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Untangling complex morphological variation: taxonomic revision of the subgenus *Crematogaster* (*Oxygyne*) in Madagascar, with insight into the evolution and biogeography of this enigmatic ant clade (Hymenoptera: Formicidae)

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Abstract. The subgenus *Crematogaster* (*Oxygyne*) shows an Old World distribution and comprises a group of ants with specialised queen morphology suggestive of temporary social parasitism. This study investigated species hypotheses for *Oxygyne* species in Madagascar by integrating morphology and mitochondrial and nuclear sequences in an iterative taxonomic approach. Phylogenetic analyses (MRBAYES and RAXML) of one mitochondrial gene (cytochrome oxidase I) and analysis of population genetic structure (STRUCTURE) based on three nuclear markers (long wavelength rhodopsin, arginine kinase, carbomoyl phosphate synthase) yielded a contrast between complex morphological variation and genetic data in six previously described species and subspecies. Thus, the taxonomy of the Malagasy *Oxygyne* is revised and three species are recognised: *Crematogaster ranavalonae* Forel, *C. agnetis* Forel and *C. marthae* Forel. The previously described *C. ranavalonae pepo* Forel, *C. ranavalonae paulinae* Forel, *C. emmae* Forel, *C. emmae laticeps* Forel, *C. inops* Forel and *C. descarpentriesi* Santschi are synonymised under *C. ranavalonae*, a species that is morphologically highly variable and shows exceptional polymorphism in the queen caste. Species descriptions and identification keys based on worker and queen ants are provided. A taxonomic synopsis, updated species list and morphological diagnosis of the entire subgenus *Oxygyne* is presented, and the subgenus *Nematocrema* is hereby synonymised under *Oxygyne*. Phylogenetic estimations (MRBAYES, RAXML, *BEAST) explore relationships between Malagasy and four other African and Asian taxa within *Oxygyne* and are based on both mitochondrial DNA and nuclear DNA sequences. The monophyly of the subgenus *Oxygyne* is strongly supported and the Malagasy *Oxygyne* form a clade that is sister to a clade of both African and an Asian taxa. Social parasitism is discussed as the cause for complex morphological variation in queens of *C. ranavalonae*, and phylogenetic results lead to the hypothesis of a single origin of this highly specialised queen caste in *Crematogaster* in the Old World. These findings highlight the need for field studies to explore the temporary parasitic behaviour and host–parasite relationships in these enigmatic ants, and to increase phylogenetic sampling to further investigate the evolution of this intriguing trait.

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Introduction

Approaches using multiple sources or types of data (e.g. morphological, molecular or behavioural) to delimit species have become increasingly common in insect systematics in recent years (e.g. Cardoso *et al.*, 2009; Padial *et al.*, 2009; Lumley & Sperling, 2010; Ross *et al.*, 2010; Schlick-Steiner *et al.*, 2010). When implemented in an explicit manner, this new modus operandi is usually referred to now as 'integrative taxonomy' and guidelines have been formalised to standardise the species delimitation process (Padial *et al.*, 2009; Schlick-Steiner *et al.*, 2010). However, criticism has been voiced that true integration over different data sources is rarely achieved in practice, as quantitative methods would be required to statistically integrate over multiple data partitions from different sources, for which currently no adequate analysis tools exist (Yeates *et al.*, 2011). Yeates *et al.* (2011) argued that most of the 'integrative' taxonomic studies are thus 'correlative' rather than integrative, as they are searching for congruence between different data sources to delimit species. These authors (Yeates *et al.*, 2011) have suggested a process of 'iterative taxonomy' that uses multiple sources of evidence to define species boundaries by testing species hypotheses erected on the basis of one type of data with another datatype. Pursuing multiple lines of evidence is especially advisable for species delimitation studies in the ants, the Formicidae, a group of insects for which intraspecific morphological variability is often high (Ward, 2007, 2009b), and cryptic species diversity frequently goes unnoticed by traditional morphological approaches (Schlick-Steiner *et al.*, 2006; Seifert & Seifert, 2009; Bernasconi *et al.*, 2011). In addition, confounding processes on the genetic level, such as hybridisation (Nonacs, 2006; Feldhaar *et al.*, 2008; Sirvio *et al.*, 2011), and recent speciation events leading to sister taxa can complicate the investigation of species boundaries in ants.

Yet species-level taxonomic studies of ants are needed for a breadth of research disciplines in ecology or conservation management that depend on reliable species identifications in this ubiquitous and ecologically dominant arthropod family. In Madagascar, ants have developed into one of the better known groups of arthropods (e.g. Fisher, 2003, 2008) in terms of distribution records and overall species richness estimations – data that are critically needed for conservation management in a biodiversity hotspot where natural habitats are steadily declining (Brooks *et al.*, 2002; Kremen *et al.*, 2008). But although recent advances in species-level taxonomy of Malagasy ants have been achieved (Fisher & Smith, 2008; Ward, 2009a; Blaimer, 2010; LaPolla *et al.*, 2010), nonetheless a majority of the larger ant clades are still in pressing need of taxonomic revision before they can reflect adequate estimates of species diversity and this information can be used in fine-scale mapping of ant diversity in Madagascar. Clarification of species identities also forms the basis for investigation of the evolutionary history of the unique Malagasy biota and its phylogenetic affinities, an intriguing area of research that has been very actively pursued for mammals (e.g. Poux *et al.*, 2008), reptiles (e.g. Raxworthy *et al.*, 2002), amphibians (e.g. Bocxlaer *et al.*, 2006) and a few groups of arthropods (e.g. Wesener *et al.*, 2010), but has not been much probed for ants because of the taxonomic impediment currently imposed on the sampling for such molecular phylogenetic studies.

The acrobat ants, genus *Crematogaster*, constitute one of the largest ant genera within the subfamily Myrmicinae and are distributed throughout the world. Similar to other large genera, the taxonomy of *Crematogaster* is heavily burdened by synonymic names (Ward, 2009b), especially in widespread species that show geographical variation in morphology, such as coloration or size. Sixteen subgenera have been erected within *Crematogaster* by several authors (Bolton, 2011), subdividing the species diversity into groupings based upon morphological characters. In Madagascar, *Crematogaster* is one of the most abundant groups of ants, often a dominant element of the forest ecosystem and nesting arboreally with polydomous colonies occupying multiple trees (Blaimer, 2010; Dejean *et al.*, 2010). *Crematogaster* species described from Madagascar have been assigned previously to five of the subgeneric groupings: *Decacrema*, *Oxygyne*, *Orthocrema*, *Mesocrema* and *Crematogaster* (sensu stricto). The genus is currently undergoing a series of taxonomic revisions for Madagascar, starting with a revision of the *Decacrema* group. A review on the natural history of *Crematogaster*, an overview of the subgenera, a summary of all described Malagasy species and a key to the Malagasy subgenera can be found in Blaimer (2010). The following study focused on a taxonomic revision of the Malagasy species of the subgenus *Oxygyne*, while establishing a phylogenetic framework to investigate the relationships of Malagasy *Oxygyne* beyond Madagascar.

The subgenus *Oxygyne* was established by Forel (1901) based on the following features: (i) masticatory margin of queen mandibles more or less completely edentate with an enlarged, pointed terminal edge (= falcate mandibles hereafter); (ii) very reduced or rudimentary developed frontal carinae in both workers and queens; (iii) overall unusual morphology and smaller size of the queens compared with queens in the rest of the genus. Emery (1922) later added the 11-segmented antenna with a weakly three-segmented club in workers to Forel's (1901) diagnosis. In practice, of the above only the falcate (sickle-shaped) mandibles of the queens can reliably identify members of this subgenus. This diagnostic mandibular character state is shared with the subgenus *Nematocrema*, which was established by Santschi (1918) and is found only in continental Africa. Due to an apparent lack of further distinctive characteristics for segregating *Nematocrema* from *Oxygyne*, an additional objective of this study was to determine whether *Nematocrema* is a synonym of *Oxygyne*.

Oxygyne is distributed in the Old World tropics, with species described from Madagascar, Africa, India and Indonesia/Malaysia. Little is known about the biology of these enigmatic ants, and they are rarely encountered compared with other *Crematogaster* species. It has been hypothesised that the queen caste in both *Oxygyne* and *Nematocrema* is adapted to temporary social parasitism [for a definition see Buschinger (2009)] in the colony founding stage, in which an inseminated

parasite queen enters the nest of a host species, where she replaces the host queen and uses the host workers to rear her own offspring (Forel, 1910; Santschi, 1934; Hölldobler & Wilson, 1990). This behaviour has been postulated on the basis of morphological characters such as the generally smaller size of these queens and the characteristic falcate mandibles, features that are seen in other known parasitic ants (Hölldobler & Wilson, 1990). A single anecdotal record exists documenting the discovery of a dealate *Oxygyne* queen in a nest of a different *Crematogaster* species; in this case, however, the host queen was still present in the nest (Forel, 1913). As deduced from the little biological data that accompanies the few collections, it appears that arboreal carton nest building is a common nesting habit of species of *Oxygyne* and *Nematocrema* (Forel, 1891, 1910, 1915; Santschi, 1934; M. Janda, personal communication; S. van Noort, personal communication; personal observation). Malagasy *Oxygyne* occur in both humid as well as in dry forest habitats and seem to nest exclusively arboreally. *Crematogaster ranavalonae* Forel is known to build carton nests housing very large colony sizes (Dejean *et al.*, 2010; personal observation), but the relatively few collections in Madagascar suggest a generally lower abundance and population sizes of *Oxygyne* species in comparison with other *Crematogaster* species.

Prior to this study, *Oxygyne* in Madagascar comprised the species and subspecies *Crematogaster ranavalonae* Forel, *C. ranavalonae pepo* Forel, *C. ranavalonae paulinae* Forel, *C. emmae* Forel, *C. emmae laticeps* Forel, *C. agnetis* Forel, *C. marthae* Forel, *C. descarpentriesi* Santschi and *C. inops* Forel (Bolton, 2011). Forel (1892) published a key, separating workers, queens and males of some of these species, but his couplets consisted of species descriptions rather than articulated diagnostic character states.

The first objective of the present study was to improve current species delimitation in Malagasy *Oxygyne*. High morphological distinctness appears to exist between queens of previously described species in a variety of characters, such as head shape, scape length, postpetiole size and pilosity. However, with the single exception of one species, *C. agnetis*, no obvious morphological characters are available to distinguish between workers. I therefore aimed to infer species boundaries in Malagasy *Oxygyne* both with morphological and molecular methods, using a multi-evidence approach that, considering recent discussions in the literature above, can best be described as 'iterative taxonomy' sensu Yeates *et al.* (2011). I specifically set out to: (i) test morphological species hypotheses against genetic variation in the mitochondrial gene cytochrome oxidase I (COI) and (ii) subsequently investigated whether genetic variation in the mitochondrial gene COI is mirrored by variation and population structure in three nuclear markers.

The second part of this study gives a review and update on the status and morphological diagnosis of the subgenera *Oxygyne* and *Nematocrema*, and further aims to provide a first insight into their evolution and biogeography by estimating a molecular phylogeny based upon four genes. Specific hypotheses hereby under scrutiny are: (iii) *Oxygyne* and *Nematocrema* are closely related and can be united into a single subgenus and (iv) the Malagasy *Oxygyne* are a monophyletic group.

Materials and methods

Morphological analysis

Specimens were examined in the following collections: CASC, California Academy of Sciences, San Francisco, CA, U.S.A.; BBBC, B. B. Blaimer Collection, University of California at Davis, CA, U.S.A.; MCZC, Museum of Comparative Zoology, Harvard, MA, U.S.A.; MHNG, Muséum d'Histoire Naturelle, Genève, Switzerland; NHMB, Naturhistorisches Museum, Basel, Switzerland; PSWC, P. S. Ward Collection, University of California at Davis, CA, U.S.A.; UCDC, Bohart Museum of Entomology, University of California, Davis, U.S.A.; ZMBH, Museum für Naturkunde der Humboldt Universität, Berlin, Germany.

All morphological observations were made with a Leica MZ12.5 stereomicroscope. Standard measurements (in mm) were taken at 50×, or rarely at 25× (some queen measurements) with a Wild M5A stereomicroscope and a dual-axis Nikon micrometer wired to a digital readout. Measurements were recorded to the nearest 0.01 mm. Ranges are always presented as minimum – maximum values. Indices are presented as decimal fractions to two decimal places. For details on standardisations and abbreviations used for measurements, indices and descriptions of sculpture and size ranges see Blaimer (2010). One index is newly established in this study, the petiole–postpetiole index, and is calculated as postpetiole width divided by petiole width. I further created scatter plots in Microsoft Excel to visualise a selection of measurements and indices.

Colour images 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. All images of ants presented here are also publicly available on AntWeb (www.antweb.org). Line drawings were produced by tracing colour images in Adobe Illustrator CS5. Species distributions were plotted with ARCMAP (v9.3) within the software ARCGIS, based on coordinates (latitude and longitude) as given on the specimen labels of all material. For material lacking this information the following sources were used to georeference collection sites: United States Board on Geographic Names (1989), Viette (1991), the GEOnet Names Server (National Geospatial-Intelligence Agency, 2010) and the Gazetteer to Malagasy Botanical Collecting Localities (Schatz & Lescot, 2003). Classification of major geographical regions in Madagascar throughout species descriptions follows Gautier & Goodman (2003). Common abbreviations within locality data are: F, Forêt; P.C., Parc Naturel Communautaire; P.N., Parc National; R.N.I., Réserve Naturelle Intégrale; R.S., Réserve Spéciale.

The International Commission on Zoological Nomenclature (1999) requires lectotypes designated after 1999 to 'contain an express statement of deliberate designation' (amended Article 74.7.3). I use the statement 'hereby designated the lectotype' to fulfil this requirement. Lectotypes have been

designated where a name lacks a holotype or lectotype and unambiguous syntypes have been identified. The purpose is to provide stability of nomenclature, and designation is done in a revisionary context in agreement with the amended Recommendation 74G of Article 74.7.3.

Taxon sampling

Locality information on all specimens examined within morphological studies is given in the context of species description (see Results S1 in electronic material) and in Table S1, and further in Table S2 for non-Malagasy taxa. Only worker and queen ants were included in the study, males were excluded as collections are sparse. Most samples did not stem from colony series collections, and thus associated worker and queen ants of the same colony and species were not available to study. Most queen ants were collected as alates from malaise trap samples. In total, 28 ant specimens belonging to the *Oxygyne* group from Madagascar were selected to be sampled for genetic data. Twenty-six individuals represented the full morphological diversity as it is seen in the syntype specimens of the previously described species *C. ranavalonae*, *C. emmae*, *C. descarpentriesi*, *C. inops* and all of their subspecies. These 26 individuals included 20 worker ants and six queen ants. The remaining two sampled worker ants represented *C. agnetis*. One of the previously described species, *C. marthae*, was not represented in the molecular study as no material of this species was available that was suitable for DNA extraction. Seven individuals representing four species currently placed within the subgenera *Oxygyne* and *Nematocrema* from Africa, India, Thailand and New Guinea, and one representative each of three Malagasy species currently placed in the subgenera *Crematogaster* (*C. sp. BBB43* and *C. sp. BBB51*) and *Mesocrema* (*C. rasoherinae* Forel) were further included in this molecular dataset (voucher images of these species have been deposited on www.antweb.org; specimen codes of these are given in Table S3). These latter outgroups were chosen to represent three separate clades emerging from a preliminary analysis estimating a broad-scale, genus-level phylogeny for *Crematogaster* (Blaimer, in preparation). These three clades range from moderately to very distantly related to the *Oxygyne* + *Nematocrema* ingroup. Details on locality data of all specimens included in the molecular analyses are given in Table S1.

Molecular data collection

DNA was extracted from all 38 specimens using a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, U.S.A.), following the manufacturer's protocol, but eluting the extract in sterilised water rather than the supplied buffer and at half the suggested volume. A nondestructive method (cuticle pierced prior to extraction) was used to enable voucher specimens to be retained and re-mounted after extractions. In cases where multiple individuals from colony series were available, a destructive technique (entire ant pulverised) was preferred. Destructive extraction was further performed on previously harvested legs of 28 of these specimens at the Biodiversity Institute of Ontario, University of Guelph, using extraction protocols as outlined in Smith & Fisher (2009).

Four genes were selected for amplification that are widely used for phylogenetic inference in ants and for which primers are available (Ward & Downie, 2005; Brady *et al.*, 2006; Moreau *et al.*, 2006; Smith & Fisher, 2009; Ward *et al.*, 2010). Among these are the three nuclear protein-coding genes long wavelength rhodopsin (LW Rh, 1095–1117 bp), arginine kinase (ArgK, 527–557 bp) and carbomoyl phosphate synthase (CAD, 747–755 bp), as well as the mitochondrial gene COI (594–604 bp). The sequence lengths given here refer to the unambiguous and aligned sequence data used in phylogenetic inference and population genetic analyses; these numbers vary due to variation in intron lengths and exclusion of ambiguous characters between different aligned taxon sets. A total of ~3000 bp of sequence data was used for phylogenetic inference. Mostly these data have been successfully obtained from all 38 specimens, except as follows. For one taxon (C_rana03_mds) COI data are lacking, and for three taxa (*C. meijerei*_1, *C. meijerei*_2, C_rana12_adn) sequence data from one gene fragment (LW Rh, COI, ArgK, respectively) was only partially obtained. All sequences have been deposited in GenBank, with accession numbers listed in Table 1.

The primers used in amplifications and sequencing are listed in Table 2. Amplifications of LW Rh, ArgK, CAD for all 38 taxa, and of COI for 10 taxa were performed using standard PCR methods outlined in Ward & Downie (2005) and sequencing reactions were analysed on an ABI 3730 Capillary Electrophoresis Genetic Analyser with ABI BigDye Terminator v3.1 Cycle Sequencing chemistry (Applied Biosystems Inc., Foster City, CA, U.S.A.). Sequence data for COI for the remaining taxa were obtained at the Biodiversity Institute of Ontario, with amplification and sequencing protocols as detailed in Smith & Fisher (2009).

Phylogenetic inference

Sequence data were assembled and edited in the program SEQUENCHER 4.6 (Gene Codes Corporation, 2006, Ann Arbor, MI, U.S.A.), aligned in CLUSTALX 2.0.12 (Thompson *et al.*, 1997; Larkin *et al.*, 2007) and unambiguous misalignments were manually realigned in MACCLADE 4.08 (Maddison & Maddison, 2000).

Phylogenetic inference was performed on two separate datasets with different taxon sampling (see Table S1). Dataset A (29 taxa) included all individuals of Malagasy *Oxygyne* and one African *Oxygyne* species (outgroup) and was used to investigate the molecular taxonomy of the ingroup. Dataset B (17 taxa) consisted of seven individuals of the Malagasy ingroup taxa, the four non-Malagasy species of the *Oxygyne* and *Nematocrema* group, and the three *Crematogaster* sp. outgroups. This dataset was assembled to infer the phylogenetic and biogeographical relationships within *Oxygyne* as a whole.

Both datasets A and B were analysed within a Bayesian framework using MRBAYES 3.1 (Ronquist & Huelsenbeck, 2003), accessed through the CIPRES science gateway (Miller *et al.*, 2010). For dataset A, analyses were performed on each of the four loci independently, and also on a concatenated datamatrix of the three nuclear loci; analysis of dataset B

Table 1. Genbank accession numbers assigned to sequences used in phylogenetic inference.

Specimen information		Genbank accession numbers			
Species	Voucher specimen	Long wavelength rhodopsin	Arginine kinase	Carbomoyl phosphate synthase	Cytochrome oxidase I
Crematogaster					
ranavalonae	CASENT0489246	JN129935	JN129899	JN129861	HM880780
ranavalonae	CASENT0073574	JN129952	JN129901	JN129873	HM880739
ranavalonae	CASENT0151874	JN129937	JN129907	JN129862	HM880756
ranavalonae	CASENT0486407	JN129950	JN129912	JN129854	HM880778
ranavalonae	CASENT0125550	JN129949	JN129904	JN129867	HM880747
ranavalonae	CASENT0156540	JN129934	JN129919	JN129878	JN129966
ranavalonae	CASENT0491513	JN129929	JN129914	JN129869	HM880715
ranavalonae	CASENT0485141	JN129954	JN129909	JN129856	HM880775
ranavalonae	CASENT0193425	JN129942	JN129891	JN129871	HQ547862
ranavalonae	CASENT0036095	JN129953	JN129903	JN129858	HM880721
ranavalonae	CASENT0060762	JN129931	JN129905	JN129876	HM880729
ranavalonae	CASENT0119122	JN129939	JN129906	JN129864	N/A
ranavalonae	CASENT0193054*	N/A	N/A	N/A	HQ925612
ranavalonae	CASENT0193164*	JN129944	JN129915	JN129879	N/A
ranavalonae	CASENT0057288	JN129943	JN129902	JN129872	HM880728
ranavalonae	CASENT0121558	JN129932	JN129918	JN129870	HM880746
ranavalonae	CASENT0067031	JN129951	JN129913	JN129877	HM880732
ranavalonae	CASENT0193531	JN129947	JN129910	JN129865	HQ547877
ranavalonae	CASENT0433728	JN129946	JN129892	JN129874	HM880773
ranavalonae	CASENT0423151	JN129940	JN129893	JN129868	HM880767
ranavalonae	CASENT0490101**	N/A	N/A	N/A	HQ925651
ranavalonae	CASENT0021249**	JN129936	JN129911	JN129875	N/A
ranavalonae	CASENT0038502	JN129930	JN129908	JN129860	HM880722
ranavalonae	CASENT0068800	JN129938	JN129916	JN129857	HM880735
ranavalonae	CASENT0136353	JN129945	JN129917	JN129859	HM880753
ranavalonae	CASENT0477030	JN129933	JN129900	JN129855	HQ925649
ranavalonae	CASENT0066530	JN129925	JN129922	JN129863	HM880730
ranavalonae	CASENT0193261	JN129948	JN129900	JN129866	HQ547828
agnetis	CASENT0051228	JN129941	JN129888	JN129885	JN129971
agnetis	CASENT0487780	JN129961	JN129898	JN129853	JN129967
aberrans	CASENT0193779	JN129955	JN129894	JN129881	JN129962
aberrans	CASENT0193687	JN129956	JN129895	JN129848	JN129963
santschii	CASENT0193640	JN129924	JN129889	JN129889	JN129970
meijerei	CASENT0193685	JN129926	JN129886	JN129851	JN129968
meijerei	CASENT0193683	JN129927	JN129887	JN129852	JN129964
stadelmanni	CASENT0193573	JN129928	JN129896	JN129880	JN129965
stadelmanni	CASENT0094528	JN129959	JN129897	JN129883	JN129969
rasoherinae	CASENT0193413	JN129958	JN129923	JN129882	JN197295
sp.BBB43	CASENT0193399	JN129960	JN129920	JN129884	HQ547861
sp.BBB51	CASENT0193264	JN129957	JN129921	JN129850	HQ547829

*and **denote cases where separate individuals from the same colony have been used in DNA extraction.

was implemented on a concatenated datamatrix of all four loci. Dataset B was also analysed under a coalescent approach to estimate a species tree from multilocus sequence data, using the *BEAST method as implemented in the program BEASTv1.6.1 (Drummond & Rambaut, 2007). *BEAST uses a multispecies coalescent model within a Bayesian framework to jointly infer a species tree topology, divergence times and gene trees from sequence data (Heled & Drummond, 2010). The three outgroup taxa were pruned prior to this analysis. Further analyses were carried out on dataset A (COI only) and B (concatenated, four-locus matrix) under a maximum likelihood (ML) framework using RAXML GUIV.0.93 (Stamatakis, 2006; Silvestro & Michalak, 2010).

