

A morphological and molecular study of some species in the *Camponotus maculatus* group (Hymenoptera: Formicidae) in Australia and Africa, with a description of a new Australian species

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Abstract

Captain Cook is recognised as the collector of *Camponotus maculatus* (FABRICIUS, 1782) from Sierra Leone and since then many subspecies have been described, most of which are from Africa. One, *Camponotus maculatus humilior* FOREL, 1902 is common in northern Australia. We describe a morphological and molecular study aimed at determining the relationship of species of the *C. maculatus* group in Australia and Africa. From this we find no close relationship between the Australian and African species examined. We raise *Camponotus maculatus humilior* to species rank, synonymise *Camponotus villosus* CRAWLEY, 1915 with *Camponotus novaehollandiae* MAYR, 1870 and describe *Camponotus crozieri* sp.n. We indicate the need for more work in defining the boundaries of *Camponotus novaehollandiae*.

Key words: Ants, Formicinae, DNA, cryptic species, *Camponotus maculatus*, *C. novaehollandiae*, *C. humilior*, *C. crozieri*, new species.

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Introduction

There are over 1400 described species and subspecies of *Camponotus* MAYR, 1861 world wide (BOLTON 1995). Their taxonomy is complicated by the wide variation in shape and colour of worker castes within a species. These divide into major and minor workers for performing the tasks associated with maintenance of a colony. The minors are the food gatherers and may be only a third of the size of majors which possess an especially large head and powerful mandibles for defending the colony.

Camponotus maculatus (FABRICIUS, 1782) has accumulated over 100 subspecies and although the major worker of the species type has been adequately described and re-described (DONISTHORPE 1922), the minor worker has not been. Characters of minor workers are most useful for defining species boundaries in *Camponotus*. A.J.M. has examined the type specimen in BMNH of a major worker. It was collected by Captain Cook and believed to be from Sierra Leone. Most of the described taxa of the *C. maculatus* group are from Africa and thirty-nine of them have been raised to species rank (BARONI URBANI 1972). It is assumed the species name derives from the Latin macula = blotch or stain because of patches of lighter colour on its mesosoma.

One subspecies, *C. maculatus humilior* FOREL, 1902 has been described from Australia and it and its relatives, *C. novaehollandiae* MAYR, 1870 and *C. villosus* CRAWLEY, 1915 are common ants of northern Australia.

These three species possess strikingly similar morphological characters to some specimens of *C. maculatus* subspecies collected from Africa and now in the Humboldt Museum, Berlin and in the Natural History Museum, Vienna.

This study has been prompted by two questions: (a) Is the visual similarity in morphological characters of some Australian and African ants suggestive of a close evolutionary relationship, and could it be supported by molecular

analysis? (b) What is the status of brownish specimens apparently closely related to *C. novaehollandiae*, common in Australia, in relation to the consistently coloured yellowish types of *C. villosus* and *C. novaehollandiae*?

A key to Australian *Camponotus* based on morphological characters has been constructed to facilitate the identification of species from Australia (MCARTHUR in press). Boundaries of most of the 101 described Australian species are obvious but a few species lack distinct characters for their separation, in particular *C. novaehollandiae* and *C. villosus*. Previous studies on Australian *Camponotus* (MCARTHUR & ADAMS 1996, MCARTHUR & al. 1997) have shown that molecular analysis is useful in separating closely related species suggesting this procedure could be helpful in this present study.

The *Camponotus* species targeted for this study possess the following distinguishing characters and are loosely referred to as the *C. maculatus* group herein:

1. The most striking character is the distinct occipital carina in minor workers (Fig. 1). This forms a ridge externally and probably serves to strengthen the anterior parts of the head (SNODGRASS 1935), it is absent in major workers.

2. Strong dimorphism, i.e., workers encountered are mostly either major or minor, medium workers are non-existent or very scarce.

3. The sides of the heads of major workers taper strongly to the front (Fig. 2) while in minor workers, the sides are mostly parallel and taper to the rear (Fig. 4).

4. The vertex in major workers is concave or flat, in minor workers it is convex.

5. The scape and tibiae have plentiful short setae, raised up, more so in Australian than in African species.

6. Biology: (a) mostly nocturnal, (b) nests are in clay soil, never in sand, (c) entrances to nests are well hidden, (c) strong attraction to honey bait at night, (d) domination

at honey bait, (e) quickly scatter when disturbed by torch light.

Other Australian species included in the *C. maculatus* group viz *C. spenseri* (CLARK, 1930), *C. extensus* (MAYR, 1876) and *C. oxleyi* (FOREL, 1902) are not considered here because they can be separated from the target species by the integument of the heads of the major workers which are covered with fine punctations and are matte whereas the target species are more smooth and glossy.

