimer, 2012a; Ward and Downie, 2005; Brady et al., 2006; Moreau et al., 2006; Ward et al., 2010), primers for Top1 have recently been published by Ward and Sumnicht (2012). The sequence lengths given here refer to the aligned sequence data included in the matrix used for phylogenetic inference. Amplifications of LW Rh, ArgK, CAD, Top1 and Wg were performed using standard PCR methods outlined in Ward and Downie (2005) and sequencing reactions were analyzed on an ABI 3730 Capillary Electrophoresis Genetic Analyzer with ABI BigDye Terminator v3.1 Cycle Sequencing chemistry (Applied Biosystems Inc., Foster City, CA). Most gene fragments were successfully