The following partitioning scheme was employed for all MRBAYES analyses on single loci and concatenated, multiple-locus data matrices, and for *BEAST analyses. Each gene was partitioned by translational pattern (exon, intron) and by codon position (first + second positions vs third position). Best-fitting models of nucleotide sequence evolution were selected for each partition using the Akaike information criterion in the program MRMODELTEST v2.3 (Posada & Crandall, 1998; Nylander, 2004), executed through PAUP* 4.0b10 (Swofford, 2000). Details on sizes of partitions and selected models can be found in Table 3. MRBAYES analyses each employed two runs of Metropolis-coupled Markov Chain Monte Carlo (MCMC) consisting of four chains and

Table 2. Primers used for amplification and sequencing of long wavelength rhodopsin (LR), arginine kinase (AK), rudimentary (CD) and cytochrome oxidase I (COI).

Primer	Sequence (5′-3′)	Source
LR-134F	ACM GTR GTD GAC AAA GTK CCA CC	A
LR-143F	GAC AAA GTK CCA CCR GAR ATG CT	A
LR-395Fcr	ATC AAC TGC TAT TAY GAG ACT TGG	G
LR-398Fcr	AAC TGC TAT TAY GAG ACT TGG GT	G
LR-482Fcr	ATA TGG ACG ATG ACR ATG ATC GC	I
LR-503Fcr	GCA TTC GAT AGG TAC AAT GTA ATY GT	I
LR-798F	GCH GCY CAY GAG AAG AAY ATG CG	C
LR-480R	GA GCC ACA TCC RAA CAG RGA ACC	A
LR-508R	GAA YGC RAT CAT CGT CAT YGT CCA	A
LR-639ER	YTTAC CG RTT CCA TCC RAA CA	A
LR-855R	GA TCG YAR VGA AGC RAC GTT CAT	C
LR-1047R	GG ATT RTA YAC RGC RTT GGC TTT BGC	C
LR-1065ER	AC CT RAT RCC RTA TAC RAT VGG ATT	C
AK-1F2	ATG GTT GAY GCY GCY GTT YTG GA	B
AK-4F2	GTT GAY GCY GCY GTT YTG GAY AA	B
AK-244Fcr	GAT CCC ATC ATC GAC GAC TAC CA	H
AK-286F	GAY AAR CAY CCG CCM AAR GAY TT	B
AK-308Rcr	AA GTC CTT GGG TGG ATG CTT GTC	H
AK-345ERcr	ACT TAC GGT GGG GTC GAG ATT GC	H
AK-446Rcr	TC TTC CAT TTC TTT ATA TTG CGC	I
AK-461Rcr	GT GCT GGA CAC CTT TTC TTC CAT	H
CD-892F	GGY ACC GGR CGT TGY TAY ATG AC	B
CD-909F	C ATG ACY TCR CAR AAT CAY GGA TTY TG	B
CD-1258Fcr	CAG GCT GGA GAA TTY GAT TAT TCG GG	H
CD-1276Fcr	TAT TCG GGY TCR CAA GCG ATT AAA GC	H
CD-1388Rcr	TAT ACT TTG TCA GCC ATT CCT TTT GA	H
CD-1491R	GCC GCA RTT NAG RGC RGT YTG YCC	B
CD-1592R	GC RAA YAT YTT YCT RTC YTC RGT	B
COI-1810F (= LF1)*	AT TCA ACC AAT CAT AAA GAT ATT GG	E
COI-2067Fcr	TAY CCN CGN WTA AAY AAY ATR AGD TT	I
COI-2185Rcr	GC TAR NGG NGG RTA RAY NGT YCA	I
COI-2518R2 (= LR1)*	TA AAC TTC AGG GTG ACC AAA AAA TCA	E
COI-TRL-3382R	TY CAWT GCAC TTAW TCTG CCAT ATTA	D
C_ANTMR1D-RonIIdeg_R**	GGRGGRTARAYAGTTCATCCWGTWCC	F
C_ANTMR1D-AMR1deg_R**	CAWCCWGTWCCKRMNCCWKCAT	F
COI-RonMWASPdeg_t1**	TGTAAAACGACGGCCAGTGGWTCWCCWGATATAKCWTTTCC	J

*COI primers were used both by the author at UC Davis and the Biodiversity Institute of Ontario.
**COI primers that were only used at the Biodiversity Institute of Ontario.
Primers that have been modified for this study (suffix 'cr') are customised to provide a better match for *Crematogaster* sequences.
Sources: A, Ward & Downie (2005); B, Ward *et al.* (2010); C, P. S. Ward (personal communication); D, P. S. Ward (personal communication) (modified from Simon *et al.*, 1994); E, Smith *et al.* (2005); F, Fisher & Smith (2008); G, this study, modified from Ward & Downie (2005); H, this study, modified from Ward *et al.* (2010); I, this study, modified from P. S. Ward (personal communication); J, M. A. Smith (unpublished data).

sampling every 1000 generations. The model parameters transition–transversion ratio, gamma shape, proportion of invariable sites, rate matrix and state frequencies were unlinked across partitions, and a variable rate prior was employed to allow for rate variation among partitions. For dataset A, single gene analyses were run for a length of 20 million generations and multilocus analyses for 50 million generations. MRBAYES analyses on dataset B involved 20 million generations, whereas *BEAST analyses were performed for 30 million generations. In TRACER v.1.5 (Rambaut & Drummond, 2007) convergence of MCMC chains was assessed visually and mixing of chains by evaluating effective sample size values for all parameters. Between 20 and 25% of sampled trees were discarded as burnin and the remaining trees were summarised as majority-rule consensus trees. The differing lengths of MCMC chains were determined by initially running each analysis for 20 million generations, assessing convergence and mixing of chains and increasing length where necessary. ML analyses uniformly applied a GTRGAMMA substitution model to both datasets and were partitioned by gene and codon position. These analyses included a joint ML and thorough bootstrap search with 1000 bootstrap replicates. Each of the analyses described above was performed twice with identical settings to confirm the stability of the results.

In PAUP* 4.0b10 (Swofford, 2000) pairwise distances were calculated from the mitochondrial sequences to assess genetic

Table 3. Data on partitions, including number of bases, number of variable characters (VC), number of parsimony-informative characters (PIC) and substitution models selected for the respective partition using the Akaike information criterion in MRMODELTEST v2.3 (Posada & Crandall, 1998; Nylander, 2004).

	Dataset A				Dataset B – 17 taxa (BI, ML)				Dataset B – 14 taxa (*BEAST)			
Partition	No. bases	No. VC	No. PIC	Substitution model	No. bases	No. VC	No. PIC	Substitution model	No. bases	No. VC	No. PIC	Substitution model
COI	594	181	142	GTR + I + G	604	244	212	GTR + I + G	604	211	182	GTR + I + G
COI position 1 + 2	396	31	25	HKY + I	403	65	47	HKY + I + G	403	46	37	HKY + I
COI position 3	198	150	117	GTR + G	201	179	165	GTR + G	201	165	145	GTR + G
LW Rh	1095	24	17	HKY + I	1117	132	50	HKY + G	1108	58	36	HKY + I
LW Rh exons position 1 + 2	580	5	3	HKY	576	27	6	GTR			n/a	
LW Rh exons position 3	289	13	9	HKY + I	289	54	26	HKY + I			n/a	
LW Rh introns	226	6	5	HKY + I	252	51	18	HKY + I			n/a	
ArgK	557	18	7	HKY	527	67	26	GTR	514	30	21	HKY
ArgK exons position 1 + 2	290	7	3	HKY	261	10	7	HKY + I			n/a	
ArgK exons position 3	146	5	1	HKY	131	32	10	HKY			n/a	
ArgK intron	121	6	3	HKY	135	25	9	HKY			n/a	
CAD	755	24	9	HKY	747	107	43	HKY + G	744	10	30	HKY + I
CAD exons position 1 + 2	366	3	2	HKY	377	22	6	HKY			n/a	
CAD exons position 3	184	10	4	HKY	189	41	18	HKY + I			n/a	
CAD introns	205	11	3	HKY	181	44	19	HKY			n/a	
All genes	3001				2995				2970			

COI, cytochrome oxidase I; LW Rh, long wavelength rhodopsin; ArgK, arginine kinase; CAD, carbomoyl phosphate synthase, ML, maximum likelihood; BI.

divergence within and between Malagasy *Oxygyne* species; both uncorrected p distances and Tamura Nei distances are reported (Tamura & Nei, 1993), which correct for multiple substitutions per site and sequence saturation.

Bayesian structure analysis

Haplotypes were reconstructed for nuclear loci of 26 Malagasy *Oxygyne* individuals using the algorithmic approach implemented in PHASE v2.1.1 (Stephens *et al.*, 2001; Stephens & Donnelly, 2003), performing five independent analyses on each locus with the default settings suggested in the program manual. Haplotypes reconstructed with $P \geq 0.9$ were summarised in a genotype matrix, reconstructions with a lower P ($n = 5$) were excluded by coding these as missing data. Analysis of population structure was then performed on this genotype matrix of the nuclear loci in the program STRUCTURE v2.3.3 (Pritchard *et al.*, 2000). I ran a series of analyses that assumed a specific number of populations (K), with a range of K from 1 to 8. This range of possible populations was derived from the maximum number of genetic clusters observed in phylogenetic analysis of the mitochondrial data. Ten independent analyses were run with a length of 1 million generations (burnin = 100 000), employing the default model and parameter settings of the program. STRUCTURE outputs include the estimated ln probability of the data (ln $\Pr(X/K)$), a 'goodness of fit' parameter that assesses the probability of the data being derived from the number of populations specified. The results were summarised graphically by plotting ln $\Pr(X/K)$ over K for all runs, and by further calculating harmonic means of ln $\Pr(X/K)$ across separate analyses for each K. A second important output of STRUCTURE is the Q-matrix, which gives the estimated membership coefficients for each individual to each cluster. Q-matrices of the K receiving the highest probability, $K = 3$, were summarised using the program CLUMPP (Jakobsson & Rosenberg, 2007), which optimally aligns n-outputs from replicate cluster analyses. This summary Q-matrix was then graphically displayed using DISTRUCT v1.1 (Rosenberg, 2004). For $K = 5$ ln $\Pr(X/K)$ was only marginally worse and results for Q were also summarised.

Results

Taxonomy of the Malagasy *Oxygyne* species

Morphological species. Queen ants were initially sorted into eight morphospecies. One of these represented the queen of *C. agnetis*, the remaining seven were further reduced to three forms (A, B, C) to which queen syntype specimens of *C. ranavalonae* and *C. emmae*, including their subspecies, were associated as follows. Queen A includes *C. ranavalonae pepo* syntypes; B includes *C. ranavalonae* syntypes; C includes *C. emmae* and *C. emmae laticeps* syntypes. These three forms are morphologically recognisable with a combination of characters (see Results S1). None of the recently collected queen specimens represented *C. marthae*; this species is quite distinct by having four teeth on the mandibular margin. The original descriptions of *C. descarpentriesi* and *C. inops* were entirely based upon workers and queens are undescribed. Morphometric plots (Figures S1–S5) of scape index and leg–body index support *C. agnetis* and *C. marthae* as well distinct from the rest of the three queen morphotypes by virtue of having shorter legs and shorter scapes. Cephalic index, petiole–postpetiole index and body size on the other hand can separate *C. agnetis* from *C. marthae*, but neither of the two with certainty from the other queen forms. All plots

further demonstrated that queen forms A, B, C show discrete variation in the above characters, but cannot be fully separated by these measurements.

All worker specimens available were separated into 13 morphological species and one of these was easily identified as *C. agnetis*. The remaining 12 forms present a wide range of continuous morphological diversity, while at the same time lacking clear-cut, diagnostic characters. Similarly, considerable variation exists between individual workers within in a syntype series, and distinct segregating characters between workers of syntype series of *C. ranavalonae* including subspecies, *C. emmae* including subspecies, *C. descarpentriesi* and *C. inops* are lacking. Morphometric plots of cephalic index, scape index, petiole width index and body size depict this range of variation and further show that values for the respective type specimens fall well within this range (Figures S6–S9). The syntype workers of *C. marthae* can be distinguished from the '*C. ranavalonae-emmae-descarpentriesi-inops*' material by a few characters (see below), but they were not represented in the examined material, apart from the syntype series and associated material in historic collections.

Molecular taxonomy. Both MRBAYES and ML analyses of the mitochondrial data showed *C. agnetis* as genetically distinct from a clade that includes all remaining worker and queen morphospecies (Fig. 1). In the topology resulting from MRBAYES analyses, this clade is further subdivided into two quite deeply diverging clades (Fig. 1, node A and B), but this initial split is not highly supported (PP node A = 0.84; node B = 0.83) and clade B is not recovered by the ML topology (see Figure S10). Here the respective taxa form a grade, and clade A receives only moderate bootstrap support of 78. The larger clade (A) further shows deep levels of mitochondrial divergence and is subdivided into a number of specimen clusters (Fig. 1, node labels I, II, III, IV, V), but these only partly correspond with queen morphology. Queen form B (associated with *C. ranavalonae* syntypes) and form C (associated with *C. emmae* and *C. emmae laticeps* syntypes) fall into well-supported clusters III and V, respectively, whereas form A appears in two separate clusters, I and IV. The 12 worker morphospecies are distributed across the genetic clusters without any obvious pattern, and multiple individuals sampled per morphospecies mostly do not belong to the same genetic cluster. Overall, mitochondrial genetic

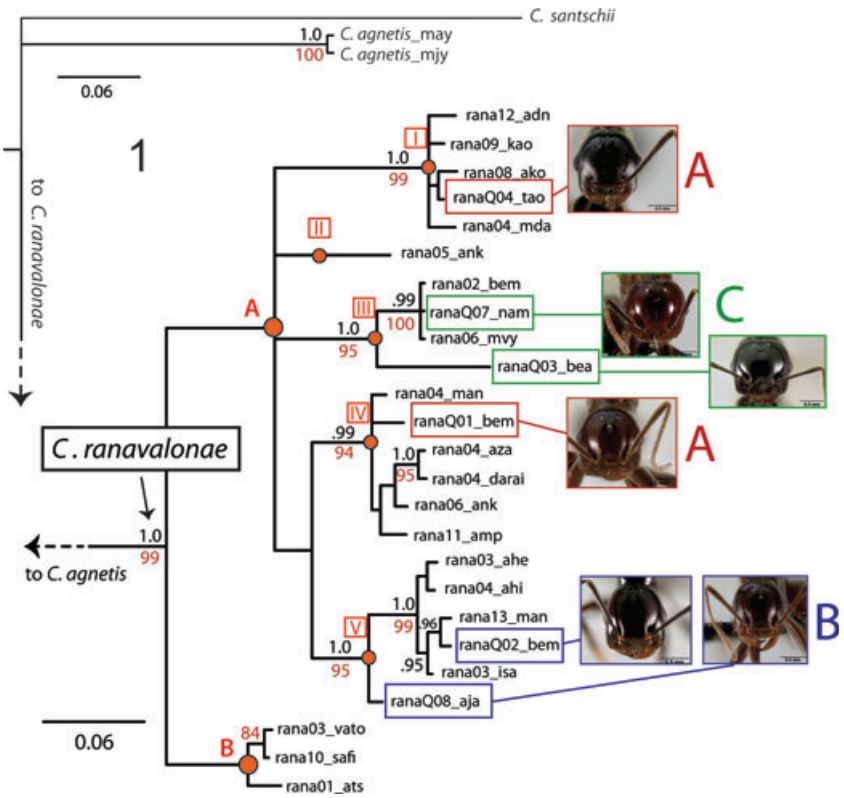


Fig. 1. Bayesian phylogeny based on 594 bp of cytochrome oxidase I (COI) and dataset A. Only posterior probabilities (in black) ≥0.95 are shown; bootstrap (in red) values from maximum likelihood analyses > 80 are mapped onto corresponding nodes. Scale bars represent estimated substitutions per site. However, these do not directly correspond with observed genetic distances (refer to Table S4 instead). The long branch leading to *Crematogaster agnetis* and *C. santschii* has been pruned and reduced in size for display. The morphological forms (A), (B), (C) of *C. ranavalonae* queens (nomenclature postrevision), as described in Results S1 (electronic material), are indicated. Labelled clades or lineages A, B and I, II, III, IV and V are discussed in the text and correspond with denotations in Table S4. Nodes A and B received support values of 0.84/78 and 0.83/−, respectively.

divergence within the entire clade (A + B) is moderate to high, with a mean distance of 6.2/6.7% and a maximum distance of 9.3/10.3% (see Table S4), referring to uncorrected p and Tamura-Nei distances, respectively. Genetic distances within and between the two deep clades and subclusters are summarised in Table S4, and distances of these to C. *agnetis* are further given. The here-observed level of mitochondrial variation within the *C. ranavalonae* clade may seem relatively high (0–9.3% uncorrected p, or 0–10.3% Tamura-Nei), but this remains comparable with levels known from other 'good' species within *Crematogaster* in Madagascar, where isolated populations within species can show COI sequence divergences of up to 12% (Blaimer, 2010; B. B. Blaimer, in preparation). It should be noted that both model-based phylogenetic methods (MRBAYES and RAXML) produced inflated estimates of substitution rates from the COI data, probably overestimating the occurrence of multiple hits, and the resulting branch lengths were therefore in disagreement with the calculated genetic distances. MRBAYES analyses of the nuclear data further showed little phylogenetic resolution between specimens sampled within *C. ranavalonae* (Figure S11), but consistently recovered *C. ranavalonae* and *C. agnetis* as distinct clades.

STRUCTURE was used to estimate population genetic structure from the nuclear data and possible correlations to genetic clustering in mitochondrial DNA (mtDNA) were investigated. Plots of the estimated log probability of the data for replicated STRUCTURE analyses showed a plateau (decrease in – ln values) at and above $K = 3$ (Figure S12), but all except one of 10 runs for $K = 5$ gave only a slightly worse probability, and values of all $K > 2$ were fairly close together. Summarised bar plots of Q (membership coefficient for each individual in each cluster) for $K = 3$ (Fig. 2) and $K = 5$ (Figure S12) revealed an almost equal assignment of each individual to each cluster. The greatest deviation from this absence of pattern can be seen for five individuals (*rana08_ako*, *rana02_bem*, *ranaQ07_nam*, *rana06_mvy*, *rana04_man*) that are assigned with a coefficient

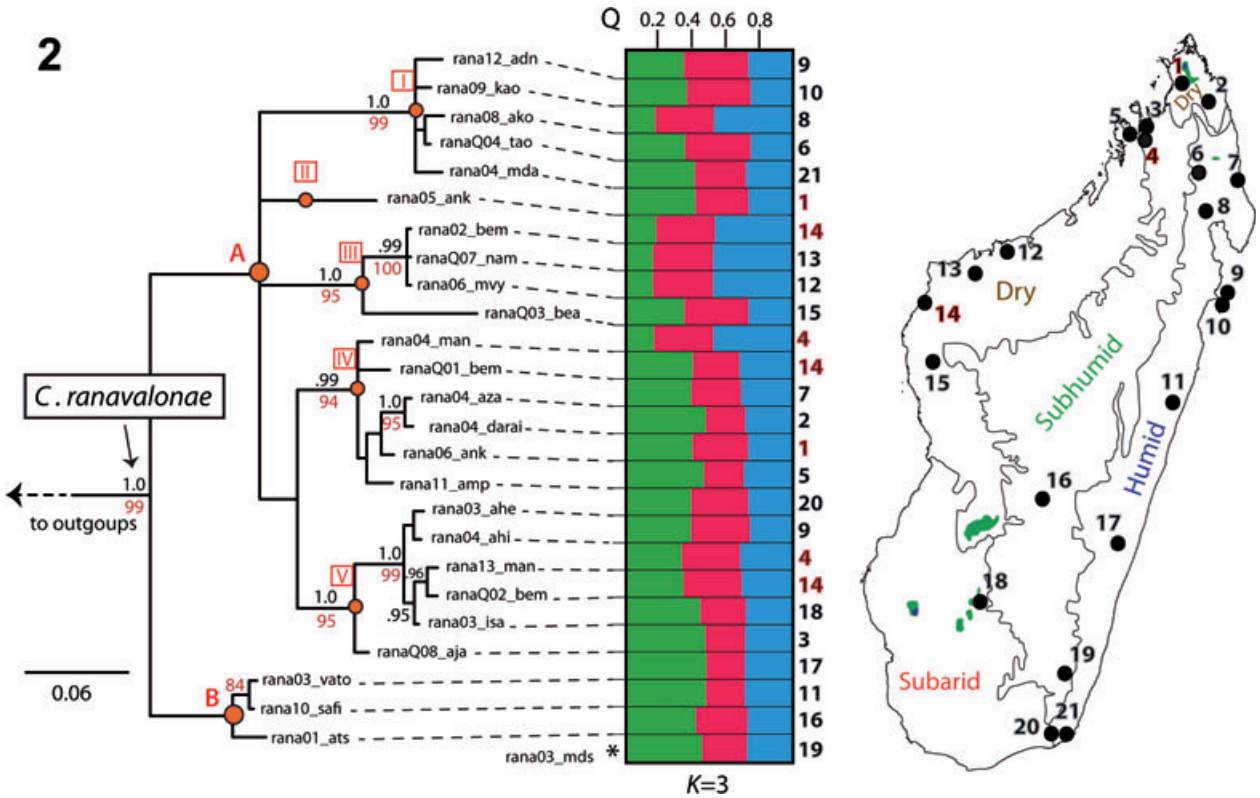


Fig. 2. Correspondence between clusters in the Bayesian mitochondrial DNA phylogeny, nuclear clusters resulting from the STRUCTURE analysis with $K = 3$ and geographical origin of populations. The phylogeny is identical to Fig. 1, but *Crematogaster agnetis* and *C. santschii* have been removed. Individual specimens in the phylogeny are mapped to corresponding individuals represented by the STRUCTURE bar plots to their right. Bar plots show summarised results from 10 independent runs. One taxon (*) is represented in the bar plot, but not in the mitochondrial DNA phylogeny. Numbers beside bars refer to localities; numbers in red highlight localities from which multiple individuals were included. K = number of populations; Q = estimated membership coefficient for each individual in each cluster. Map of sampling localities is based upon GPS collection data as listed in Table S1. Locality codes are: 1, P.N. Ankarana; 2, Daraina; 3, Ambanja; 4, R.S. Manongarivo; 5, Ampasindava; 6, Betaolana; 7, Ambanitaza; 8, Mt. Akirindro; 9, Ambohidena; 10, Kalalao; 11, Sahafina; 12, Mahavavy River; 13, P.N. Namoroka; 14, P.N. Bemarivo; 15, Rés. Beanka; 16, Atsirakambiaty; 17, F.C. Vatovavy; 18, P.N. Isalo; 19, P.N. Befotaka-Midongy; 20, P.N. Andohahela; 21, Mandena. Major habitat types in Madagascar are depicted as humid, subhumid, dry and subarid. Isolated patches of habitat correspond to the colour coding used for labels of the broad regions.

of almost 0.5 to one cluster in analyses for both *K*s (Fig. 2; Figure S12 in blue). Overall, these results demonstrate only minor population genetic structure, and this little structure is not in alignment with the genetic clusters observed in the mtDNA data, as highlighted by Fig. 2. Contrasting locality data with the STRUCTURE bar plots and the mtDNA phylogeny (Fig. 2) further revealed that some of the genetic clustering corresponds with geographical proximity of sampling. The best examples therefore present the cluster A-III, which is entirely drawn from localities in the west of Madagascar, and the divergent cluster B, which consists of central-eastern localities. However, geography cannot fully explain the clustering seen in the mitochondrial data. This is illustrated by the three incidences where multiple specimens have been sampled per locality, but these do not group as each other's closest relative (see Fig. 2, locality numbers 1, 4, 14 in red).