Materials and methods

Preliminary to this study A.J.M. examined types of *C. novaehollandiae* in NHMW and *C. villosus* in BMNH and failed to find characters to separate them. We consider them synonymous and use only the name *C. novaehollandiae* in the rest of this paper (see Systematics). In late 2005, A.J.M. and A.A. Simpson collected ants from the vicinity of Broome in Western Australia, between Townsville and Cairns in Queensland and in Western Cape and Natal in South Africa. Bait consisting of 90 % honey and 10 % water was distributed during the last few hours of daylight either by painting it on trees at eye level or by placing rolled up toilet paper dipped in honey on the ground. Beginning around 2100 hrs the baits were visited and the ants from each bait were brushed into vials of 100 % ethanol, and labelled. Whenever a few target specimens could be recognised using a hand lens, attempts were made to find nests (by so doing there was a better chance of collecting a series of worker castes of the species as the baits were often attended by only minor workers). Next day, the previous night's catch was examined under a microscope and searches were made for the nests of target species. Eventually, in the South Australian Museum, a leg was taken from each of the 80 odd specimens of workers for DNA analysis. The remainders of these specimens were glued to points for morphological study and retained as vouchers. African species herein referred to were identified in the Iziko Museum, Cape Town, South Africa or named *Camponotus* A, *Camponotus* D or *Camponotus* E. Voucher specimens of African ants collected for the study were placed in the Iziko Museum.

Morphological analysis

Measurements were carried out using a Mitutoyo 209116 micrometer attached to an Olympus XZ microscope fitted with cross hairs at 20 to 80 ×. Measurements were transmitted to MS Excel 2000 via George Link Wedge (SPLat Controls Pty. Ltd). Measurements of head width, head length, pronotal width, frontal carinae width and mid tibia length were taken from representatives of the groups in the molecular analyses P Q R S T & U (Fig. 3, Tab. 1). Specimens were measured thus: head width HW = maximum distance between head sides with underside of head horizontal; head length HL = distance between anterior margin of clypeus and vertex with both in a horizontal plane; frontal carinae width = maximum distance between carinae ignoring any abrupt curvature at posterior ends with underside of head horizontal; tibia length = overall length of a mid-tibia in horizontal plane; pronotal width PW = maximum width of pronotum in dorsal view.

In "Systematics", measurements are given in mm, as minimum - maximum.



Fig. 1: Photograph of the top of the head of a minor worker of *C. crozieri* sp.n., indicating the occipital carinae.

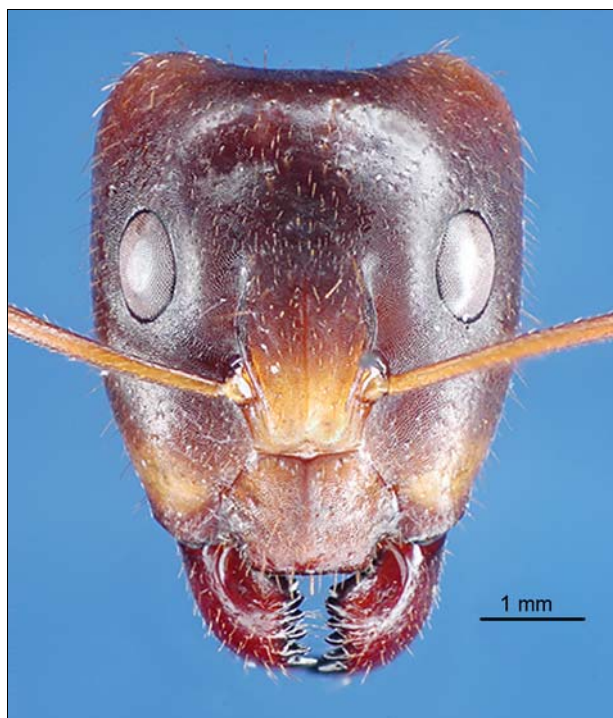


Fig. 2: Photograph of the front of the head of a major worker in the target group showing the sides tapering to the front.

Statistical analyses

Morphological data of the Australian specimens of the *C. maculatus* group were analysed using canonical discriminant analyses in SPSS© version 11.0 (2001). In order to compare overall morphological data with the results from

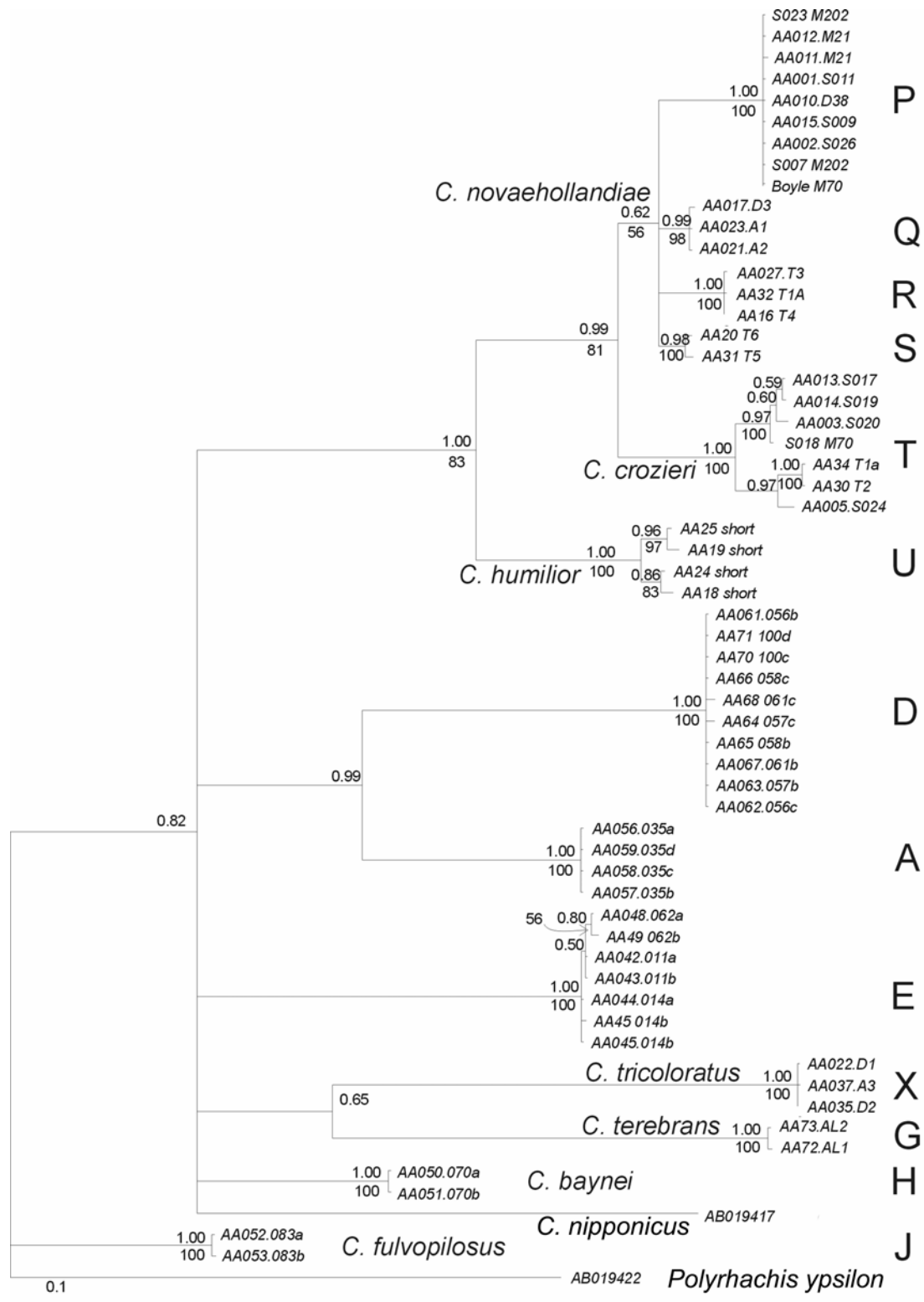


Fig. 3: Phylogenetic relationships presented as a Bayesian consensus tree of Australian and African *Camponotus* species, based on sequence data derived from the cytochrome c oxidase (CO1) gene. Posterior probabilities are shown above the branches and parsimony bootstrap values (10000 replicates) below the branches. Clades **P** = yellow *C. novaehollandiae* from Western Australia, **Q** = brownish, originally thought to be near *C. novaehollandiae* from Queensland, **R** = yellow *C. novaehollandiae* from Queensland, **S** = brownish, originally thought to be near *C. novaehollandiae* from Queensland, **T** = brown, *C. crozieri* sp.n. from Queensland and Western Australia, **U** = *C. humilior* st.n. from eastern Australia, **D** = *Camponotus* sp. D from Africa, **A** = *Camponotus* sp. A from Africa, **E** = *Camponotus* sp. E from Africa, **X** = *C. tricoloratus* from Queensland, **G** = *C. terebrans* from South Australia, **H** = *C. baynei* from Africa, and **J** = *C. fulvopilosus* from Africa.

Tab. 1: Showing collection data for the taxa used in the molecular analysis with Ref. the sample identifier, n the number of specimens examined morphologically, and GenBank accession numbers for CO1 and CytB. The ants were collected by A.J.M. and A.A. Simpson during late 2005.