Species delimitation. Based on the absence of a pattern correlating the observed genetic differentiation with the morphological variation and the lack of morphological characters to identify worker ants, the existing species names *C. ranavalonae pepo*, *C. ranavalonae paulinae*, *C. emmae*, *C. emmae laticeps*, *C. inops* and *C. descarpentriesi* are therefore in the following treatment synonymised under *C. ranavalonae*. *Crematogaster marthae* is retained as a separate species, as queens show very distinct mandibular characteristics (see below).

Synonymic list of Malagasy *Crematogaster (Oxygyne)* species, as established by this study

agnetis Forel 1892
marthae Forel 1892
ranavalonae Forel 1887
= *descarpentriesi* Santschi 1928 **syn.n.**
= *emmae* Forel 1891 **syn.n.**
= *emmae* var. *laticeps* Forel 1892 **syn.n.**
= *inops* Forel 1892 **syn.n.**
= *ranavalonae* subsp. *paulinae* Forel 1892 **syn.n.**
= *ranavalonae* var. *pepo* Forel 1922 **syn.n.**

Lectotype designations

Crematogaster agnetis Forel, A. 1892: 531 (q), 533 (w.), 534 (m.) (diagnoses in keys). Worker, queen and male syntypes from Madagascar: Amparafarafantsiv (= Mangoroufer on labels) (M. Sikora) (MHNG, examined). One worker syntype (CASENT0101731, top specimen of 2w. on one pin, image on AntWeb) is hereby designated the LECTOTYPE.

Crematogaster marthae Forel, 1892: 529 (q.), 534 (w.) (diagnoses in key). Queen and worker syntypes from Madagascar: Mangoroufer (= Amparafarafantsiv) (M. Sikora) (MHNG, examined). One worker syntype (CASENT0101802, top specimen of 3w on one pin; image on AntWeb) hereby designated the LECTOTYPE.

Crematogaster ranavalonis Forel, A. 1887: 388. (Justified emendation of spelling to *ranavalonae*: Forel, 1891: 184.) Worker syntypes from Madagascar: Bois l'Ivondro près de Tamatave (C.Keller) (MHNG, examined). One worker syntype (CASENT0101762, top specimen of 3w on one pin, image on AntWeb) hereby designated the LECTOTYPE.

Identification keys to Malagasy *Oxygyne*

Key to the workers of the Crematogaster (Oxygyne) group in Madagascar.

1. Antennal scapes shorter (scape index 0.74–0.85) and not or only just surpassing head margin; head sculpture aciculate to weakly reticulate 2
– Antennal scapes usually longer (scape index 0.82–1.00) and easily surpassing head margin; head sculpture mostly finely to coarsely reticulate, sometimes aciculate . . . . . *ranavalonae*
2(1). Mesonotum distinctly raised with respect to pronotum and propodeum (Fig. 3a); promesonotal suture very distinct (Fig. 4) . . . . . *agnetis*
– Mesonotum only slightly raised with respect to pronotum (Fig. 3b); promesonotal suture indistinct (Fig. 5) . . . . *marthae*

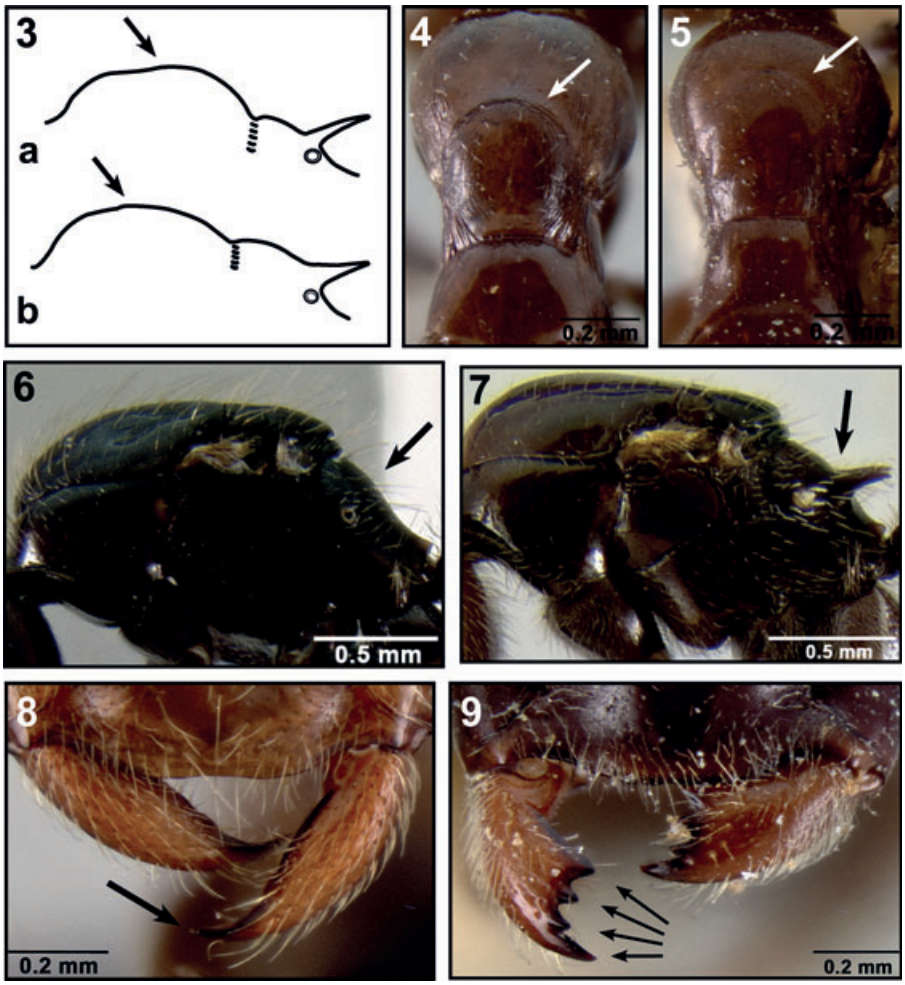
Key to the queens of the Crematogaster (Oxygyne) group in Madagascar.

1. Propodeal spines absent (Fig. 6) . . . . . *agnetis*
– Propodeal spines present (Fig. 7) 2
2(1). Mandibles falcate, apical tooth enlarged and acute, masticatory margin without teeth except one min tooth may be present (Fig. 8); erect pilosity present on eyes . . . . . *ranavalonae*
– Mandibles of normal shape, apical tooth enlarged and acute, and three smaller teeth along masticatory margin of mandibles (Fig. 9); erect pilosity absent from eyes . . . . . *marthae*

For full species descriptions, measurements, lists of material examined, taxonomic synopses, distribution maps and species images for Malagasy *Oxygyne* refer to Results S1 in the electronic supporting material and Figures S13–S41.

Synopsis, distribution and diagnosis of the subgenus *Crematogaster* (*Oxygyne*)

Oxygyne Forel, 1901: 375 (as subgenus of *Crematogaster*). Type species: *Crematogaster* (*Oxygyne*) *daisyi*, by subsequent designation of Wheeler, W.M. 1911: 169.
Oxygyne raised to genus: Soulié, 1964: 398.
Oxygyne junior synonym of *Crematogaster*: Hölldobler & Wilson, 1990: 13.
Oxygyne subgenus of *Crematogaster*: Bolton, 1995: 40.
= *Crematogaster* (*Nematocrema*) Santschi, 1918: 182 (as subgenus of *Crematogaster*). Type species: *Crematogaster stadelmanni*, by original designation. **syn.n.**



Figs 3–9. 3(a), mesonotum distinctly raised with respect to pronotum and propodeum; (b), mesonotum only slightly raised with respect to pronotum; 4, promesonotal suture very distinct; 5, promesonotal suture indistinct; 6, propodeal spines absent; 7, propodeal spines present; 8, mandibles falcate, apical tooth enlarged and acute; 9, mandibles of normal shape, apical tooth enlarged and acute.

This study recognises species and subspecies of the *Crematogaster* (*Oxygyne*) group as follows (modified list from Bolton, 2011). Madagascar: *C. agnetis*; *C. marthae*; *C. ranavalonae*. Africa: *C. breviventris*; *C. donisthorpei*; *C. magitae*; *C. margaritae* including subspecies *brevarmata*, *cupida*, *lujae*; *C. oscaris*; *C. santschii* including subspecies *clymene* (re-transferred); *C. stadelmanni* including subspecies *anguliceps*, *angustata*, *dolichocephala*, *gracilenta*, *intermedia*, *ovinodis*, *schereri*, *spissata*; *C. trautweini* (re-transferred). India, South-East Asia: *C. aberrans* including subspecies *assmuthi* and *inglebyi*; *C. augusti*; *C. butteli*; *C. daisyi*; *C. dalyi* including subspecies *sikkimensis*; *C. ebenina* including subspecies *corax*; *C. pia* including subspecies *soengeiensis* and *taivanae* (transferred); *C. soror*; *C. tumidula*. Papua New Guinea: *C. meijerei* (transferred). *Crematogaster travancorensis* is hereby excluded from *Oxygyne*, and transferred provisionally to the subgenus *Crematogaster*. For an illustration of distribution records refer to Fig. 17. In total, 21 nominal species are hence currently included in *Oxygyne*, of which 17 have been examined in the context of this study. For a comprehensive list of species examined see Table S2. Measurement ranges given below were based upon 59 Malagasy and 26 non-Malagasy queen, and 121 Malagasy and 40 non-Malagasy worker ant specimens.

Queen diagnosis of *Crematogaster* (*Oxygyne*)

1 Mandibles with apical tooth enlarged and acute (Fig. 8), other teeth, if present, relatively reduced (Fig. 9).

2 Anterior portion of clypeus flat (Fig. 10) and at least slightly projecting over mandibles (Fig. 11).

3 Head significantly longer than wide or about equal length and width, cephalic index 0.84–1.11.

4 Mesosoma with the following characteristics: thorax reduced in size, shortened, mesonotal index 0.74–1.12. Propodeum in contrast elongate, its dorsum meeting metanotum at ~30° angle (Fig. 13a, in contrast to Fig. 13b). Propodeal spines present or absent.

5 Postpetiole with distinct median impression, and width usually significantly exceeding petiole width, petiole–postpetiole index 0.94–1.69.
6 Leg length highly variable, very long to short, leg–body index 1.05–2.45, length of hind tibia 0.61–2.55 mm.
7 Body size highly variable, head width 0.81–1.82 mm and Weber's length 1.16–2.98 mm.
8 Erect pilosity often abundant.

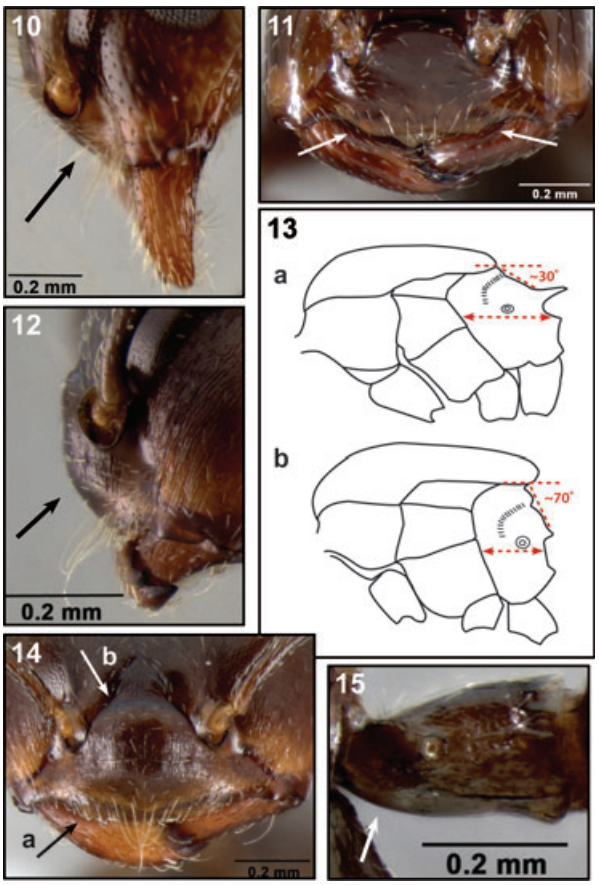
Worker diagnosis of *Crematogaster* (*Oxygyne*)

1 In lateral view, median portion of clypeus more or less prominently convex (Fig. 12) and in full-face view medially protruding over mandibles (Fig. 14a).
2 Masticatory margin of mandibles with four teeth.
3 Fronto-clypeal suture impressed (Fig. 14b), often anterior portion of frons (above suture) transversely concave.
4 Head usually rounded and equally long as wide or slightly wider than long, cephalic index 0.97–1.18.
5 Promesonotal suture often complete and distinct.
6 Propodeal spines present.
7 Subpetiolar process absent (Fig. 15).
8 Postpetiole with median impression.
9 Leg length variable, fairly long to moderately short, leg–body index 1.10–1.43, length of hind tibia 0.59–1.25 mm.
10 Body size highly variable, small to very large, head width 0.73–1.26 mm and Weber's length 0.80–1.40 mm.

Phylogeny and biogeography of *Oxygyne*

The subgenera *Oxygyne* and *Nematocrema* are strongly supported to form one clade by all phylogenetic analyses (Fig. 16A, B). *Crematogaster stadelmanni*, representing *Nematocrema*, is nested within *Oxygyne* in all MRBAYES and ML analyses (Fig. 16B), but species tree estimation under the coalescent model in *BEAST reconstructs a sister-group relationship between *C. meijerei* and *C. stadelmanni* (Fig. 16A). In any case, either method of reconstruction supports *Nematocrema* and *Oxygyne* as closely related and their combination into a single subgenus *Oxygyne*. MRBAYES and ML analyses further demonstrate high phylogenetic resolution between species with maximum node support (Fig. 16B). *Crematogaster meijerei* is sister to the remaining five species in these analyses, and the Malagasy *Oxygyne* (*C. ranavalonae* and *C. agnetis*) form a monophyletic group that is sister to the South African *C. santschii* and the Asian *C. aberrans*. Species tree reconstruction performed in *BEAST estimated similar species relationships, except with regard to the clade formed by *C. meijerei* and *C. stadelmanni* (Fig. 16A), which received low posterior support values. Decreased support is also seen for the clade comprising the remaining four species *C. aberrans*, *C. santschii*, *C. agnetis* and *C. ranavalonae*. Individual gene trees produced by *BEAST depict identical interspecific relationships, but with varying support across genes for the mentioned clades (Figure S42).

Phylogenetic relationships across the biogeographical distribution of *Oxygyne* are illustrated in Fig. 17 (for locality data refer to Table S2) and appear to be of a complex nature.



Figs 10–15. 10, anterior portion of clypeus flat; 11, clypeus at least slightly projecting over mandibles; 12, median portion of clypeus more or less prominently convex; 13(a), propodeum elongate, its dorsum meeting metanotum at ~30° angle; 13(b), propodeum short, its dorsum meeting metanotum at ~70° angle; 14(a), clypeus in full-face view medially protruding over mandibles; 14(b), fronto-clypeal suture impressed; 15, subpetiolar process absent.

Within this small snapshot of the entire *Oxygyne* species diversity, only few, but nonetheless interesting, inferences about biogeographical relationships can be made. First, the Malagasy *Oxygyne* are equally closely related to the Central and South African *C. santschii* and the Asian *C. aberrans*. Second, the New Guinea endemic *C. meijerei* shows equally close affinities to Asian, African and Malagasy species and is not more closely related to the geographically more proximate *C. aberrans*. Lastly, the two African species, *C. santschii* and *C. stadelmanni*, do not form a clade, as one might expect given their distribution.

Discussion

Malagasy *Oxygyne*

Patterns of complex morphological variation. When Forel (1892) worked on his descriptions and key to the Malagasy

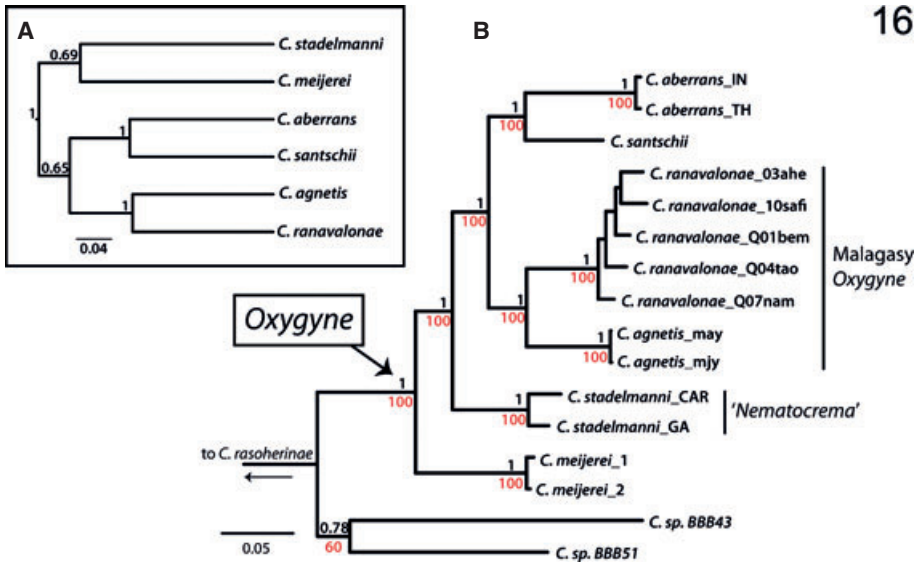


Fig. 16. Phylogeny of *Oxygyne*. (A) Species tree as estimated by *BEAST based upon four genes: long wavelength rhodopsin (LW Rh; 1108 bp), arginine kinase (ArgK; 514 bp), carbomoyl phosphate synthase (CAD; 744 bp) and cytochrome oxidase I (COI; 604 bp). Outgroups were excluded from this analysis. (B) MRBAYES phylogeny, based upon 2995 bp of nuclear and mitochondrial data. Maximum likelihood bootstrap values are mapped upon respective nodes. The subgenus *Oxygyne*, the Malagasy *Oxygyne* clade and the position of the previous subgenus *Nematocrema* are indicated. The branch leading to *Crematogaster rasoherinae* has been removed.

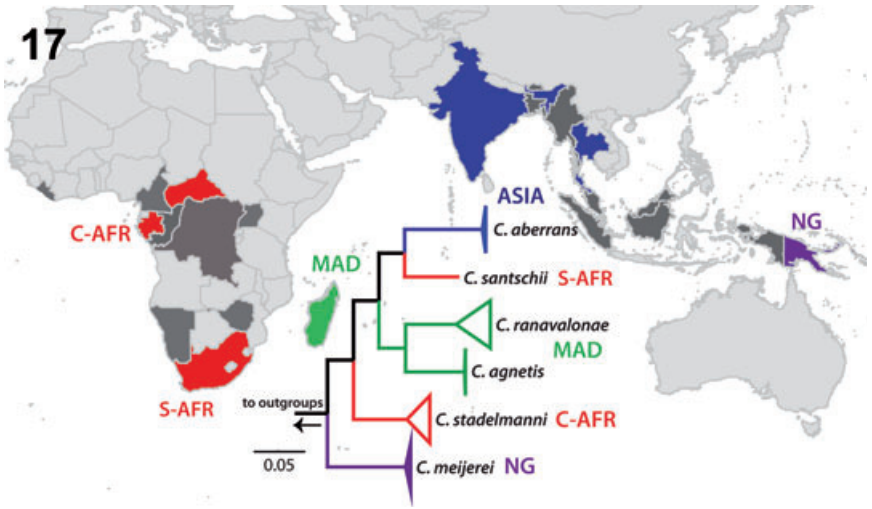


Fig. 17. Biogeography and distribution of *Oxygyne*. Coloured countries on the map represent sources of molecular samples, with colours corresponding to branches in the MRBAYES phylogeny. The tree is identical to that of Fig. 16B, but outgroups have been removed and species nodes have been collapsed. Dark grey coloured countries represent distribution records for the subgenus *Oxygyne* as a whole; these are based either upon locality data of specimens included in the present morphological studies, or upon type descriptions (for species where no material was examined). Additional literature and collection records may exist from other countries. C-AFR, Central Africa; MAD, Madagascar; NG, New Guinea; S-AFR, South Africa.