Clade	Taxa	Ref.	n	Country	Locality	Latitude	Longitude	Date	CO1	CytB
P	<i>C. novaehollandiae</i>	SO23		Australia	Roebuck Plains	17°56'09"S	122°21'25"E	12.IX.2005	DQ915762	
P	<i>C. novaehollandiae</i>	m21	10	Australia	Minyirr	17°53'03"S	122°21'31"E	13.IX.2005	DQ915768	
P	<i>C. novaehollandiae</i>	m21	3	Australia	Minyirr	17°53'03"S	122°21'31"E	14.IX.2005	DQ915768	DQ915728
P	<i>C. novaehollandiae</i>	SO11		Australia	Derby Road	17°49'23"S	122°36'34"E	12.IX.2005	DQ915767	
P	<i>C. novaehollandiae</i>	D38		Australia	Dampier Creek	17°55'31"S	122°14'40"E	16.IX.2005	DQ915766	
P	<i>C. novaehollandiae</i>	S009	2	Australia	Derby Road	17°49'24"S	122°35'33"E	17.IX.2005	DQ915765	
P	<i>C. novaehollandiae</i>	S026		Australia	Roebuck Plains	17°58'25"S	122°21'10"E	17.IX.2005	DQ915757	DQ915740
P	<i>C. novaehollandiae</i>	S007	2	Australia	Derby Road	17°49'24"S	122°35'09"E	13.IX.2005	DQ915759	
P	<i>C. novaehollandiae</i>	Boyle	4	Australia	Broome	17°55'33"S	122°13'01"E	15.IX.2005	DQ915763	
Q	nr <i>C. novaehollandiae</i>	D3		Australia	Dead Horse Crk.	18°16'40"S	145°59'04"E	29.IX.2005	DQ915748	DQ915731
Q	nr <i>C. novaehollandiae</i>	A1	9	Australia	Attie Ck	18°16'48"S	145°59'01"E	29.IX.2005	DQ915770	
Q	nr <i>C. novaehollandiae</i>	A2		Australia	Attie Ck	18°16'52"S	145°59'54"E	29.IX.2005	DQ915769	
R	<i>C. novaehollandiae</i>	T3	10	Australia	Castle Hill	19°15'16"S	146°48'14"E	28.IX.2005	DQ915753	DQ915736
R	<i>C. novaehollandiae</i>	T1A		Australia	James Cook Uni.	19°20'01"S	146°45'23"E	28.IX.2005	DQ915755	DQ915738
R	<i>C. novaehollandiae</i>	T4		Australia	Castle Hill	19°15'16"S	146°48'14"E	28.IX.2005	DQ915747	DQ915730
S	nr <i>C. novaehollandiae</i>	T6		Australia	Castle Hill	19°15'16"S	146°48'14"E	28.IX.2005	DQ915800	
S	nr <i>C. novaehollandiae</i>	T5		Australia	Castle Hill	19°15'16"S	146°48'14"E	28.IX.2005	DQ915754	DQ915737
T	<i>C. crozieri</i> sp.n.	SO17		Australia	Derby Road	17°49'09"S	122°42'30"E	16.IX.2005	DQ915744	DQ915727
T	<i>C. crozieri</i> sp.n.	SO19		Australia	Derby Road	17°46'51"S	122°50'44"E	16.IX.2005	DQ915746	DQ915729
T	<i>C. crozieri</i> sp.n.	SO20	20	Australia	Derby Road	17°46'27"S	122°52'11"E	16.IX.2005	DQ915743	DQ915726
T	<i>C. crozieri</i> sp.n.	S018		Australia	Roebuck Plains	17°56'27"S	122°21'32"E	17.IX.2005	DQ915759	DQ915742
T	<i>C. crozieri</i> sp.n.	T1a	10	Australia	James Cook Uni.	19°19'44"S	146°45'39"E	28.IX.2005	DQ915756	DQ915739
T	<i>C. crozieri</i> sp.n.	T2		Australia	James Cook Uni.	19°19'44"S	146°45'39"E	28.IX.2005	DQ915799	
T	<i>C. crozieri</i> sp.n.	SO24		Australia	Roebuck Plains	17°56'27"S	122°21'32"E	17.IX.2005	DQ915758	DQ915741
U	<i>C. humilior</i> stat.n.	S2		Australia	Stoney Creek	16°54'15"S	145°45'12"E	1.X.2005	DQ915752	DQ915735
U	<i>C. humilior</i> stat.n.	W2		Australia	Mt. Whitfield	16°52'30"S	145°44'18"E	2.X.2005	DQ915750	DQ915733
U	<i>C. humilior</i> stat.n.	S1		Australia	Stoney Creek	16°52'32"S	145°40'01"E	1.X.2005	DQ915749	DQ915732
U	<i>C. humilior</i> stat.n.	W3	10	Australia	Mt. Whitfield	16°53'55"S	145°44'58"E	3.X.2005	DQ915751	DQ915734
D	<i>Camponotus</i> D	56b		S. Africa	Vernon Crookes	30°16'27"S	30°36'34"E	27.X.2005	DQ915787	
D	<i>Camponotus</i> D	100d		S. Africa	Umlalazi	28°57'24"S	31°46'19"E	1.XI.2005	DQ915798	
D	<i>Camponotus</i> D	100c		S. Africa	False Bay	28°02'08"S	32°21'42"E	30.X.2005	DQ915797	
D	<i>Camponotus</i> D	58c		S. Africa	Umlalazi	28°57'25"S	31°46'39"E	1.XI.2005	DQ915796	
D	<i>Camponotus</i> D	61c		S. Africa	False Bay	28°01'38"S	32°21'09"E	29.X.2005	DQ915795	
D	<i>Camponotus</i> D	57c		S. Africa	Umlalazi	28°57'24"S	31°46'19"E	1.XI.2005	DQ915794	
D	<i>Camponotus</i> D	58b		S. Africa	Umlalazi	28°57'24"S	31°46'19"E	1.XI.2005	DQ915793	
D	<i>Camponotus</i> D	61b		S. Africa	Umlalazi	28°57'24"S	31°46'19"E	1.XI.2005	DQ915790	
D	<i>Camponotus</i> D	57b		S. Africa	Umlalazi	28°57'24"S	31°46'19"E	1.XI.2005	DQ915789	
D	<i>Camponotus</i> D	56c		S. Africa	Umlalazi	28°57'24"S	31°46'19"E	1.XI.2005	DQ915788	
A	<i>Camponotus</i> A	35a		S. Africa	False Bay	28°01'38"S	32°21'09"E	30.X.2005	DQ915783	
A	<i>Camponotus</i> A	35d		S. Africa	False Bay	28°01'38"S	32°21'09"E	30.X.2005	DQ915786	
A	<i>Camponotus</i> A	35c		S. Africa	False Bay	28°01'38"S	32°21'09"E	30.X.2005	DQ915785	
A	<i>Camponotus</i> A	35b		S. Africa	Hluhluwe-Umfolozi	28°04'11"S	32°02'18"E	31.X.2005	DQ915784	