Oxygyne species, he remarked, on the one hand, that there was exceptional morphological variation between the queens of these species and, on the other hand, a contrasting morphological uniformity of the workers. The present study illuminates that much of these morphological differences between queens constitute intraspecific variation in *C. ranavalonae*. Queen polymorphism in ants is known to occur in a suite of other unrelated ant genera, such as, for example, *Ectatomma* (Lenoir *et al.*, 2011), *Pogonomyrmex* (Johnson *et al.*, 2007), *Leptothorax* (Rueppell *et al.*, 2001), *Polyrhachis* (van Zweden *et al.*, 2007) and *Mystrium* (Molet *et al.*, 2007). In most of these cases, however, the polymorphism is manifested as a bimorphism of 'macrogynes', regular-sized winged queens, and 'microgynes', which are of isometrically reduced size to

normal queens and often wingless; these two different forms can perform different dispersal strategies under different environmental conditions (Heinze, 2007) and often occur in the same colony (Johnson *et al.*, 2007; van Zweden *et al.*, 2007; Lenoir *et al.*, 2011). If a gradual polymorphism, such as reduction of wings and other thoracic characters, is seen, these forms are usually termed 'intercastes'. Intercastes are known for instance in *Myrmecina* (Buschinger & Schreiber, 2002; Steiner *et al.*, 2006) and also in *Crematogaster* (Heinze *et al.*, 1999; Oettler *et al.*, 2008). In contrast to the above examples, the queen polymorphism in *C. ranavalonae* is not restricted to size or thorax reduction and does not involve wing reduction, and appears to represent an exceptional and until now undescribed case in the ants. Queen morphs A, B and C of *C. ranavalonae* vary discretely in characters such as head shape, scape length, size of subpetiolar process and postpetiole width. It is an open question whether one colony exclusively produces one of the three forms, or mixed morphs can occur in one colony. Some evidence for the former is based on a single observation of one colony containing only one queen morph in high numbers (B. B. Blaimer, personal observation). Within a population more than one morph appears to occur, although generally the most abundant form found is A, and C is the rarest.

Considering that the three queen morphs may be separable by an identification key, this could suffice as enough evidence to accept these ants as separate species – despite the inability to align this morphological variation with the observed pattern of genetic variation. My interpretation is that worker morphology contradicts such a multispecies hypothesis. Worker polymorphism in *C. ranavalonae* is gradual, as morphometric plots of cephalic index and body size show (Figures S6–S9) and does not demonstrate any relationship with the observed variation in queen morphology, or with mtDNA variation. Nonetheless, the existence of multiple cryptic species within *C. ranavalonae*, which are not diagnosable by worker morphology, remains a possibility – especially in the light of other taxonomic work that finds worker morphology problematic in species delimitation and identification of ants (e.g. genus *Azteca*; Longino, 2007). Worker variation in *C. ranavalonae* is further reminiscent of the morphological variation in the recently described *C. hova* complex (Blaimer, 2010) in Madagascar. Relative to the *C. hova* complex, both morphological and genetic variation in *C. ranavalonae* workers is a magnitude lower (B. B. Blaimer, personal observation). For the *C. hova* complex, a complex evolutionary history of either incipient speciation or hybridisation was suggested to underlie the observed pattern (Blaimer, 2010). Similarly, distinctive lineages could be evolving within *C. ranavalonae* that are diverging in queen morphology, and these could eventually become separate, reproductively isolated species.

Social parasitism in Oxygyne. The subject of temporary social parasitism particularly invites speculation as a possible cause of morphological patterns in *C. ranavalonae* and the Malagasy *Oxygyne*. This mode of colony founding has been suggested as a probable habit in the entire *Oxygyne* clade, based on the falcate mandibles of the queens (Fig. 8) and their relatively small size (e.g. Forel, 1910; Hölldobler & Wilson, 1990). Parasites may experience elevated selection pressures and increased rates of evolution as a consequence of adapting to their hosts (Murphy *et al.*, 2008; Krueger *et al.*, 2009). Assuming a parasitic habit of *Oxygyne* queens, high selection pressures could be exerted on the queen caste and on adaptive characters that enable a successful invasion and takeover of a host colony. Morphological diversity in *C. ranavalonae* queens could be correlated with such advantageous characters and represent 'host races' – comparable with adaptations in the common cuckoo (*Cuculus canorus*), in which so-called 'gentes' (host races) are adapted to parasitic egg mimicry of different host species (e.g. de L. Brooke & Davies, 1988; Takasu *et al.*, 2009). This parallel between parasitic ants and cuckoos has been drawn before by Davies *et al.* (1989), who suggested that similar selection pressures could be affecting both systems. Huang & Dornhaus (2008) found that temporary social parasites in ants often had multiple closely related host species, and one could thus imagine each of the three *C. ranavalonae* queen morphs to be morphologically adapted to parasitise a different host. Unfortunately, information on possible host ant species for *C. ranavalonae*, or *C. agnetis* and *C. marthae* does not exist. 'Emery's rule' (Emery, 1909; Le Masne, 1956) predicts social parasites in ants to be phylogenetically closely related to their host species. When this prediction was tested for multiple degrees of social parasitism in ants by Huang & Dornhaus (2008), the authors found Emery's rule to loosely comply with most known cases of temporary social parasitism, as host and parasite species were often closely related species. The current consensus is that host and parasite species mostly belong to the same genus, although exceptions exist (Hölldobler & Wilson, 1990; Buschinger, 2009; Jansen *et al.*, 2010). If *Oxygyne* is indeed a monophyletic group as the present results suggest (Fig. 16B) and all species share a temporary parasitic habit, their host species in contrast should consequently be found in rather distantly related species groups of *Crematogaster*. In the case of the Malagasy *Oxygyne*, the present phylogenetic estimations show *C. ranavalonae* and *C. agnetis* to form a monophyletic group (Fig. 16A, B) and this clade is phylogenetically distantly related to other *Crematogaster* clades in Madagascar (B.B. Blaimer, in preparation). Any putative host species from the remaining *Crematogaster* species pool in Madagascar therefore could only be a distant relative.

Species delimitation approach. The first iteration of the species delimitation process aimed to reconcile the morphological species hypotheses with clustering of sequences in the mitochondrial data (Fig. 1). This step did not provide sufficient information to resolve species boundaries in Malagasy *Oxygyne*. Most morphospecies, as well as previously described species, are not or only partly in agreement with mtDNA clusters, although overall genetic divergence within the group is quite high. Nonetheless, COI data were able to confirm the identity of *C. agnetis*, a species that is also morphologically

well supported. An important conclusion of the integration over morphological and mitochondrial data was a reconsideration of species hypotheses. Given the absence of a uniting pattern between the morphological variation and the variation in mtDNA data within all sampled specimens except for *C. agnetis*, the hypothesis of a morphologically variable species (*C. ranavalonae*) was subsequently investigated using a nuclear DNA dataset.

The program STRUCTURE has been developed to infer population structure from multilocus genotype data (Pritchard *et al.*, 2000), but is used increasingly as an analysis tool in species delimitation, especially when working at the interface between species and populations. A recent exploration of lineage diversity in Malagasy mouse lemurs was able to define cryptic phylogenetic lineages as candidate species using this approach (Weisrock *et al.*, 2010). In ants, nuclear population structure was inferred in STRUCTURE with the aim of illuminating species boundaries in a difficult species complex of South American fire ants (genus *Solenopsis*) (Ross *et al.*, 2010). STRUCTURE was used to infer population genetic structure from three nuclear loci and to test whether inferred nuclear genetic clustering, or more specifically the absence hereof, lends support to the presence of a widespread, variable species that could be united under the name *C. ranavalonae*. The results showed slight support for a population structured into either three or five clusters ($K = 3$ or 5), but all numbers of predefined clusters greater than one ($K = 2–8$) received very similar support values (Figure S12). Membership coefficients that have been plotted for $K = 3$ (Fig. 2) and $K = 5$ (Figure S12) are very evenly distributed across individuals, a sign that there is actually little population structure and all sampled individuals stem from a fairly admixed population (STRUCTURE documentation, Pritchard *et al.*, 2010). These results are additionally supported by phylogenetic analyses performed on the three nuclear markers that show low resolution (Figure S11). Thus, genetic exchange between populations appears to be high, even between geographically very isolated localities on the west coast (e.g. 14 = R.S. Bemarivo) and the northern tip of Madagascar (e.g. 4 = R.S. Manongarivo) that cluster together, whereas sympatric specimens sampled from the same or adjacent localities are not placed within the same mitochondrial cluster (Fig. 2). These conclusions seem at odds with the considerable elusiveness and rarity of these ants, but could mean that dispersal abilities of *C. ranavalonae* reproductives are quite high, and that alates travel substantial distances.

In synthesis, these results warrant the recognition of only one species, *C. ranavalonae*, which summarises most of the morphological diversity within Malagasy *Oxygyne*. It has become common practice to increase the number of molecular markers (e.g. Ross *et al.*, 2010), or add different types of data (e.g. Schlick-Steiner *et al.*, 2006) when confronted with a complex species problem. Although this will increase the depth and rigor of the analysis, it does not necessarily lead to higher success in species delimitation – as exemplified by Ross *et al.* (2010), who employed 68 genetic markers together with morphological and ecological data and still refrained from drawing conclusions leading to a taxonomic decision. In the present study, one could argue that the further addition of nuclear markers (of appropriate levels of variability) to the genotype dataset may have resulted in higher support for distinct genetic clusters within *C. ranavalonae*. However, the three nuclear markers used in this study are highly informative for species delimitation within ants, usually reliably recovering species within this genus, and the primary goal of this paper was not a population-level genetic study, but the implementation of an iterative taxonomic approach to revise species boundaries within Malagasy *Oxygyne*. Three 'disciplines', i.e. types of data, were suggested by Schlick-Steiner *et al.* (2010) to provide a well-grounded approach to modern species delimitation. The results of this study agree with this general guideline. Although the third type of data (nuclear markers) did not strictly provide novel evidence to the case, they were in fact crucial in adding support to the hypotheses generated by the first two types of data (morphological and mitochondrial) and thus aided in successful species delimitation.

Crematogaster marthae. One limitation of this study was the lack of suitable material from *C. marthae* for genetic analysis. At the same time, this is an excellent example to illustrate that integrative taxonomic approaches are not always feasible for all involved taxa and one has to settle on a provisional solution. Fortunately in the context of species delimitation in Malagasy *Oxygyne*, the *C. marthae* queens are morphologically quite distinct from the morphological variation of *C. ranavalonae* queens, by virtue of their four-toothed mandibles (Fig. 9) instead of the characteristic falcate mandibles (Fig. 8). It seems unlikely that this feature and thus *C. marthae* are also part of the complex intraspecific variation within *C. ranavalonae*, but in the absence of additional data this possibility cannot be wholly excluded.

Phylogeny and biogeography of the subgenus *Oxygyne*

Molecular phylogenetic results provide a first glimpse into the complex evolutionary history of the enigmatic and elusive *Crematogaster* (*Oxygyne*) group (Figs 16, 17). The group is recovered as monophyletic with high support, and the placement of *Nematocrema* within *Oxygyne* further supports the hypothesis of a close phylogenetic relationship and the formal synonymy of *Nematocrema* under *Oxygyne*. In a way this result is surprising, despite the strong shared morphological characteristics. If this remains true throughout further analyses, this confines the evolution of the unusual queen morphology in *Oxygyne* (falcate mandibles or at least apical tooth acute, reduced body size, large postpetiole) and thus the evolution of (hypothesised) temporary social parasitism in this group to a single origin. Phylogenetic studies investigating social parasitism in ants as well as bees more often find the parasitic species not belonging to the same clade (Ward, 1996; Smith *et al.*, 2007; Jansen *et al.*, 2010), and superficially similar morphological traits linked to parasitism are known to have evolved convergently across different groups of ants. For example, the falcate mandibles

of *Oxygyne* queens resemble very much the mandibular characters of workers of the parasitic genera *Polyergus* and *Strongylognathus* (Buschinger, 1986). Another group of species of *Crematogaster* ants (placed in the subgenera *Eucrema* and *Neocrema*) in the Neotropical region shares similar queen characters with *Oxygyne*, but again only rare anecdotal evidence exists regarding the parasitic behaviour of these species (see Longino, 2003). At least some part of these species is phylogenetically very distantly related to the Paleotropical *Oxygyne* in a larger, ~120 taxon phylogeny of *Crematogaster* (B. B. Blaimer, in preparation). This evidence points to at least two independent cases of evolution of the parasitic behaviour in *Crematogaster*.

Biogeographical hypotheses. Inferences on biogeographical relationships in this study are impeded by the rarity of the study subjects and the resulting limitations in taxon sampling. Of the 21 nominal species included in *Oxygyne*, molecular data were obtained from only six. With regard to the hypothesis of a monophyletic Malagasy *Oxygyne* and its closest relatives, results can therefore only be indicative and must remain inconclusive until most or all remaining species are re-collected and included in a more comprehensive phylogenetic analysis. Nevertheless, these preliminary phylogenetic results are very useful in generating a suite of subsequent hypotheses, some of which are worth sketching out in the following.

Crematogaster meijerei and *C. stadelmanni* apparently represent sister lineages to the other sampled species within *Oxygyne*, but with the exact relationships differing between analyses (see below). This already evidences a complex biogeographical history of the subgenus involving biotic exchange between New Guinea/Malaysia and Africa in the earlier evolution of the group. Based upon queen morphology of the examined species, I hypothesise that the Malaysian *Oxygyne* are closely related to *C. meijerei*, *C. stadelmanni* and probably the two African species formerly also classified under *Nematocrema*. The remainder of the African species I expect to be related to the current clade containing *C. santschii* and *C. aberrans*, as all of their queens, as far as known, appear close to *C. aberrans* (the queen of *C. santschii* is undescribed). The Indian and Malagasy species further represent both of these two morphological groupings, and I surmise that sampling on the Indian subcontinent will resume a central position in any further investigation of this biogeographical puzzle. It is well established that India and Madagascar have had a prolonged shared evolutionary history during the break-up of Gondwana, yet have attained complete isolation from each other ca. 85–90 Ma (Storey *et al.*, 1995; Torsvik *et al.*, 2000). The fossil record of the genus *Crematogaster* currently gives evidence of its presence only as early as the Miocene (Heer, 1850; Brown, 1973), and molecular dating analyses depict a timeframe of ant evolution that is not compatible with a vicariant origin of any ant clade within the subfamily Myrmicinae in Madagascar (Brady *et al.*, 2006; Moreau *et al.*, 2006). General patterns of biogeographical relationships of Malagasy taxa usually agree with long-distance dispersal scenarios from Africa [review by Yoder & Nowak (2006)], although less frequently phylogenetic relationships between Madagascar and Asia are observed (Ward *et al.*, 2010; review by Zakharov *et al.*, 2004; Warren *et al.*, 2010). For the ants, this area remains a highly interesting topic largely open for investigation, and especially species-rich and widespread genera such as *Crematogaster* will probably reveal very complex biogeographical histories.

Evaluation of *BEAST *performance.* Because the multispecies coalescent approach integrated in *BEAST is a fairly novel method with little practical exploration, it is worthwhile to compare and discuss the observed variation in tree topology and support values between this gene tree – species tree approach and the 'standard' MRBAYES and ML methods using concatenation of loci. All interspecies relationships receive maximum support under the concatenation approach (Fig. 16B), whereas the *BEAST species tree returns very low posterior probabilities for deeper phylogenetic relationship (Fig. 16A). An obvious reason for this discrepancy could be underlying conflict in gene trees, but when examining these individually this seems not the case: tree topologies here are identical concerning the respective nodes (Figure S42). However, support values do vary and are especially low for the gene tree based on ArgK. Heled & Drummond (2010) discussed the need for multiple samples per species to perform coalescent-based species estimation, as this method relies on accurate estimation of population size about which a single sample will give no information. They further stated that as few as two samples per species (representing separate populations) are sufficient – if 'enough' loci are included in the analyses. The present dataset used in *BEAST analyses comprised four loci and at least two individuals per species, except for *C. santschii*, which was only represented by a single individual (outgroups were pruned prior to analysis). Simulations show an increase in accuracy of the method well beyond four loci and two individuals per species, and the authors point out that having only a single sample per species could have a detrimental effect on the inference of speciation times or topology (Heled & Drummond, 2010). For these reasons, the observed deviations in support values can probably be attributed to violating the multispecies coalescent model implemented in species tree inference in *BEAST, either due to insufficient gene or taxon sampling.

Conclusions

Previous species diversity estimates for Malagasy *Crematogaster* (*Oxygyne*) ants have been confounded by remarkable variation in queen morphology. The iterative taxonomic approach using morphological, mitochondrial and nuclear genetic data chosen here to delimit species of *Oxygyne* in Madagascar circumscribes three species, *C. ranavalonae*, *C. agnetis* and *C. martha*e, with high confidence, in contrast to six previously described species and three subspecies. Morphological variation in queens of *C. ranavalonae* may be evolving as an adaptation to a temporary social parasitic behaviour. The Malagasy *Oxygyne* appear to form a monophyletic group,

at least with regard to the taxon sampling achieved in this study, and are sister to a clade containing an African and an Indo-Asian *Oxygyne* species. One *Oxygyne* species from New Guinea is estimated as sister to all other *Oxygyne* species sampled here. The phylogeny of *Oxygyne* further indicates monophyly for the subgenus and warrants the inclusion of *Nematocrema* in the former. These findings suggest a single origin of morphologically highly derived queens in *Crematogaster* in the Old World, and much highlight the need for field studies to explore the supposed parasitic behaviour and to further increase molecular phylogenetic sampling to thoroughly investigate the evolution of this intriguing trait.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-3113.2011.00609.x

Results S1. Species Accounts.

Figures S1–S5. Morphometric plots of selected queen measurements, showing morphological variation between and among Malagasy *Oxygyne* species as revised. 1, cephalic index; 2, scape index; 3, body size; 4, petiole–postpetiole index; 5, leg–body index. Values are based upon data presented in the context of species descriptions (see Results S1).

Figures S6–S9. Morphometric plots of selected worker measurements, showing morphological variation between and among Malagasy *Oxygyne* species as revised. Where available for morphometric study, the associated syntype specimens to *Crematogaster ranavalonae* have been highlighted as separate data series. 6, cephalic index; 7, scape index; 8, body size; 9, petiole width index. Values are based upon data presented in the context of species descriptions (see Results S1).

Figure S10. Maximum likelihood phylogeny of Malagasy *Oxygyne* based on 594 bp of cytochrome oxidase I (COI) and dataset A bootstrap values are shown in red.

Figure S11. MRBAYES phylogenies of Malagasy *Oxygyne* based on separate analyses of nuclear loci for dataset A; A, long wavelength rhodopsin (LW Rh) gene tree; B, arginine kinase (ArgK) gene tree; C, carbomoyl phosphate synthase (CAD) gene tree; D, LW Rh; ArgK, CAD concatenated. For information on the number of nucleotides for each loci, partitions and model settings, refer to Table 3.

Figure S12. (A) The estimated ln probability of data, Ln P(D), plotted over the number of populations $K = 1–8$, as estimated in STRUCTURE. Different colours represent different runs ($n = 10$). (B) Structure bar plots for *Crematogaster ranavalonae* individuals for $K = 5$; results are summarised across 10 runs. Individuals are grouped by locality.

Figures S13–S19. *Crematogaster agnetis* (w, CASENT0107473; q, CASENT0112155); 13, worker profile view; 14, worker dorsal view; 15, worker full-face view; 16, queen profile view; 17, queen full-face view; 18, queen dorsal view; 19, distribution.

Figures S20–S26. *Crematogaster marthae* (w, CASENT0101802; q, CASENT0171185); 20, worker profile view; 21, worker full-face view; 22, worker dorsal view; 23, queen profile view; 24, queen full-face view; 25, queen dorsal view; 26, distribution.

Figures S27–S31. Morphological diversity of *Crematogaster ranavalonae* worker ants; (a) full-face view; (b) profile view; (c) dorsal view (CASENT0491513, CASENT0193205, CASENT0141548, CASENT0193277, CASENT0423149).

Figures S32–S35. *Crematogaster ranavalonae* queen ants. 32, 33, queen A (CASENT0485160, CASENT0066769); 34, 35, queen B (CASENT0496495, CASENT0193642); (a) full-face view; (b) profile view; (c) dorsal view.

Figures S36–S41. *Crematogaster ranavalonae* queen ants. 36, 37, queen C (CASENT0114271, CASENT0193239); (a) full-face view; (b) profile view; (c) dorsal view. 38, distribution of *C. ranavalonae*; 39, *C. ranavalonae* carton nest; 40, internal structure of the same nest; 41, position of the same nest in young tree.

Figure S42. Gene trees as reconstructed jointly with the species tree (Fig. 16A) in *BEAST. (A) long wavelength rhodopsin (LW Rh), 1108 bp; (B) arginine kinase (ArgK), 514 bp; (C) carbomoyl phosphate synthase (CAD), 744 bp; (D) cytochrome oxidase I (COI), 604 bp.

Table S1. Species names, taxon identification, specimen identification, locality data and dataset of specimens used in the molecular study. In cases where two CASENT numbers are given, two different specimens from the same collection have been used for DNA extraction and sequence generation (*COI; **LR, AK and CD). Locality numbers refer to Fig. 2.

Table S2. Comprehensive list of species included in *Oxygyne*. For each species, the localities (coordinates where applicable), collectors and sources of all examined specimens are given. In cases where no material was examined, type localities are given. Localities in this table form the basis of *Oxygyne* distribution as shown in Fig. 17. Species that have been transferred or re-transferred to *Oxygyne* are indicated. *Crematogaster travancorensis* is excluded from the subgenus *Oxygyne* and thus not listed.