E	<i>Camponotus</i> E	62a		S. Africa	Vernon Crookes	30°16'43"S	30°35'45"E	27.X.2005	DQ915778	
E	<i>Camponotus</i> E	62b		S. Africa	Vernon Crookes	30°16'27"S	30°36'34"E	27.X.2005	DQ915777	
E	<i>Camponotus</i> E	11a		S. Africa	Vernon Crookes	30°16'27"S	30°36'34"E	27.X.2005	DQ915774	
E	<i>Camponotus</i> E	11b		S. Africa	False Bay	28°01'38"S	32°21'09"E	30.X.2005	DQ915775	
E	<i>Camponotus</i> E	14a		S. Africa	Grootvadersbos	33°59'08"S	20°42'36"E	24.X.2005	DQ915776	
E	<i>Camponotus</i> E	14b		S. Africa	Grootvadersbos	33°59'08"S	20°42'36"E	24.X.2005	DQ915792	
E	<i>Camponotus</i> E	14b		S. Africa	Grootvadersbos	33°59'08"S	20°42'36"E	24.X.2005	DQ915792	
X	<i>C. tricoloratus</i>	d1		Australia	Dead Horse Crk.	18°16'40"S	145°59'04"E	29.IX.2005	DQ915771	
X	<i>C. tricoloratus</i>	a3		Australia	Attie Creek	18°16'48"S	145°59'01"E	29.IX.2005	DQ915773	
X	<i>C. tricoloratus</i>	d2		Australia	Dead Horse Crk.	18°16'40"S	145°59'04"E	29.IX.2005	DQ915772	
G	<i>C. terebrans</i>	a12		Australia	Aldinga	35°16'01"S	138°27'00"E	12.XII.2005	DQ915760	
G	<i>C. terebrans</i>	a11		Australia	Aldinga	35°16'01"S	138°27'00"E	12.XII.2005	DQ915761	
H	<i>C. baynei</i>	70a		S. Africa	Brenton on Sea	34°04'23"S	23°02'37"E	25.X.2005	DQ915779	
H	<i>C. baynei</i>	70b		S. Africa	Brenton on Sea	34°04'23"S	23°02'37"E	25.X.2005	DQ915780	
J	<i>C. fulvopilosus</i>	83a		S. Africa	Gamkaskloosf	33°20'29"S	22°01'57"E	25.X.2005	DQ915781	
J	<i>C. fulvopilosus</i>	83b		S. Africa	Gamkaskloosf	33°20'29"S	22°01'57"E	25.X.2005	DQ915782	

the molecular analyses, specimens that shared clades based on molecular evidence were treated as groups.