Table S3. List of specimen codes for voucher specimen images deposited on AntWeb (www.antweb.org). Images can be found by searching for the CASENT number on AntWeb or via a search engine.

Table S4. Genetic distances within Malagasy *Oxygyne*, calculated as uncorrected p and Tamura-Nei distances from cytochrome oxidase I (COI) sequence data. Distances are averaged across members of the two deep clades A and B and five subclusters within A (I, II, III, IV, V) as recovered by MRBAYES analyses and shown in Fig. 1. Numbers in italics give mean distances within clusters or clades; bold numbers show distances of the various subsets to *Crematogaster agnetis*; maximum distances within *C. ranavalonae* are in bold and italic. Averages represent arithmetic means rounded to the fourth decimal.

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Results S1: SPECIES ACCOUNTS

Crematogaster agnetis (Figs S13–19)

Crematogaster agnetis Forel, A. 1892: 531 (q), 533 (w.), 534 (m.) (diagnoses in keys). Worker, queen and male syntypes from Madagascar: Amparafarafantsiv [=Mangoroufer on labels] (M. Sikora) [MHNG, examined]. Three of these syntypes are imaged on AntWeb: CASENT0101731 (top specimen of 2 w. on one pin), CASENT0101581 (bottom specimen of 3 aq. on one pin), CASENT0101760 (top specimen of 2 m. on one pin). One worker syntype (CASENT0101731, top specimen) is hereby designated the **LECTOTYPE**.
Combination in *C. (Oxygyne*): Wheeler, W.M. 1922: 1024.

Material examined.—(CASC, MHNG, MCZ) MADAGASCAR: *Antsirananana:* R.S. Manongarivo: -13.96167, 48.43333, 400m (B.L.Fisher); Ampasindava, Ambilanivy: -13.79861, 48.16167, 600m (B.L.Fisher et al.); F Antsahabe: -13.21167, 49.55667, 550m (B.L.Fisher et al.); P.N. Marojejy: -14.43500, 49.76000, 775m (B.L.Fisher et al); P.N. Marojejy [Manantenina]: -14.43333, 49.76667, 750m (B.L.Fisher et al); *Antananarivo*: NE Andranomay: -18.47333, 47.96000, 1300m (B.L.Fisher et al.); Andrangoloaka: -19.03333, 47.91667 (Sikora); *Fianarantsoa*: P.N. Ranomafana: -21.26650, 47.42017, 1020m; -21.29000, 47.43333, 1100m; -21.25000, 47.36667, 1130m (B.L.Fisher et al); -21.25, 47.36667, 1160m (A.Pauly); *Toamasina*: Mangoroufer [Amparafarafantsiv]: -18.88330, 48.11670 (M.Sikora); F Torotorofotsy: -18.87000, 48.34670, 1070m (B.L.Fisher et al.); F Ambatovy: -18.85083, 48.32000, 1075m; Analamay: -18.80623, 48.33707, 1068m (B.L.Fisher et al.); P.N. Mantadia: -18.79167, 48.42667, 895m (B.L.Fisher et al.); La Mandraka: -18.92500, 47.91940, 1280m (W.L.&D.E.Brown); *Toliara*: F Petriky: -25.06167, 46.87000, 10m (B.L.Fisher).

Worker measurements (n=11) HW 0.94 - 1.10 , HL 0.92 - 1.06, EL 0.18 - 0.21, SL 0.72 - 0.84, WL 1.01 - 1.21, SPL 0.13 - 0.25, PTH 0.19 - 0.23, PTL 0.27 - 0.32, PTW 0.25 - 0.33, PPL 0.18 - 0.24, PPW 0.26 - 0.33, LHT 0.79 - 0.87, CI 1.02 - 1.11, OI 0.17 - 0.21, SI 0.74 - 0.83, SPI 0.13 - 0.23, PTHI 0.65 - 0.77, PTWI 0.83 - 1.04, PPI 1.29 - 1.57, PPPI 0.93 - 1.18, LBI 1.28 - 1.43.

Worker description

Medium to large species (HW 0.94 - 1.10 mm, WL 1.01 - 1.21 mm); colour from reddish brown, medium brown, dark brown to black.

Masticatory margin of mandibles with four teeth; posterior margin of head in full-face view with suboval or subangular corners, often medially depressed; antennal scapes not or barely reaching posterior margin of head (SI 0.74 - 0.83); eyes situated above midline of head in full face view.

Pronotum laterally rounded; promesonotal suture very distinct; mesonotum raised with respect to pronotum and propodeum, dorsal face of mesonotum convex, lateral portions rounded or subangular, mesonotum postero-laterally rounded and with a distinct posterior face; dorsal face of propodeum long, almost equal length to posterior face; propodeal spines small - medium sized (SPI 0.13 - 0.20), between half and full the width between their bases, slender, straight or down-curved, in dorsal view weakly diverging (<20º); propodeal spiracle round; petiole in dorsal view variable, from slender and suboval to weakly flared anteriorly; postpetiole wider than long, often wider than petiole, with feeble median impression.

Head sculpture aciculate to weakly reticulate; promesonotum dorsally reticulate, sometimes weakly costulate; mesopleuron and metapleuron reticulate or costulate; dorsal and posterior face

of propodeum shiny; petiole and postpetiole dorsally mostly shiny, ventrally reticulate; face with 0-4 erect setae, and sparse appressed pubescence; head ventrally with regular appressed pubescence, but suberect pilosity absent; erect pilosity on mesosoma usually absent; petiole lacking erect pilosity; postpetiole usually lacking long erect pilosity, suberect short setae may be present.

Queen measurements (n=11) HW 0.94-1.11, HL 0.95-1.23 , EL 0.25-0.30, SL 0.69-0.82, MSNW 0.76-0.85, MSNL 0.80-0.98, WL 1.60-1.90, SPL 0.00, PTH 0.28-0.37, PTL 0.32-0.38, PTW 0.41-0.54, PPL 0.22-0.27, PPW 0.46-0.54, LHT 0.73-0.87, CI 0.89-0.92, OI 0.23-0.26, SI 0.63-0.70, MSNI 0.80-0.99, SPI 0.00, PTHI 0.81-1.02 , PTWI 1.14-1.49, PPI 1.85-2.75, PPPI 0.94-1.15, LBI 2.09-2.23.

Queen description

Very small (HW 0.94-1.11 mm, WL 1.60-1.90 mm); colour dark brown to black. Clypeus medially slightly projecting over mandibles; mandibles falcate, with apical tooth enlarged and pointed and a second, very small pre-apical tooth present on masticatory margin; head usually longer than wide, head width tapering from anterior to posterior margin, posterior margin of head in full-face view oval; antennal scapes short (SI 0.63-0.70), not surpassing posterior margin of head; eyes barely emarginate, situated at midline of head in full face view.

Mesosoma short (MSNI 0.80-0.99, WL 1.60-1.90 mm); mesoscutum in dorsal view oval; propodeal suture shallow; dorsal face of propodeum very short, indistinct from posterior face; propodeal spines absent; petiole distinctly wider than long, oval, lobed anteriorly; subpetiolar process absent; postpetiole more than twice as wide as long, lacking antero-ventral tooth; legs short (LBI 2.09–2.23).

Sculpture on head, meso-and metasoma largely absent, at most aciculate; face with abundant long, flexuous, golden setae on vertex and frons, regular appressed pilosity throughout; head ventrally with abundant appressed pubescence; erect setae absent on eyes; mesonotum with abundant long, flexuous, golden setae, absent from median portion; petiole and postpetiole with abundant long suberect golden setae; abdominal segments 4-7 with abundant appressed golden pubescence, a row of decumbent longer setae at posterior margins of segments; a patch of short, stiff setae present anterior to frontal tibial spur.

Distribution and biology

Crematogaster agnetis is found in low- and mid-elevation rainforest habitats in north and north-west Madagascar and on the Central Plateau (Fig. 16). An isolated record exists from a small patch of littoral rainforest (Forêt de Petriky) in the far south of Madagascar. However, it seems likely that the currently available material does not represent the actual distribution of this species. I assume that *C. agnetis* is nesting arboreally and probably a carton-builder as most *Oxygyne* species, but I know of only one arboreal collection of workers from a dead twig, while most other collections consist of foraging workers or queens captured in malaise traps.

Crematogaster marthae (Figs S20–26)

Crematogaster marthae Forel, A. 1892:529 (q.), 534 (w.) (diagnoses in key). Queen and worker syntypes from Madagascar: Mangoroufer [=Amparafarafantsiv] (M. Sikora) [MHNG, examined]; Wheeler, W.M. 1922:1025. Combination in *C. (Oxygyne*). Three of these syntypes are imaged on AntWeb: CASENT0101802 (top specimen of 3 w. on one pin), CASENT0101858 (bottom specimen of 2 aq. on one pin), CASENT0171185 (top specimen of 2aq. on one pin). One worker syntype (CASENT0101802, top specimen of 3w on one pin) hereby designated the **LECTOTYPE**.

Material examined.—(MHNG, MCZ) MADAGASCAR: *Toamasina*: Mangoroufer [Amparafarafantsiv]: -18.88330, 48.11670 [approximation] (M.Sikora); Madagascar (unknown collector).

Worker measurements (n=5) HW 0.87 - 0.98, HL 0.85 - 0.95, EL 0.16 - 0.19, SL 0.69 - 0.78, WL 0.92 - 1.09, SPL 0.18 - 0.24, PTH 0.17 - 0.19, PTL 0.25 - 0.27, PTW 0.27 - 0.30, PPL 0.20 - 0.23, PPW 0.30 - 0.33, LHT 0.73 - 0.79, CI 1.01 - 1.05, OI 0.19 - 0.20, SI 0.78 - 0.85, SPI 0.20 - 0.24, PTHI 0.69 - 0.72, PTWI 1.06 - 1.12, PPI 1.41 - 1.62, PPPI 1.05-1.14, LBI 1.20 - 1.39.

Worker description

Medium size (HW 0.92 - 0.98 mm, WL 0.94 - 1.09 mm); colour reddish brown, gaster dark brown.

Masticatory margin of mandibles with four teeth; posterior margin of head in full-face view with subangular corners, medially depressed; antennal scapes not or barely surpassing posterior margin of head (SI 0.78 - 0.85); eyes situated above midline of head in full face view.

Pronotum laterally subangular; promesonotal suture indistinct; mesonotum only slightly raised with respect to pronotum and propodeum, dorsal face of mesonotum convex, lateral portions rounded or subangular, mesonotum postero-laterally rounded, without distinct posterior face; dorsal face of propodeum long, equal length as posterior face; propodeal spines small - medium sized (SPI 0.15 - 0.20), between half and full the width between their bases, stout and slightly swollen at base, then quickly tapering, straight and in dorsal view weakly diverging (<20º); propodeal spiracle oval; petiole flared anteriorly; postpetiole wider than petiole (PPPI 1.05 - 1.10), with feeble median impression.

Head sculpture aciculate to weakly reticulate; promesonotum dorsally weakly reticulate; mesopleuron and metapleuron weakly reticulate or costulate; dorsal and posterior face of propodeum shiny; petiole and postpetiole dorsally mostly shiny, ventrally reticulate; face with 0–4 erect setae, and regular appressed pubescence; head ventrally with regular appressed pubescence, and sparse suberect pilosity; 0–2 erect setae on mesosoma; petiole and postpetiole with or without a pair of erect dorso-posterior setae, other suberect shorter setae may be present [the condition of the syntypes is poor, and setae may be abraded rather than absent].

Queen measurements (n=4) HW 1.24 - 1.32, HL 1.12 - 1.26 , EL 0.27 - 0.30, SL 0.69 - 0.78, MSNW 0.92 - 1.05, MSNL 0.87 - 1.01, WL 1.73 - 1.81, SPL 0.29 - 0.33, PTH 0.40 - 0.48, PTL 0.33 - 0.44, PTW 0.59 - 0.67, PPL 0.25 - 0.38, PPW 0.83 - 0.90, LHT 0.71-0.79, CI 1.05 - 1.10, OI 0.22 - 0.26, SI 0.62 - 0.63, MSNI 0.93 - 1.12, SPI 0.17 - 0.19, PTHI 0.97 - 1.21 , PTWI 1.34 - 1.84, PPI 2.39 - 3.45, PPPI 1.34–1.40, LBI 2.26–2.45.

Queen description

Small (HW 1.27 - 1.32 mm, WL 1.75 - 1.81 mm); colour brown.

Clypeus medially slightly projecting over mandibles; mandibles normal-shaped, four teeth along masticatory margin, apical tooth enlarged and acute; head wider than long, head width increasing from anterior to posterior margin, posterior margin of head in full-face view with subangular corners, straight; antennal scapes short (SI 0.62 - 0.63), not surpassing posterior margin of head; eyes moderately emarginate, situated somewhat above midline of head in full face view.

Mesosoma short (MSNI 0.93 - 1.03, WL 1.75 - 1.81 mm); mesoscutum in dorsal view oval, scutellum enlarged, scuto-scutellar suture reduced; dorsellum (postscutellum) flattened, flush with propodeum; propodeal suture distinct; dorsal face of propodeum very short, indistinct from posterior face; propodeal spines very stout, swollen (SPI 0.18–0.19); petiole wider than long, flared anteriorly, swollen; subpetiolar process absent; postpetiole wide, more than twice as wide as long, and distinctly wider than petiole (PPPI 1.34–1.40), with antero-ventral tooth large and acute; legs short (LBI 2.26–2.45).

Sculpture on head, meso-and metasoma largely absent, at most aciculate; mandibles with dense, silken pubescence; erect pilosity absent from eyes; face lacking pilosity except for silken short pubescence and a few longer setae close to anterior clypeal margin; head ventrally with very sparse appressed pubescence; pilosity on mesonotum absent; erect pilosity absent on petiole and postpetiole; dorsal and ventral erect or suberect pilosity lacking on abdominal segments 4 - 7, sparse appressed pilosity present.

Distribution and biology

Crematogaster marthae is known only from its type locality Amparafarafantsiv, near Moramanga and Andasibe National Park in the Central Region of Madagascar (Fig. 23).

Crematogaster ranavalonae (Figs S27–41)

Crematogaster ranavalonis Forel, A. 1887: 388. Worker syntypes from Madagascar: Bois l'Ivondro près de Tamatave (C.Keller) [MHNG, examined]. One worker syntype (CASENT0101762, top specimen of 3w on one pin, image on antweb) hereby designated the **LECTOTYPE**.
[Justified emendation of spelling to *ranavalonae*: Forel, 1891:184.]

Crematogaster ranavalonae Forel, Forel, A. 1891:185. Male described.

Crematogaster ranavalonae Forel, Emery, C. 1897:13. Queen described.

Crematogaster ranavalonae Forel, Forel, A. 1901:375. Combination in *C. (Oxygyne*).

= *Crematogaster paulinae* Forel, A. 1892: 530. Queen diagnosis in key. Queen syntypes from Madagascar: Andrangoloaka (M.Sikora) [NOT examined, specimens could not be located in MHNG]; Emery, C. 1897:14 and Forel, A. 1903:254. *C. paulinae* as subspecies of *C. ranavalonae*; Forel, A. 1903: 254. Worker described [this specimen and the associated queens have been examined]; Combination in *C. (Oxygyne*): Wheeler, W.M. 1922:1025. **NEW SYNONYMY**

= *Crematogaster ranavalonae* var. *pepo* Forel, A. 1922:96. Worker, queen and male syntypes from Madagascar: St-Marie de Madagascar [=Ile St.Marie] (unknown collector) [MHNG, examined]. **NEW SYNONYMY**

= *Crematogaster* (*Oxygyne*) *descarpentriesi* Santschi, F. 1928:68. Worker syntypes from Madagascar: Fianarantsoa (Descarpentries) [NHMB, examined]. **NEW SYNONYMY**

= *Crematogaster emmae* Forel, A. 1891:227. Queen holotype [by monotypy] from Madagascar: Forêt d'Andrangoloaka (M.Sikora) [MHNG, examined]; Santschi, F. 1928: 69. Worker described; Forel, A.1901: 375. Combination in *C. (Oxygyne*). **NEW SYNONYMY**

= *Crematogaster emmae* var. *laticeps* Forel, 1892:529(q.), 534(w.), 535(m.) (diagnoses in key). Queen, worker and male syntypes from Madagascar: Forêt d'Andrangoloaka (M.Sikora) [MHNG, examined]; Emery, 1922:156. Combination in *C. (Oxygyne*). **NEW SYNONYMY**

= *Crematogaster inops* Forel, 1892:254. Worker holotype [by monotypy] from Madagascar: Environs de la ville d'Anosibé (province de Bezanozano) (M.Sikora) [MHNG, examined; note that this specimen is in very poor condition: head, mesosoma and metasoma are detached, covered in glue and partly crushed]; Wheeler, W.M. 1922:1024. Combination in *C. (Oxygyne*). **NEW SYNONYMY**

Material examined.—(BBBC, CASC, PSWC, MHNG, NHMB, MCZ) MADAGASCAR: *Antsirananana:* Sakalava Beach: -12.26278, 49.39750, 10m (R. Harin'Hala); Montaigne Français: -12.32500, 49.33333, 150m (R. Harin'Hala); R.S. Ambre: -12.46889, 49.24217, 325m (B.L.Fisher et al.); P.N. Montagne d'Ambre: -12.52028, 49.17917, 1125m (B.L.Fisher et al.); Nosy Bé, R.N.I. Lokobé: -13.41944, 48.33117, 30m (B.L.Fisher et al.); Nosy Bé: -13.39875, 48.29609, 5 m (B.L.Fisher et al.); Nosy Bé Airport: -13.32017, 48.31083, 25m (B.L.Fisher et al.); Nosy Bé, 4km ESE Andoany (=Hellville): -13.41670, 48.30000, 50-200m (P.S.Ward); Nosy Bé, R.N.I. Lokobé: -13.41670, 48.30000, 0-400m (D.M.Olson); Nosy Bé, Lokobe Forest: -13.41640, 48.30720, 50m (G.Alpert); Nosy Bé, 5km E Marodokana: -13.36670, 48.30000, 50m (G.Alpert); R.S. Manongarivo: -13.96167, 48.43333, 400m; -13.97667, 48.42333, 780m (B.L.Fisher et al.); R.S. Manongarivo: -13.92950, 48.45320, 275m, -13.93023, 48.45285, 285m; -13.93165, 48.45053, 390m (B.B.Blaimer); Ampasindava, Ambilanivy: -13.79861, 48.16167, 600m (B.L.Fisher et al.); Ambanja: -13.68268, 48.45245, 30m (B.L.Fisher et al.); F Binara: -13.25500, 49.61667, 375m (B.L.Fisher et al.); F Analamazava: -13.25546, 49.61850, 80m (B.B.Blaimer); F Bekaraoka: -13.16667, 49.71000, 150m (B.L.Fisher et al.); F Ampondrabe: -12.97000, 49.70000, 175m (B.L.Fisher et al.); .); F Andavakoera: -13.11833, 49.23000, 425m (B.L.Fisher et al.); Rés. Analamerana: -12.80467, 49.37383, 225m (B.L.Fisher et al.); F Antsahabe: -13.21167, 49.55667, 550m (B.L.Fisher et al.); Ambondrobe: -13.71533, 50.10167, 10m (B.L.Fisher et al.); P.N. Ankarana: -12.90889, 49.10983, 80m; -12.86361, 49.22583, 210m (B.L.Fisher); Rès. Ankarana, 7km SE Matsaborimanga, -12.90000, 49.11670, 150m (P.S.Ward); Ankarana: -12.93110, 49.12250, 130m (G.Alpert); Mont. d'Akirindro: -15.28833, 49.54833, 600m (B.L.Fisher et al.); Mont. d'Anjanaharibe: -15.18833, 49.61500, 470m (B.L.Fisher et al.); F Betaolana: -14.52996, 49.44039, 880m (B.L.Fisher et al.); P.N. Marojejy [Manantenina]: -14.43333, 49.76667, 750m; -14.43333, 49.75000, 1225m (B.L.Fisher et al); 2km W Antalaha: -14.89440, 50.26970, 1m (G.Alpert); 5km SW Antalaha: -14.93810, 50.26170, 50m (G.Alpert); 30km N Antalaha, Amboangy, -14.66480, 50.19070, 130m (G.Alpert); 36km N Antalaha, Andrapengy: -14.66670, 50.21670, 250m (G.Alpert); 55km S Antalaha, Nosy Ngontsy: -15.26440, 50.48930, 50m (G.Alpert); Nosy Komba: -13.45000, 48.35000, 25m (G.Alpert); P.N. Masoala: -15.71333, 49.97167 (B.L.Fisher et al.); -15.72667, 49.95667, 150m (A.Dejean et al.); 84km SW Sambava on road to Andapa: -14.57730, 49.73940, 160m (W.L.&D.E.Brown); *Antananarivo*: Andrangoloaka: -19.03333, 47.91667 (M.Sikora); *Fianarantsoa*: Fianarantsoa: -21.45000, 47.07500 (Descarpentries); F Atsirakambiaty: -20.59333, 46.56333, 1550m (B.L.Fisher et al.); P.N. Ranomafana: -21.26650, 47.42017, 1020m; -21.25000, 47.36667, 1130m; (R.Harin'Hala); P.N. Ranomafana: -21.24833, 47.42667, 900m (C.E.Griswold et al.); P.N. Ranomafana (A.Pauly); 9km ESE Ranomafana, nr Ifanadiana: -21.28330, 47.53330, 600m (P.S.Ward); P.N. Ranomafana, Tsarahonenana: -21.16670, 47.59580, 420m (G.Alpert); 8km E Kianjavato: -21.38860, 47.94360, 145m (G.Alpert); F.C. Vatovavy: -21.40000, 47.94000, 175m (B.L.Fisher et al.); R.S. Manombo: -23.01580, 47.71900, 30m (B.L.Fisher et al.); Mahabo [Rés. Forestière d'Agnalazaha]: -23.19383, 47.72300, 20m (B.L.Fisher et al.); P.N. Isalo: -22.48167, 45.46167, 725m (B.L.Fisher et al.); F.C. Analavelona: -22.67500, 44.19000, 1100m (B.L.Fisher et al.); *Mahajanga*: F Sohisaka: -18.10322, 47.18692, 1470m (B.L.Fisher et al.); Rés. Bemarivo: -16.92500, 44.36833, 30m (B.L.Fisher et al.); P.N. Namoroka: -16.37667, 45.32667; -16.40667, 45.31000, 100m (B.L.Fisher et al.); Mahavavy River: -16.05167, 45.90833, 20m (B.L.Fisher et al.); Rés. Forestière Beanka: -18.06009, 44.54086, 260m (B.L.Fisher et al.); F Tsimembo: -18.99528, 44.44350, 50m (B.L.Fisher et al.); P.N. Tsingy de Bemeraha: -19.13222, 44.81467, 50m (B.L.Fisher et al.); S.F. Ampijoroa: -16.31670, 46.81670, 80m; *Toamasina*: Mangoroufer [Amparafarafantsiv] : -18.88330, 48.11670 (M.Sikora) ; Bois de l'Ivondro près de Tamatave: -18.23333, 49.36667 (C.Keller); Environs de la ville d'Anosibé (province de Bezanozano): -18.91670, 48.05000 (M.Sikora); St-Marie de Madagascar [Ile St.Marie] : -16.88330, 49.88330 (unknown collector); Fenerive, Ost Madagaskar (unknown collector); F Kalalao [Ile St.Marie]: -16.92250, 49.88733, 100m (B.L.Fisher et al.); Rés. Ambodiriana: -16.67233, 49.70117, 125m (B.L.Fisher et al.); F Ambohidena [Ile St.Marie] : -16.82433, 49.96417, 20m (B.L.Fisher et al.); R.S. Ambatovaky: -16.77468, 49.26551, 355m; -16.81745, 49.29250, 400m; -16.77550, 49.26427, 430m; -16.77274, 49.26551, 450m (B.L.Fisher et al.); F Sahafina: -18.81445, 48.96205, 140m (B.L.Fisher et al.); Morarano-Chrome b: -17.75000, 47.98333, 1270m (A.Pauly); P.N.Andasibe: -18.92639, 48.40783, 1025m (B.L.Fisher et al.); Perinet: -18.93330, 48.41670, 930m (W.L. Brown); Rés. Perinet-Analamazoatra: -18.93330, 48.43330, 950m (D.M.Olson); F Ambatovy: -18.85083, 48.32000, 1075m; P.N.Zahamena: -17.75244, 48.85320, 760m (B.L.Fisher et al.); Mahavelona (Foulpointe): -17.66667, 49.50000, (A.Pauly); 11km SE Ampasimanolotra (=Brickaville): -18.90000, 49.13330, 5m (P.S.Ward) ; *Toliara*: P.N. Befotaka-Midongy: -23.84080, 46.95750, 940m (B.L.Fisher et al.); F Petriky: -25.06167, 46.87000, 10m (B.L.Fisher); Ranobe: -23.03975,