Molecular analysis

Two regions of the mitochondrial genome were amplified: a 822 bp region of the 3' end of the cytochrome c oxidase subunit 1 (CO1) gene using primers M202 (forward, 5' - CAA CAT TTA TTT TGA TTT TTT GG - 3', alias Jerry, SIMON & al. 1994) and M70 (reverse, 5' - TCC ATT GCA CTA ATC TGC CAT ATT A - 3') (UEA9 and 10: LUNT & al. 1996). In some cases instead of primer M70 internal primer M81 (reverse 5' - AAA AAT GTT GAG GGA AAA ATG TTA - 3' (UEA8, LUNT & al. 1996) resulted in better amplification.

A 384 bp fragment of the cytochrome-b (CytB) gene was amplified using the primers M107 (forward, 5' - TAT GTA CTA CCA TGA GGA CAA ATA TC - 3' (alias CB1) and M108 (reverse, 5' - ATT ACA CCT CCT AAT TTA TTA GGA AT - 3' (alias CB2), designed by Y.C. Crozier (CROZIER & al. 1992).

Standard PCR amplifications included 1× reaction buffer (Perkin Elmer), 0.2 mM of each dNTP, 5 pM of each primer, 1 unit of Amplitaq Gold (Perkin Elmer) and 2 mM of MgCl₂ in a 25 µl reaction volume. PCR-amplifications were carried out on either an OMN-E 500 (Hybaid) thermocycler or a PC-960G Gradient thermal cycler (Corbett Research) for 1 cycle of 92 °C for 9 min and 36 cycles (94 °C, 45 s; 48 - 55 °C, 45 s; and 72 °C, 60 s), followed by a final incubation step at 72 °C for 6 min. PCR products were purified using the UltraClean™ PCR Clean-up DNA purification kit (MoBio Laboratories Inc.) according to the manufacturer's protocol.

Sequencing was performed using the ABI Prism™ Big Dye Terminator Cycle sequencing kit (PE Applied Biosystems) in 20 µl reaction volumes according to the manufacturer's instructions. PCR primers were used as sequencing primers and each fragment was sequenced on both strands. Reaction products were purified by isopropanol precipitation (as specified by ABI) and sequenced on ABI 3700 (version 3.7) automated DNA sequencers. SeqEd

version 1.0.3 (Applied Biosystems) was used to edit chromatogram files, to determine a consensus of bi-directional sequencing, and to manually align sequences across specimens. Sequences have been deposited at GenBank (Acc. numbers DQ915726 - DQ915800).

Phylogenetic analyses

Phylogenetic analyses of aligned sequence data were carried out using the programs PAUP* version 4.0b8 (SWOFFORD 2001) and MrBayes version 3.1 (HUELSENBECK & RONQUIST 2001). As outgroup taxa we used *Polyrhachis ypsilon* EMERY, 1887, *Camponotus nipponicus* WHEELER, 1928 (GenBank accession respectively AB019422 and AB019417), and *C. fulvopilosus* DE GEER, 1778. PAUP* was used to calculate pairwise uncorrected sequence divergences, and was used for parsimony tree reconstruction using heuristic search and TBR-branch swapping, including bootstrapping using the fast-step method. Bayesian analyses were performed using MrBayes implementing one cold and three heated chains, which were run for 10⁶ generations. The general time reversible model of sequence evolution was used with invariable site proportions and gamma shaped rates across sites (GTR-InvGamma), with unlinked parameters for the separate codon positions. Trees were sampled each 10 generations. Summarisation of the tree topologies and parameter values was done with the first 10000 trees discarded.

Depositories of type material

ANIC	Australian National Insect Collection, Canberra, Australia
BMNH	The Natural History Museum, London, U.K.
NHMW	Naturhistorisches Museum, Vienna, Austria
QM	Queensland Museum, Brisbane, Australia
SAMA	South Australian Museum, Adelaide, Australia

Results

We were able to amplify a 814 bp region of the CO1-gene for 58 specimens collected in Australia and South Africa. We failed to PCR amplify DNA extracted from

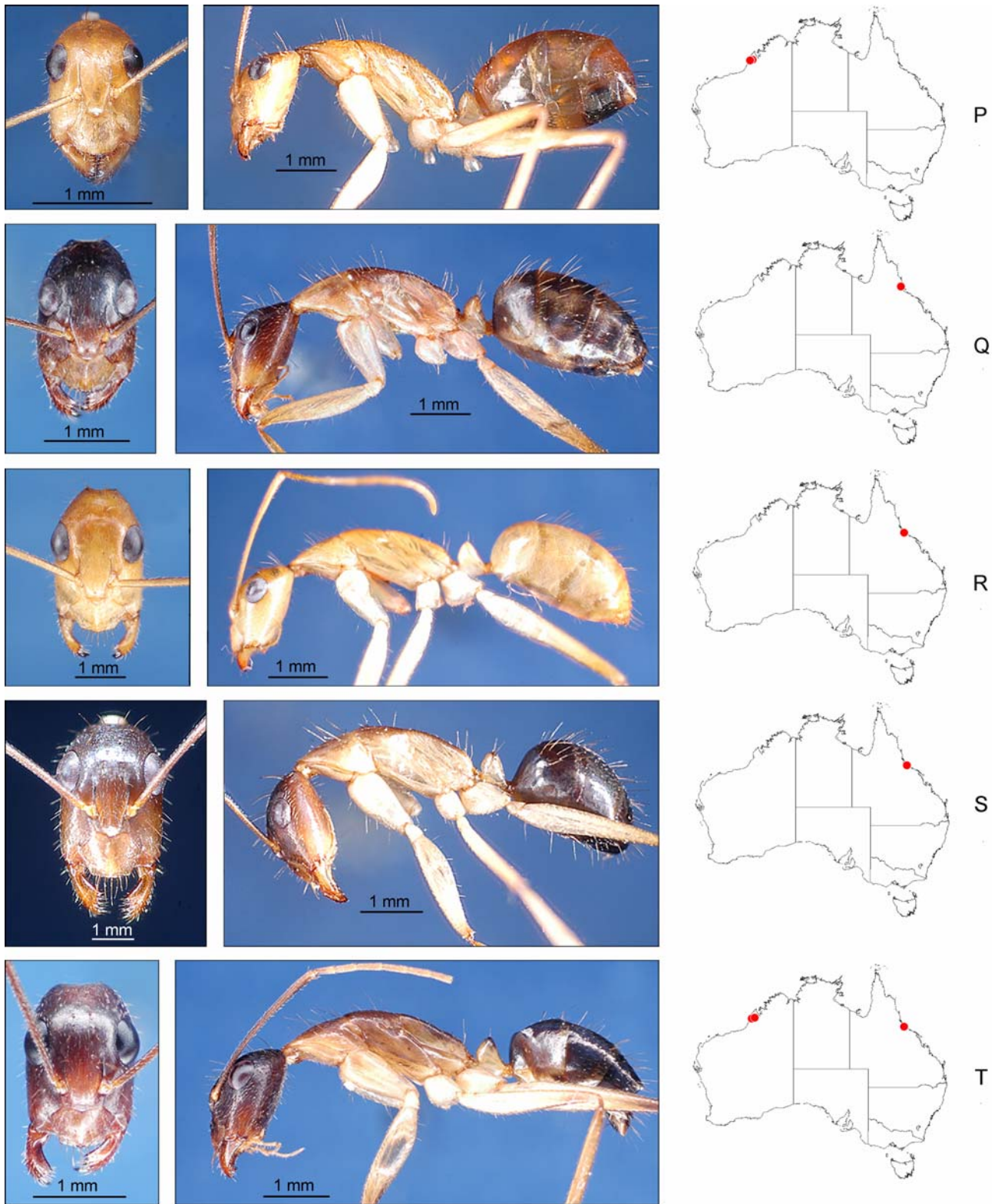


Fig. 4: Minor workers, lateral views and fronts of heads, in clades P, Q, R, S, T and their collection sites.

17 specimens belonging to two South African species and two Australian species. Bayesian and parsimony analyses were largely congruent. A Bayesian consensus tree with posterior probabilities and parsimony bootstraps indicated at the branches is presented in Fig. 3. Images of the spe-

cimens examined and their collection localities are given (Figs. 4 - 6). The phylogram (Fig. 3) shows that most of the Australian specimens in the *C. maculatus* group, as defined above, are monophyletic with the exception of *C. tricoloratus* CLARK, 1941. The African specimens allocat-