43.61090, 30m (Frontier Project, MGF); Rés. Cap St.Marie: -25.59444, 45.14683, 160m (B.L.Fisher et al.); 18km NNW Betroka: -23.16330, 45.96860, 5m (M.A.Ivie&D.A.Pollock); F Mikea: -22.90367, 43.47550, 35m (R. Harin'Hala); Libanona Beach: -25.03883, 46.99600, 20m (B.L.Fisher et al.); F Ivohibe: -24.56900, 47.20400, 200m (B.L.Fisher et al.); F Mandena: -24.95167, 47.00167, 20m (B.L.Fisher); P.N. Andohaela [Andohahela]: -24.83483, 46.48683, 60m (R. Harin'Hala); P.N. Andohahela, Col de Tanatana: -24.76420, 46.85610, 275m (B.L.Fisher et al.); P.N.Andohahela/parcel1: -24.94683, 46.67625, 440m; -24.94562, 46.68045, 470m (B.B.Blaimer); 6km SSW Eminiminy, Rès. Andohahela: -24.73330, 46.80000, 330m (P.S.Ward); 2.7km WNW 302º St.Luce: -24.77167, 47.17167, 20m (B.L.Fisher et al.).

Worker measurements (n=105) HW 0.77–1.08, HL 0.75–1.06, EL 0.11–0.22, SL 0.69–1.01, WL 0.83–1.28, SPL 0.12–0.23, PTH 0.13–0.21, PTL 0.20–0.34, PTW 0.17–0.35, PPL 0.15–0.26, PPW 0.19–0.37, LHT 0.67–1.06, CI 0.97–1.07, OI 0.17–0.23, SI 0.82–1.00, SPI 0.12–0.23, PTHI 0.55–0.79, PTWI 0.73–1.26, PPI 1.20–1.71, PPPI 0.98–1.26, LBI 1.10–1.35.

Worker description

Small to large (HW 0.77–1.08 mm, WL 0.83–1.28 mm); colour reddish brown, brown, dark brown or black.

Masticatory margin of mandibles with 4 teeth; posterior margin of head in full-face view with suboval or subangular corners, sometimes medially depressed; antennal scapes usually easily surpassing posterior head margin, rarely only barely surpassing (SI 0.82 - 1.00); eyes situated slightly above midline of head in full face view.

Pronotum laterally rounded or subangular; promesonotal suture from distinct and complete to indistinct or incomplete; promesonotal outline variable: mesonotum flush with pronotum or mesonotum moderately raised with respect to pronotum, dorsal face of mesonotum convex, lateral portions rounded or subangular; mesonotum postero-laterally rounded or sometimes with postero-lateral tubercules, with or without a distinct posterior face; metanotum often visible; dorsal face of propodeum usually long, almost equal length as posterior face, sometimes shorter or indistinct from posterior face; propodeal spines small - medium sized (SPI 0.12 - 0.23), between half and full the width between their bases, slender, straight or down-curved, in dorsal view weakly diverging (<20º); propodeal spiracle oval; petiole in dorsal view variable, usually slender and suboval or rectangular, sometimes weakly to strongly flared anteriorly; postpetiole wider than long, with broad median impression.

Head sculpture mostly finely to coarsely reticulate, sometimes aciculate; promesonotum dorsally reticulate, sometimes weakly costulate; mesopleuron and metapleuron reticulate or costulate; dorsal and posterior face of propodeum shiny; petiole and postpetiole and ventrally reticulate; erect pilosity on face highly variable, 0–20 setae, sparse appressed pubescence; head ventrally with regular appressed pubescence, and usually also suberect pilosity present; erect pilosity on mesosoma usually present, $\leq$ 8 humeral and $\leq$ 6 mesonotal setae; 0–4 long erect dorso-posterior setae present on petiole; postpetiole with 0–8 long erect to suberect dorso-posterior setae.

Queen measurements (n=44) HW 1.03 - 1.40, HL 1.11 - 1.17, EL 0.26 - 0.35, SL 0.89 - 1.38, MSNW 0.69 - 0.99, MSNL 0.82 - 1.11, WL 1.60 - 1.92, SPL 0.14 - 0.38, PTH 0.27 - 0.39, PTL 0.35 - 0.47, PTW 0.32 - 0.57, PPL 0.21 - 0.35, PPW 0.44 - 0.85 , LHT 0.90 - 1.34, CI 0.91 - 1.09, OI 0.23 - 0.28, SI 0.73 - 1.10, MSNI 0.75 - 1.00, SPI 0.09 - 0.15, PTHI 0.70 - 1.08 , PTWI 0.92 - 1.56, PPI 1.57 - 3.12, PPPI 1.09 - 1.69, LBI 1.32 - 1.87.

Queen description

Size small to very small (HW 1.03 - 1.40 mm, WL 1.60 - 1.92 mm); colour brown, dark brown or black.

Clypeus medially slightly projecting over mandibles; mandibles falcate, apical tooth enlarged and pointed and a second, very small pre-apical tooth present on masticatory margin; head shape variable, posterior margin of head in full-face view oval, suboval or subangular; antennal scape length variable (SI 0.73 - 1.10), but usually easily surpassing or at least reaching posterior margin of head; eyes distinctly emarginate, situated at or below midline of head in full face view.

Mesosoma short (MSNI 0.75 - 1.00, WL 1.60 - 1.92 mm); mesoscutum in dorsal view oval; propodeal suture shallow; dorsal face of propodeum variable in length; propodeal spines present; petiole shape highly variable, antero-ventral tooth absent; postpetiole more than twice as wide as long, with broad median impression, antero-ventral tooth present, although sometimes minute; legs long (LBI 1.32 - 1.87).

Sculpture on head, meso-and metasoma largely absent, at most aciculate or finely reticulate, somewhat more pronounced on petiole and postpetiole; face usually with long erect pilosity and shorter suberect to decumbent pilosity, both highly variable in abundance; appressed pilosity on head usually lacking; head ventrally usually with short suberect pilosity; erect or suberect setae present on eyes; erect pilosity on mesonotum usually present, but abundance variable; petiole and postpetiole with abundant long suberect pilosity; erect and appressed pilosity on abdominal segments 4 - 7 highly variable.

Morphological variation

Worker ants of this species vary continuously in size, head shape, promesonotal structure, petiole shape, pilosity and sculpture (see plots of worker measurements, Figs S6–9). Queens also show high morphological variation, but in contrast this variation is discontinuous and more pronounced than in workers (see plots of queen measurements, Figs S1–5). Three distinct morphological forms are distinguished below, which have also been investigated with molecular data (see above, and Fig. 1).

Queen morph A (n=23) (Figs S32–33) – Head more or less quadrate (CI 0.95–1.03); antennal scape very long (SI 0.98–1.12); postpetiole width moderate (PPPI 1.09–1.35); antero-ventral tooth small to minute; legs usually long (LBI 1.32–1.64); face with fairly abundant erect pilosity of short and medium length, and dense shorter suberect to decumbent pilosity on genae and below eyes; erect pilosity on mesonotum sparse anteriorly, but more abundant posteriorly and on scutellum. Similar to *C. ranavalonae paulinae* specimens and *C. ranavalonae pepo* syntypes.

Queen morph B (n=11) (Figs S34–35) – Head usually longer than wide and oval (CI 0.91 - 0.99), antennal scape shorter, but still surpassing head margin (SI 0.84 - 1.05); postpetiole width moderate (PPPI 1.16 - 1.40); antero-ventral tooth small to minute; legs short to moderately long (LBI 1.42 - 1.87); erect pilosity on face generally short, with few longer setae; from sparse to moderately dense suberect to decumbent pilosity on genae and below eyes; erect pilosity on mesonotum very much reduced, a few very short setae present. Similar to *C. ranavalonae* specimens.

Queen morph C (n=10) (Figs S36–37) – Head more or less quadrate (CI 0.95 - 1.08); antennal scape shorter, reaching or slightly surpassing head margin (SI 0.73 - 0.90); pronotum

with anterior angular shoulders; postpetiole width moderate to large (PPPI 1.20 - 1.69); antero-ventral tooth large; legs short to moderately long (LBI 1.52 - 1.83); face with abundant erect pilosity of short and medium length; erect pilosity on mesonotum variable, from nearly absent to abundant throughout. Similar to *C. emmae* holotype and *C. emmae laticeps* syntypes.

Distribution and biology

Crematogaster ranavalonae occurs throughout Madagascar in both rainforest and dry forest habitats (Fig. 35). Relatively scarce collections suggest generally low population sizes of this species. *C. ranavalonae* is a carton-nest builder, with nest sizes attaining much larger dimensions than the *Crematogaster* (*Decacrema*)-group that also builds carton-nests in Madagascar. Personally obtained nest collections of this species included two very large carton nests (ca. 40x30cm and 30x30cm, the former shown in Figs 36–38), which were constructed around a thick branch or around the trunk of a young tree. One of the colonies contained large numbers of both males and alate queens, while the other one only had males and queen pupae; collections were made in the months of February and March respectively. One nest further housed beetles of the families Staphilinidae (Pselaphinae) and Brentidae (subfamily Brentinae). *C. ranavalonae* workers are very aggressive towards intruders (pers. observ.) and highly territorial towards conspecific workers from neighboring colonies or other ant species (Dejean et al., 2010).

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Wheeler, W.M. (1922) Ants of the American Museum Congo expedition. A contribution to the myrmecology of Africa. IX. Keys to the genera and subgenera of ants. *Bulletin of the American Museum of Natural History*, **45**, 1005–1055.

TABLE S1: Specimen information			**Data set**	**Collection data**				
Species	**Taxon ID**	**Voucher specimen ID**		**Country**	**#**	**Locality**	**GPS coordinates**	
							Lat.	**Long.**
Crematogaster								
ranavalonae	C_ranaQ01_bem	CASENT0489246	**A, B**	Madagascar	**14**	Mahajanga: R.S. Bemarivo, 30m	-16.92500	44.36833
ranavalonae	C_ranaQ02_bem	CASENT0073574	**A**	Madagascar	**14**	Mahajanga: R.S. Bemarivo, 30m	-16.92500	44.36833
ranavalonae	C_ranaQ04_tao	CASENT0151874	**A, B**	Madagascar	**6**	Antsiranana: Betaolana Forest, 880m	-14.52996	49.44039
ranavalonae	C_ranaQ07_nam	CASENT0486407	**A, B**	Madagascar	**13**	Mahajanga: P.N. Namoroka, 100m	-16.40667	45.31000
ranavalonae	C_ranaQ08_aja	CASENT0125550	**A**	Madagascar	**3**	Antsiranana: Ambanja, 30m	-13.68268	48.45245
ranavalonae	C_ranaQ03_bea	CASENT0156540	**A**	Madagascar	**15**	Mahajanga: Rés.forestière Beanka, 260m	-18.06009	44.54086
ranavalonae	C_rana01_ats	CASENT0491513	**A**	Madagascar	**16**	Fianarantsoa: Forêt d'Atsirakambiaty, 1550m	-20.59333	46.56333
ranavalonae	C_rana02_bem	CASENT0485141	**A**	Madagascar	**14**	Mahajanga: R.S. Bemarivo, 30m	-16.92500	44.36833
ranavalonae	C_rana03_ahe	CASENT0193425	**A, B**	Madagascar	**20**	Toliara: P.N. Andohahela, parcel 1, 470m	-24.94562	46.68045
ranavalonae	C_rana03_isa	CASENT0036095	**A**	Madagascar	**18**	Fianarantsoa: P.N. Isalo, 725m	-22.48167	45.46167
ranavalonae	C_rana03_vato	CASENT0060762	**A**	Madagascar	**17**	Fianarantsoa: Forêt Classée Vatovavy, 175m	-21.40000	47.94000
ranavalonae	C_rana03_mds	CASENT0119122	**A**	Madagascar	**19**	Fianarantsoa: P.N. Befotaka-Midongy, 940m	-23.83517	46.96367
ranavalonae	C_rana04_darai	CASENT0193054*; CASENT0193164**	**A**	Madagascar	**2**	Antsiranana: Forêt d'Analamazava, 340m	-13.25550	49.61850
ranavalonae	C_rana04_aza	CASENT0057288	**A**	Madagascar	**7**	Antsiranana: Forêt Ambanitaza, 240m	-14.67933	50.18367
ranavalonae	C_rana04_mda	CASENT0121558	**A**	Madagascar	**4**	Toliara: Forêt Mandena, 20m	-24.95267	47.00250
ranavalonae	C_rana04_ahi	CASENT0067031	**A**	Madagascar	**9**	Toamasina: Ile St. Marie, Forêt Ambohidena, 20m	-16.82433	49.96417
ranavalonae	C_rana04_man	CASENT0193531	**A**	Madagascar	**4**	Antsiranana: R.S. Manongarivo, 330m	-13.93115	48.45237
ranavalonae	C_rana05_ank	CASENT0433728	**A**	Madagascar	**1**	Antsiranana: P.N. Ankarana, 210m	-12.86361	49.22583
ranavalonae	C_rana06_ank	CASENT0423151	**A**	Madagascar	**1**	Antsiranana: P.N. Ankarana, 210m	-12.86361	49.22583
ranavalonae	C_rana06_mvy	CASENT0490101*; CASENT0021249**	**A**	Madagascar	**12**	Mahajanga: Mahavavy River, 20m	-16.05167	45.90833
ranavalonae	C_rana08_ako	CASENT0038502	**A**	Madagascar	**8**	Toamasina: Mont. d'Akirindro, 600m	-15.28833	49.54833
ranavalonae	C_rana09_kao	CASENT0068800	**A**	Madagascar	**10**	Toamasina: Ile St.Marie, Forêt Kalalao, 100m	-16.92250	49.88733
ranavalonae	C_rana10_safi	CASENT0136353	**A, B**	Madagascar	**11**	Toamasina: Sahafina Forest, 140m	-18.81445	48.96205
ranavalonae	C_rana11_amp	CASENT0477030	**A**	Madagascar	**5**	Antsiranana: Ampasindava, F. d'Ambilanivy, 600m	-13.79861	48.16167
ranavalonae	C_rana12_adn	CASENT0066530	**A**	Madagascar	**9**	Toamasina: Ile St. Marie, Forêt Ambohidena, 20m	-16.82433	49.96417
ranavalonae	C_rana13_man	CASENT0193261	**A**	Madagascar	**4**	Antsiranana: R.S. Manongarivo, 275m	-13.92950	48.45320
agnetis	_may	CASENT0051228	**A, B**	Madagascar		Toamasina: Analamay, 1068m	-18.80623	48.33707
agnetis	_mjy	CASENT0487780	**A, B**	Madagascar		Antsiranana: P.N. Marojejy, 775m	-14.43500	49.76000
aberrans	_IN	CASENT0193779	**B**	India		Maharashtra: Sanjai Ghandi NP	19.21380	72.91990
aberrans	_TH	CASENT0193687	**B**	Thailand		Chaiyaphum: Tat Ton National Park, 290m	15.97870	102.03720
santschii	_ZA	CASENT0193640	**A, B**	South Africa		Kwazulu-Natal: Lake Sibaya, 43m	-27.41240	32.71140
meijerei	_1	CASENT0193685	**B**	New Guinea		Madang: Baitabag village, 90m	-5.13820	145.75370
meijerei	_2	CASENT0193683	**B**	New Guinea		Sandaun: Utai village, 220m	-3.38450	141.58760
stadelmanni	_GA	CASENT0193573	**B**	Gabon		Ogooué-Maritime: Rés. de Monts Doudou, 350m	-2.22250	10.40580
stadelmanni	_CAR	CASENT0094528	**B**	Centr. Afr. Rep.		Sangha-Mbaéré: P.N. Dzanga-Ndoki, 350m	2.36000	16.05333
rasoherinae	N/A	CASENT0193413	**B**	Madagascar		Toliara: P.N. Andohahela/parcel 3, 170m	-25.01790	46.65175
sp.BBB43	N/A	CASENT0193399	**B**	Madagascar		Toliara: P.N. Andohahela/parcel 3, 160m	-25.01367	46.64650

sp.BBB51	N/A	CASENT0193264	**B**	Madagascar	Antsiranana: R.S. Manongarivo, 360m	-13.93145	48.45183

TABLE S2

Species name		Locality information	Coordinates	Collector	Collection
Crematogaster aberrans		India, Thana	n/a	Gleadow	MHNG; NHMB; MCZ
		Thailand, Chaiyaphum, Tat Ton NP	15.9787, 102.0372	Jaruphan & Budsawong	CASC
		India, Maharashtra: Sanjai Ghandi NP	19.2138, 72.9199	S. Hosoishi	BBBC
C. aberrans assmuthi		described from: India, Bombay		**NOT EXAMINED**	
C. aberrans inglebyi		described from: India, Travancore		**NOT EXAMINED**	
C. agnetis		MADAGASCAR	Refer to species description		
C. augusti		described from: Sumatra, Marang		**NOT EXAMINED**	
C. butteli		Sumatra, Soengei Bamban	n/a	Buttel	MHNG; NHMB
C. breviventris	Transferred	described from: Cameroun [Cameroon], Molundu		**NOT EXAMINED**	
C. daisyi		Borneo, Sarawak	n/a	Haviland	MHNG; NHMB
C. dalyi		India, Coonoor		Daly	MNHG
C. dalyi sikkimensis		Sikkim	n/a	unknown	NHMB
C. donisthorpei		Sud-Rhodesia, Mashonaland [Zimbabwe]	n/a	Donisthorpe	NHMB
C. ebenina		India, Kanara	n/a	Wroughton	NHMB
		India, Poona	n/a	Wroughton	MCZ
C. ebenina corax		described from: Birmanie, Moulmain [Myanmar]		**NOT EXAMINED**	
C. magitae	Transferred	described from: Westafrika [Namibia]		**NOT EXAMINED**	
C. margaritae		Congo, Brazzaville	n/a	Weiss	NHMB
C. margaritae brevarmata		Congo belge [DR Congo], Kasai, Kondue	n/a	Luja	NHMB
C. margaritae cupida		described from: DR Congo, Luebo, Macaco		**NOT EXAMINED**	
C. margaritae lujae		described from: DR Congo, Kasai, Kondue		**NOT EXAMINED**	
C. marthae		MADAGASCAR	Refer to species description		
C. meijerei	Transferred	New Guinea, Huon Peninsula, lower Busu Range	n/a	E.O.Wilson	MCZ
		Papua, Karema, Brown Range	n/a	E.O.Wilson	MCZ
		PNG, Madang, Baitabag vill.,	-5.1333, 145.7833	M.Janda	MJC, BBBC
		PNG, Sandaun prov., Utai vill., SE from Vanimo,	-3.3833, 141.5833	M.Janda	MJC, BBBC
C. oscaris		W-Africa, Petit Namaland [Namibia]	n/a	unknown	MHNG;NHMB
		S-W Africa, Kamaggar [Namibia]	n/a	unknown	MCZ
C. pia	Transferred	Malacca, Negeri Sembilan	n/a	R.Martin	MCZ
C. pia soengeiensis	Transferred	described from: Sumatra, Soengei Bamban		**NOT EXAMINED**	
C. pia taivanae	Transferred	described from: Taiwan, Taihorin		**NOT EXAMINED**	
C. ranavalonae		MADAGASCAR	Refer to species description		
C. santschii	Transferred	Belg. Kongo [DR Congo], Stanleyville	n/a	Kohl	ZBMH

		Congo, Kasai	n/a	unknown	MCZ
		South Africa, Kwa-Zulu Natal, Lake Sibaya,	27.4124, 32.7114	S.van Noort	BBBC
C. santschii clymene	Transferred	described from: South Africa, Natal, Durban		**NOT EXAMINED**	
C. soror		Bombay	n/a	Rothney	MHNG; NHMB
		Phuntsholing, Bhutan	n/a	Rothney	NHMB
C. trautweini	Transferred	Kamerun [Cameroon]	n/a	unknown	ZMBH
C. tumidula		described from: Sumatra, Pangherang Pisang		**NOT EXAMINED**	
C. stadelmanni	Transferred	Liberia	n/a	Benson	ZMBH
		Uganda, Nagunga	n/a	C.C.Gowdey	MCZ
		Gabon, Woleu-Ntem,	2.0800, 12.4067	B.L.Fisher	CASC
		Gabon, Ogooué-Maritime: Rés. de Monts Doudou, 350m	2.2267, 10.3950	B.L.Fisher	CASC
		Gabon, Ogooué-Maritime: Rés. de Monts Doudou, 350m	2.2233, 10.4067	B.L.Fisher	CASC
		Gabon, Ogooué-Maritime: Rés. de Monts Doudou, 370m	2.2225, 10.4058	S.van Noort	CASC
		Central African Rep., Sangha-Mbaéré: P.N. Dzanga-Ndoki, 350m	3.0335, 16.4095	S.van Noort	CASC
		Central African Rep., Sangha-Mbaéré: P.N. Dzanga-Ndoki, 350m	2.3667, 16.0500	B.L.Fisher	CASC
		Central African Rep., Sangha-Mbaéré: Rés. Dzanga-Sangha, 420m	3.0000, 16.2000	B.Fisher	CASC
C. stadelmanni gracilenta	Transferred	Kamerun [Cameroon], Rio del Rey	n/a	Viehmeyer	ZMBH
C. stadelmanni dolichocephala	Transferred	Congo: Manamama, Benagmisa, Kwamouth	n/a	H.O.Lang	MCZ
C. stadelmanni anguliceps	Transferred	described from: Kamerun [Cameroon], Ossidinge		**NOT EXAMINED**	
C. stadelmanni angustata	Transferred	described from: Camerun		**NOT EXAMINED**	
C. stadelmanni intermedia	Transferred	described from: Camerun		**NOT EXAMINED**	
C. stadelmanni ovinodis	Transferred	described from: Belgisch-Kongo, Duma, Ubangi-Distrikt		**NOT EXAMINED**	
C. stadelmanni schereri	Transferred	described from: Liberia, Bendov		**NOT EXAMINED**	
C. stadelmanni spissata	Transferred	described from: Congo belge [DR Congo], Sankuru, Kondue		**NOT EXAMINED**	
*C. (Oxygyne) sp. undet.*1		Central African Rep., P.N. Dzanga-Ndoki	3.0335, 16.4095	S.van Noort	CASC

TABLE S3

Species	Voucher specimen	Caste
C. aberrans	CASENT0193779	worker
C. aberrans	CASENT0129432	queen
C. meijerei	CASENT0193685	worker
C. meijerei	CASENT0193793	queen
C. santschii	CASENT0193683	worker
C. stadelmanni	CASENT0193787	worker
C. stadelmanni	CASENT0193573	queen
C. rasoherinae	CASENT0193412	worker
C. sp.BBB43	CASENT0193399	worker
C. sp.BBB51	CASENT0193264	worker

TABLE S4

Uncorrected p distances

	clade A	**clade A-I**	**A-II**	**clade A-III**	**clade A-IV**	**clade A-V**	**clade B**	***C. ranavalonae* (A+B)**	**maximum**
***C. ranavalonae* (A+B)**	/	/	/	/	/	/	/	*0.0618*	***0.0934***
clade A	*0.0592*								
clade A-I	/	*0.0123*							
A-II	/	0.0698	/						
clade A-III	/	0.0729	0.0676	*0.0248*					
clade A-IV	/	0.0801	0.0630	0.0755	*0.0188*				
clade A-V	/	0.0745	0.0633	0.0726	0.0528	*0.0124*			
clade B	0.0731	0.0806	0.0705	0.0756	0.0680	0.0708	*0.0120*		
agnetis	**0.1583**	**0.1553**	**0.1552**	**0.1599**	**0.1585**	**0.1627**	**0.1384**	**0.1566**	

Tamura-Nei distances

	clade A	**clade A-I**	**A-II**	**clade A-III**	**clade A-IV**	**clade A-V**	**clade B**	***C. ranavalonae* (A+B)**	**maximum**
***C. ranavalonae* (A+B)**	/	/	/	/	/	/	/	*0.0671*	***0.1035***
clade A	*0.0643*								
clade A-I	/	*0.0125*							
A-II	/	0.0761	/						
clade A-III	/	0.0788	0.0729	*0.0263*					
clade A-IV	/	0.0891	0.0679	0.0824	*0.0193*				
clade A-V	/	0.0819	0.0682	0.0789	0.0563	*0.0127*			
clade B	0.0795	0.0887	0.0761	0.0818	0.0735	0.0768	*0.0122*		
agnetis	**0.1877**	**0.1815**	**0.1771**	**0.188**	**0.1887**	**0.1932**	**0.1584**	**0.1841**	

SUPPLEMENTARY TABLE AND FIGURE CAPTIONS

Table S1. Species names, Taxon IDs, specimen IDs, locality data and dataset of specimens used in the molecular study. In cases where two CASENT numbers are given, two different specimens from the same collection have been used for DNA extraction and sequence generation (*=COI; **= LR, AK and CD). Locality numbers refer to Figure 2.

Table S2. Comprehensive list of species included in *Oxygyne*. For each species, the localities (coordinates where applicable), collectors and sources of all examined specimens are given. In cases where no material was examined, type localities are given. Localities in this table form the basis of *Oxygyne* distribution as shown in Fig. 17. Species that have been transferred or re-transferred to *Oxygyne* are indicated. *C. travancorensis* is excluded from the subgenus *Oxygyne* and thus not listed.

Table S3. List of specimen codes for voucher specimen images deposited on AntWeb (www.antweb.org). Images can be found by searching for the CASENT number on AntWeb or via a search engine.

Table S4. Genetic distances within Malagasy *Oxygyne*, calculated as uncorrected p- and Tamura Nei-distances from COI sequence data. Distances are averaged across members of the two deep clades A and B and five subclusters within A (I, II, III, IV, V) as recovered by Mr.Bayes analyses and shown in Fig. 1. Numbers in italics give mean distances within clusters or clades; bold numbers show distances of the various subsets to *Crematogaster agnetis*; maximum distances within *C. ranavalonae* are in bold and italic. Averages represent arithmetic means rounded to the 4th decimal.

Figs S1–5. Morphometric plots of selected queen measurements, showing morphological variation between and among Malagasy *Oxygyne* species as revised. 1, cephalic index; 2, scape index; 3, body size; 4, petiole-postpetiole index, 5, leg-body index. Values are based upon data presented in the context of species descriptions (see electronic supplementary text).

Figs S6–9. Morphometric plots of selected worker measurements, showing morphological variation between and among Malagasy *Oxygyne* species as revised. Where available for morphometric study, the associated syntype specimens to *C. ranavalonae* have been highlighted as separate data series. 6, cephalic index; 7, scape index; 8, body size; 9, petiole width index. Values are based upon data presented in the context of species descriptions (see electronic supplementary text).

Fig. S10. ML-phylogeny of Malagasy *Oxygyne* based on 594bp of cytochrome oxidase I (COI) and dataset A. Bootstrap values are shown in red.

Fig. S11. BI phylogenies of Malagasy *Oxygyne* based on separate analyses of nuclear loci for Dataset A; A, LR Wh gene tree, B, ArgK gene tree, C, CAD gene tree, D, LR Wh, ArgK, CAD concatenated. For information on number of nucleotides for each loci, partitions and model settings, refer to Table 3.

Fig. S12. A, The estimated ln probability of data, Ln P(D), plotted over the number of populations *K* = 1-8, as estimated in STRUCTURE. Different colors represent different runs (n=10). B, Structure bar plots for *C. ranavalonae* individuals for *K*=5; results are summarized across 10 runs. Individuals are grouped by locality.

Figs S13–19. *Crematogaster agnetis* (w, CASENT0107473; q, CASENT0112155); 13, worker profile view; 14, worker dorsal view; 15, worker full-face view; 16, queen profile view; 17, queen full-face view; 18, queen dorsal view; 19, distribution.

Figs S20–26. *Crematogaster marthae* (w, CASENT0101802; q, CASENT0171185); 20, worker profile view; 21, worker full-face view; 22, worker dorsal view; 23, queen profile view; 24, queen full-face view; 25, queen dorsal view; 26, distribution.

Figs S27–31. Morphological diversity of *Crematogaster ranavalonae* worker ants; a, full-face view; b, profile view; c, dorsal view (CASENT0491513, CASENT0193205, CASENT0141548, CASENT0193277, CASENT0423149).

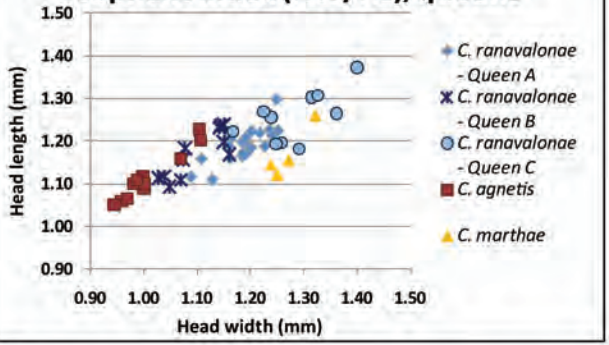
Figs S32–35. *Crematogaster ranavalonae* queen ants; 32–33, Queen A (CASENT0485160, CASENT0066769); 34-35, Queen B (CASENT0496495, CASENT0193642); a, full-face view; b, profile view; c, dorsal view.

Figs S36–41. *Crematogaster ranavalonae* queen ants; 36–37, Queen C (CASENT0114271, CASENT0193239); a, full-face view; b, profile view; c, dorsal view; 38, distribution of *C. ranavalonae*; 39, *C. ranavalonae* carton nest; 40, internal structure of the same nest; 41, position of the same nest in young tree.

Fig. S42. Gene trees as reconstructed jointly with the species tree (Fig. 16A) in *BEAST. A, LR Wh, 1108bp; B, ArgK, 514bp; C, CAD, 744bp; D, COI, 604bp.

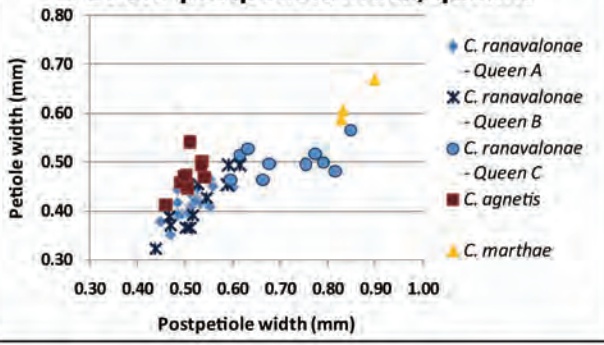
S1

Cephalic index (HW/HL), queens



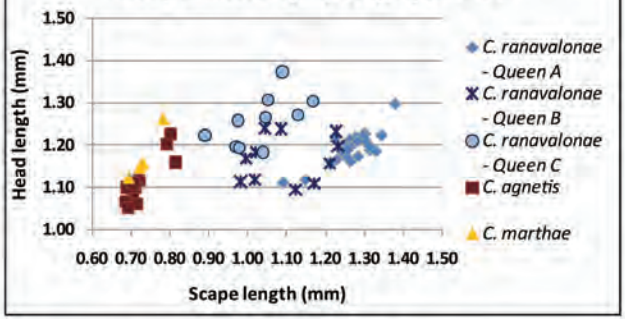
S4

Petiole-postpetiole index, queens



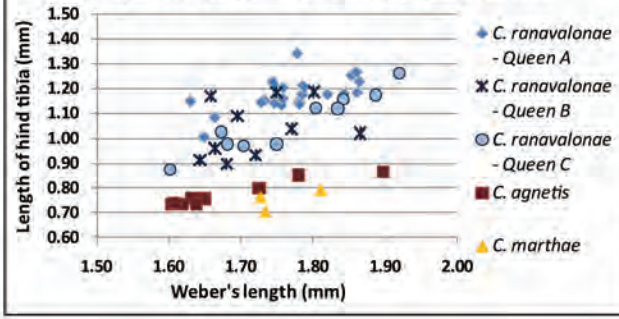
S2

Scape index (SL/HL), queens



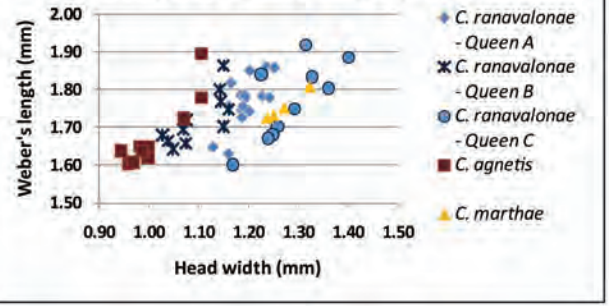
S5

Leg-body index (WL/LHT), queens

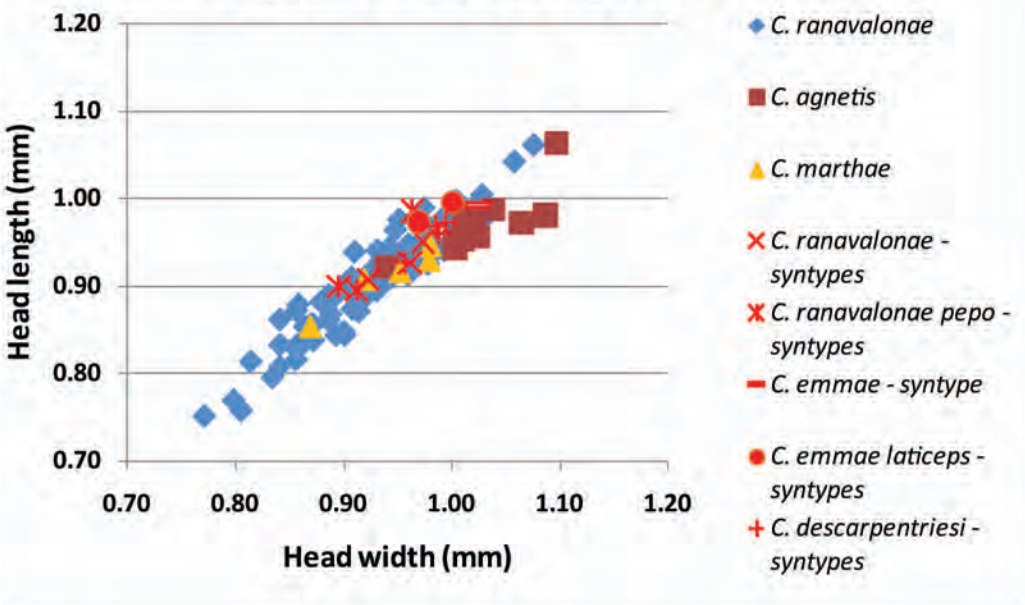


S3

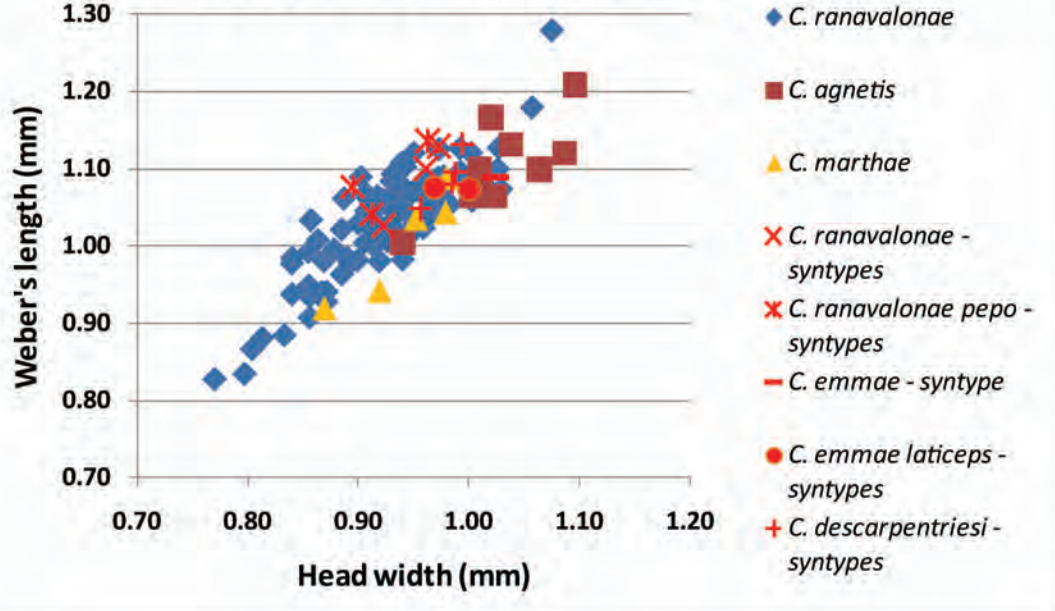
Body size (HW/WL), queens



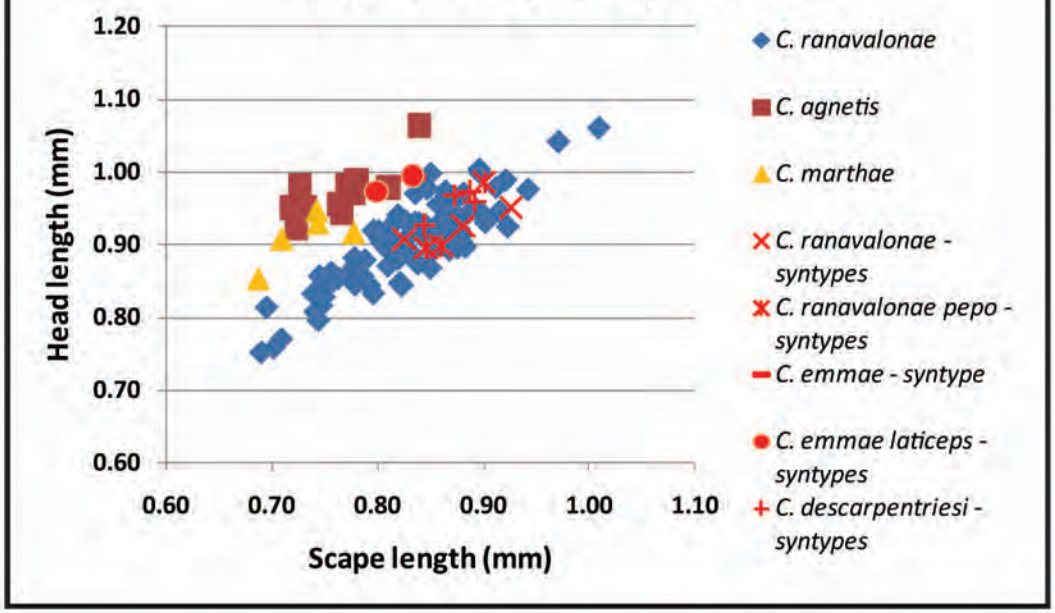
S6


Cephalic index (HW/HL), workers
Head length (mm)
1.20
1.10
1.00
0.90
0.80
0.70
0.70
0.80
0.90
1.00
1.10
1.20
Head width (mm)
C. ranavalonae
C. agnetis
C. marthae
C. ranavalonae - syntypes
C. ranavalonae pepo - syntypes
C. emmae - syntype
C. emmae laticeps - syntypes
C. descarpentriesi - syntypes

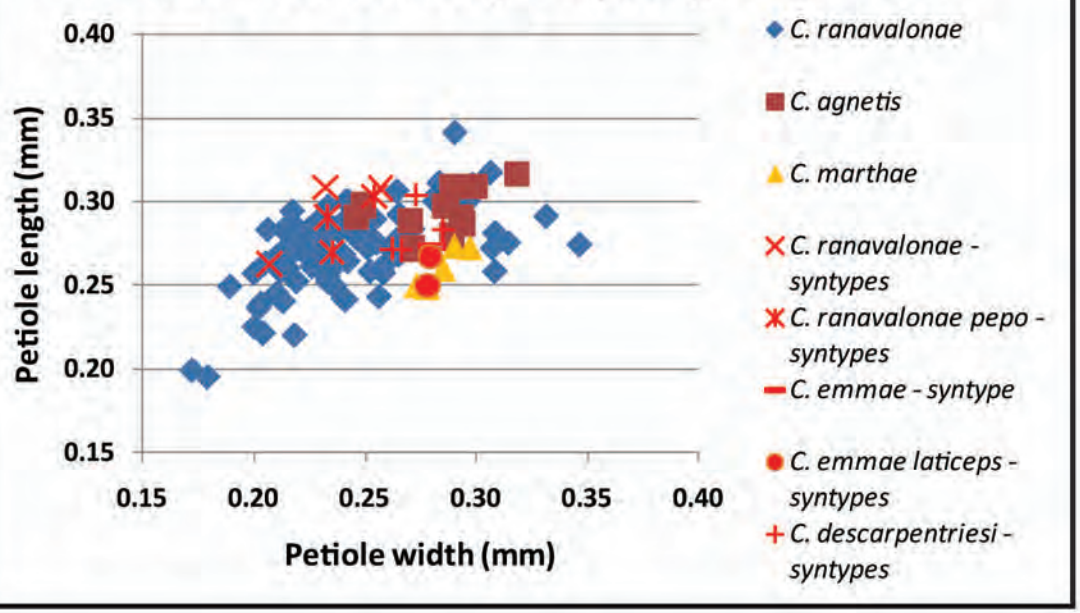
S8

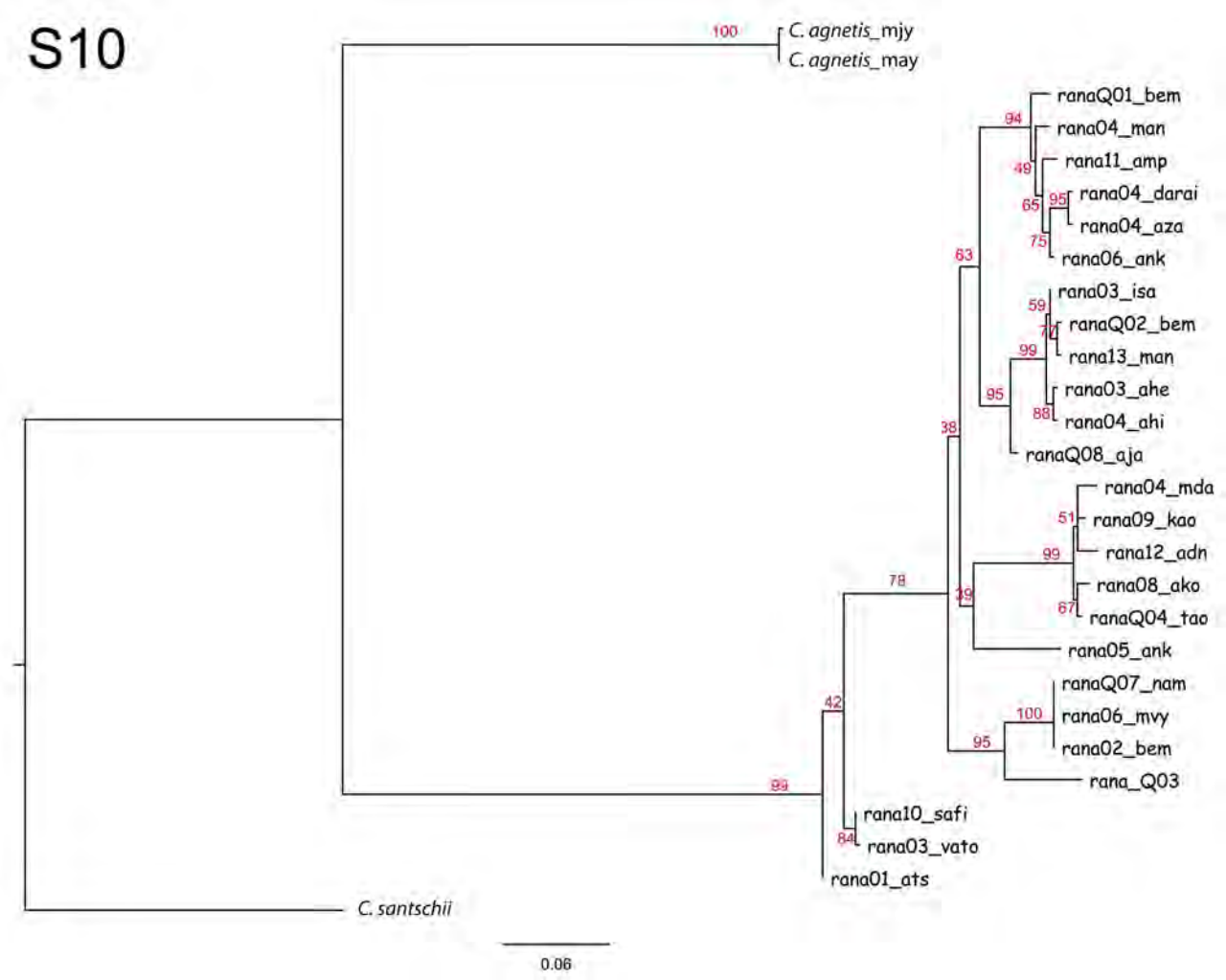

Body size (HW/WL), workers
Weber's length (mm)
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.70
0.80
0.90
1.00
1.10
1.20
Head width (mm)
C. ranavalonae
C. agnetis
C. marthae
C. ranavalonae - syntypes
C. ranavalonae pepo - syntypes
C. emmae - syntype
C. emmae laticeps - syntypes
C. descarpentriesi - syntypes

S7

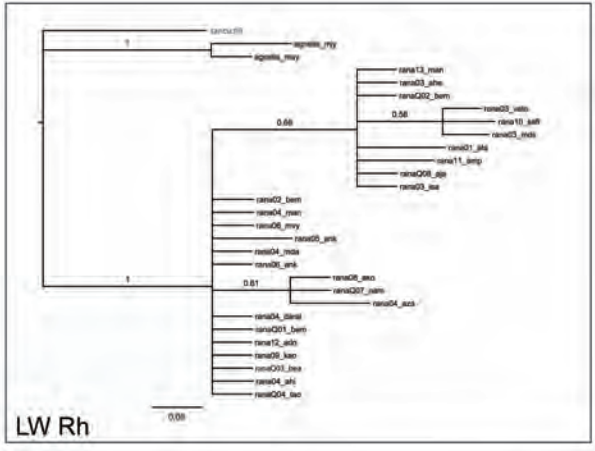

Scape index (SL /HL), workers
Head length (mm)
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.60
0.70
0.80
0.90
1.00
1.10
Scape length (mm)
C. ranavalonae
C. agnetis
C. marthae
C. ranavalonae - syntypes
C. ranavalonae pepo - syntypes
C. emmae - syntype
C. emmae laticeps - syntypes
C. descarpentriesi - syntypes

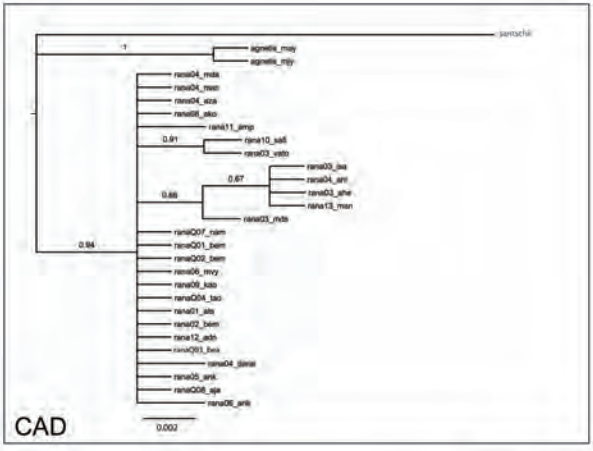
S9

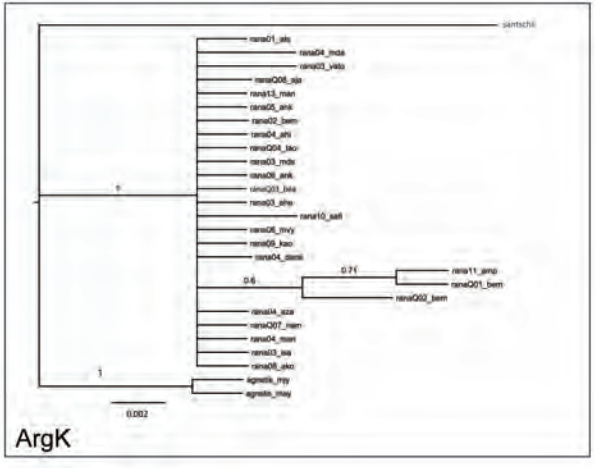

Petiole width index (PTW/PTL), workers
Petiole length (mm)
0.40
0.35
0.30
0.25
0.20
0.15
0.15
0.20
0.25
0.30
0.35
0.40
Petiole width (mm)
C. ranavalonae
C. agnetis
C. marthae
C. ranavalonae - syntypes
C. ranavalonae pepo - syntypes
C. emmae - syntype
C. emmae laticeps - syntypes
C. descarpentriesi - syntypes

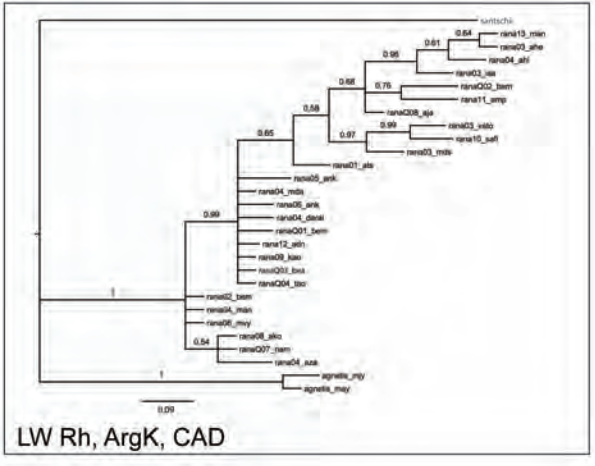

S10
C. agnetis_mjy
C. agnetis_may
ranaQ01_bem
rana04_man
rana11_amp
rana04_darai
rana04_aza
rana06_ank
rana03_isa
ranaQ02_bem
rana13_man
rana03_ahe
rana04_ahi
ranaQ08_aja
rana04_mda
rana09_kao
rana12_adn
rana08_ako
ranaQ04_tao
rana05_ank
ranaQ07_nam
rana06_mvy
rana02_bem
rana_Q03
rana10_safi
rana03_vato
rana01_ats
C. santschii
100
94
49
65
95
75
63
59
77
99
95
88
38
51
99
78
39
67
42
100
95
99
84
0.06

S11

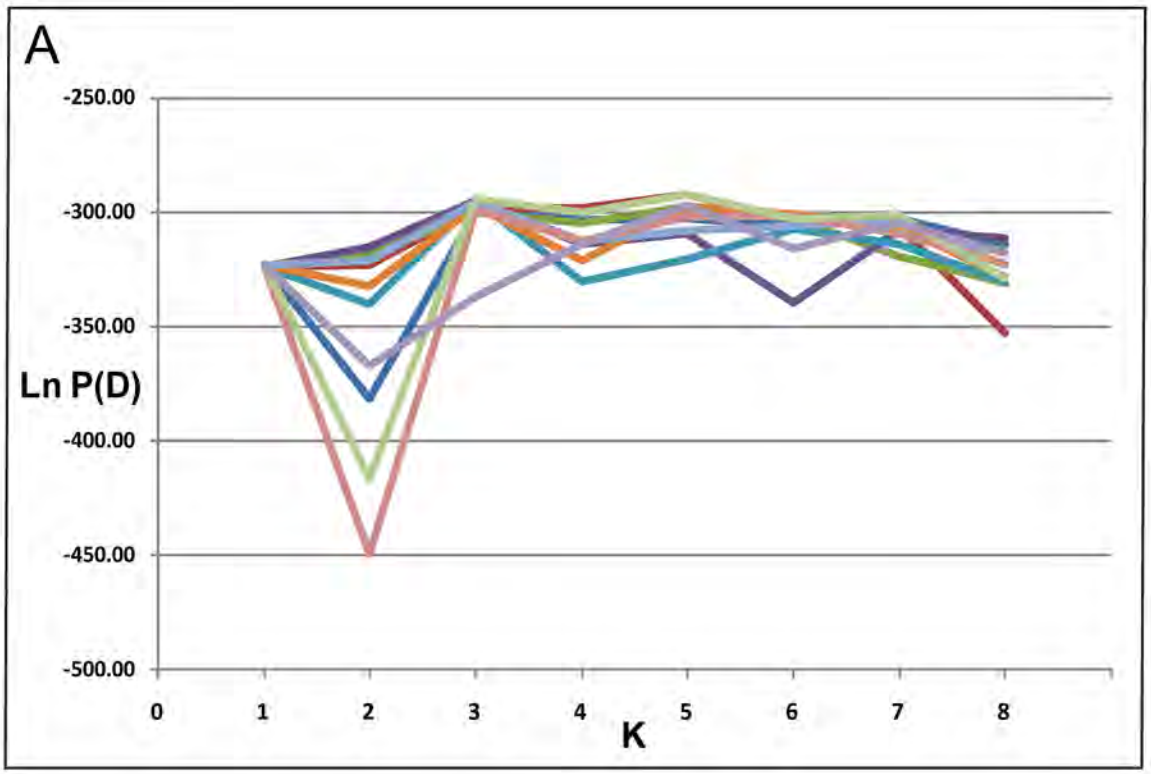


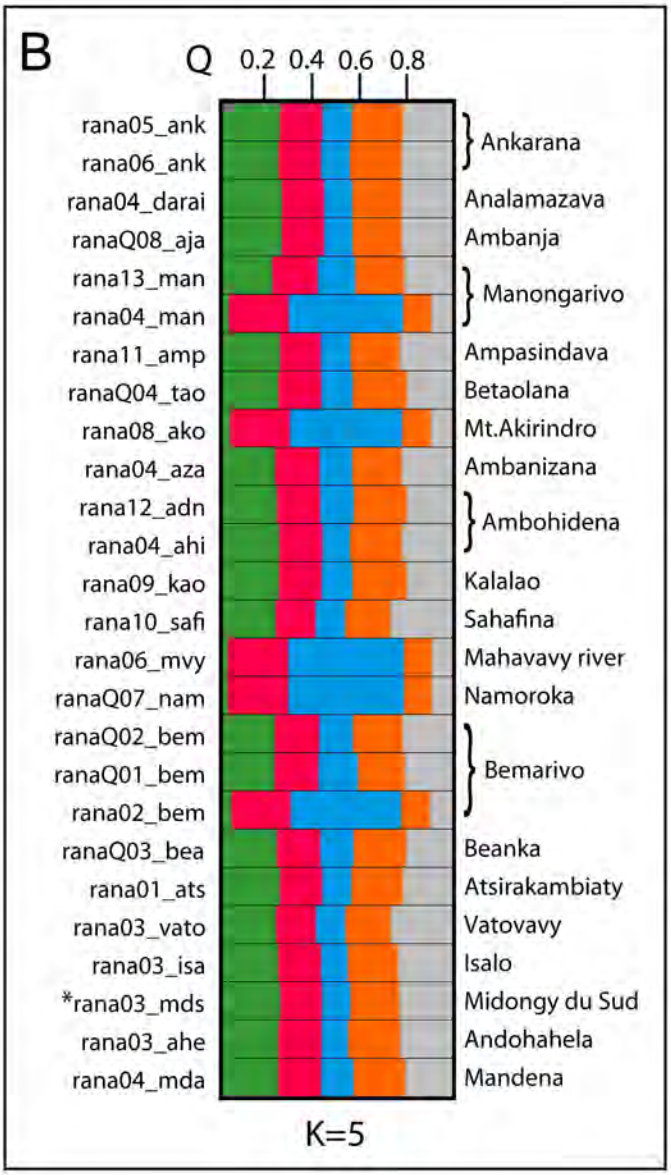






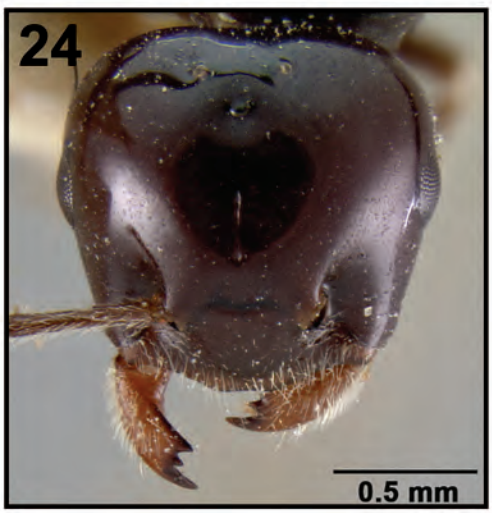
S12


A
-250.00
-300.00
-350.00
Ln P(D)
-400.00
-450.00
-500.00
0
1
2
3
4
5
6
7
8
K

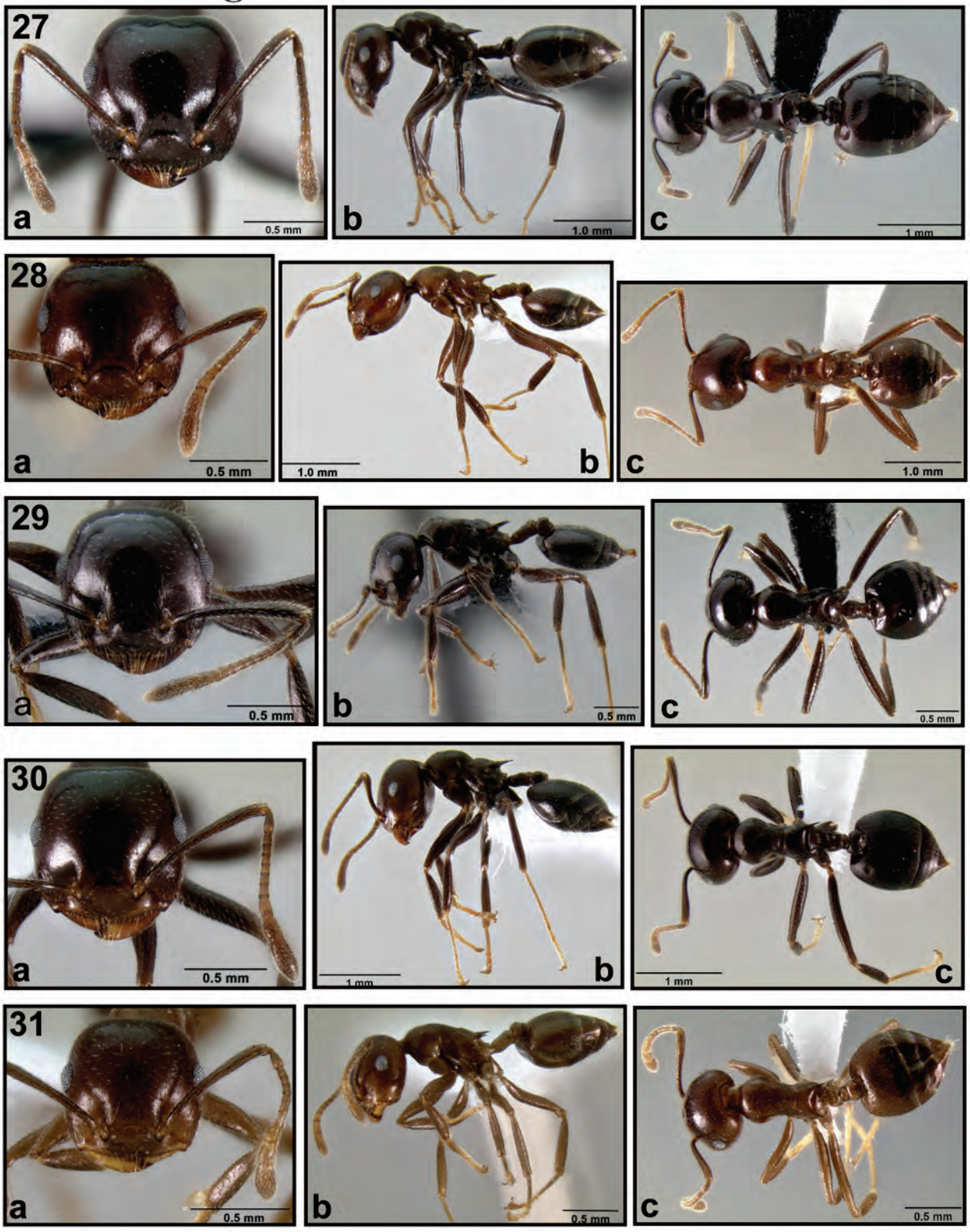

B
Q
0.2
0.4
0.6
0.8
rana05_ank
rana06_ank
rana04_darai
ranaQ08_aja
rana13_man
rana04_man
rana11_amp
ranaQ04_tao
rana08_ako
rana04_aza
rana12_adn
rana04_ahi
rana09_kao
rana10_safi
rana06_mvy
ranaQ07_nam
ranaQ02_bem
ranaQ01_bem
rana02_bem
ranaQ03_bea
rana01_ats
rana03_vato
rana03_isa
*rana03_mds
rana03_ahe
rana04_mda
Ankarana
Analamazava
Ambanja
Manongarivo
Ampasindava
Betaolana
Mt.Akirindro
Ambanizana
Ambohidena
Kalalao
Sahafina
Mahavavy river
Namoroka
Bemarivo
Beanka
Atsirakambiaty
Vatovavy
Isalo
Midongy du Sud
Andohahela
Mandena
K=5

S *Crematogaster agnetis*

S *Crematogaster marthae*

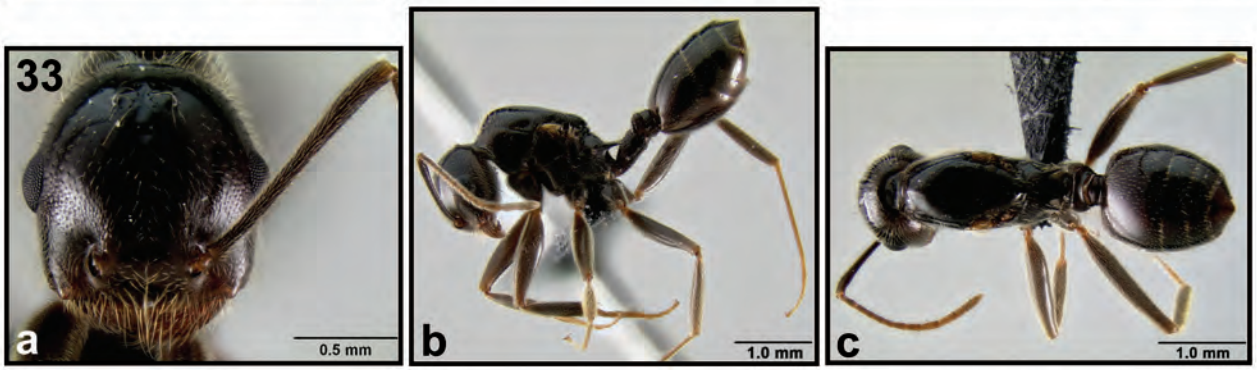


S *Crematogaster ranavalonae*

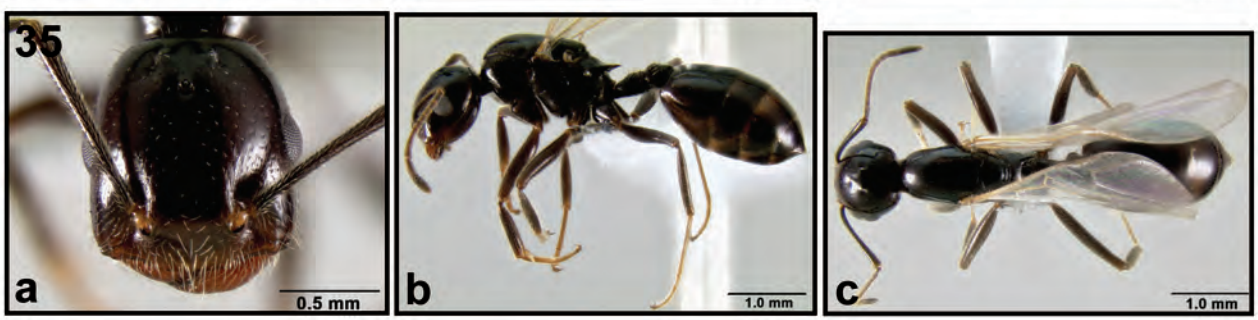


S *Crematogaster ranavalonae*

Queen A



Queen B



S *Crematogaster ranavalonae*

Queen C