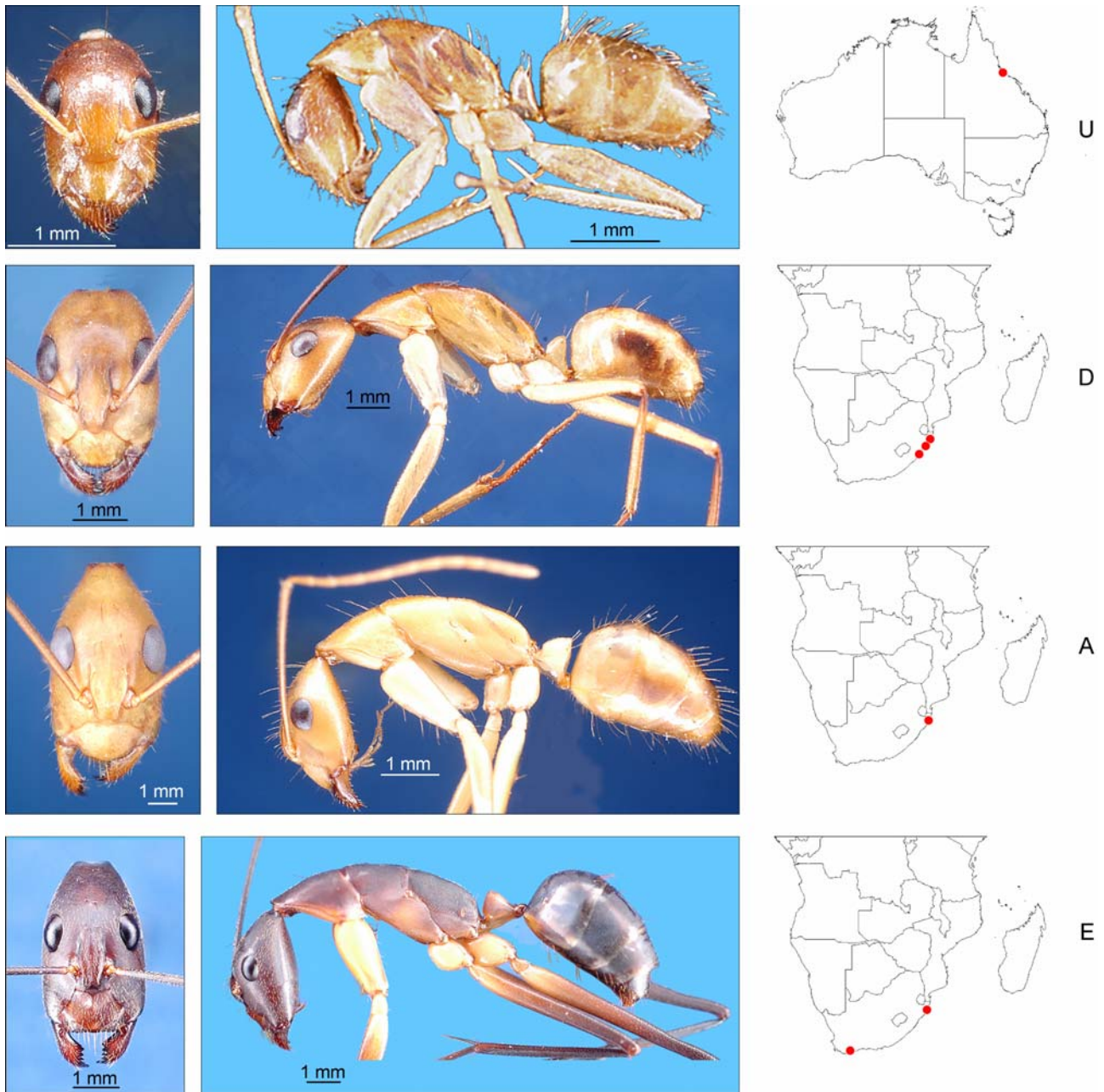


Fig. 5: Minor workers, lateral views and fronts of heads, in clades U, D, A, E and their collection sites.

ed to the *C. maculatus* group with a similar set of characters, are not monophyletic, but appear in an unresolved cluster together with the Australian *C. maculatus* group specimens, an African taxon that does not belong to the *C. maculatus* group viz *C. baynei* ARNOLD, 1922 and two Australian taxa of which one is considered to be a *C. maculatus* group member viz *C. tricoloratus*, while the other viz *C. terebrans* LOWNE, 1865 is not considered a *C. maculatus* group member. Pairwise sequence divergence among the unresolved clades at the base of the phylogram ranges from 12.1 - 18.4 %. Pairwise sequence divergence among the Australian clades assigned to the *C. maculatus* group ranges from 7.4 - 10.5 %.

In order to better resolve the relationships among the Australian groups we added partial sequences of a 457 bp

product of the CytB gene to a subset of the Australian *C. maculatus* group-specimens ($n = 17$, Tab. 1). Phylogenetic reconstructions of the CO1 and CytB combined dataset were performed using Bayesian and parsimony methods. Although the majority of the clades remained intact the additional sequences did not result in a better resolution. Clade U consistently appeared as the sister group of the remaining clades with high support (posterior probability 0.99). Clade T, which appeared in the CO1-only analysis (Fig. 3) as the sister clade of the remaining specimens with the exclusion of clade U, appeared at the tip of tree in the combined analysis, but had low support. The pairwise sequence divergence among specimens of clades T and PQRS is large (6.0 - 7.8 %). The position in the tree of the remaining clades is unresolved.

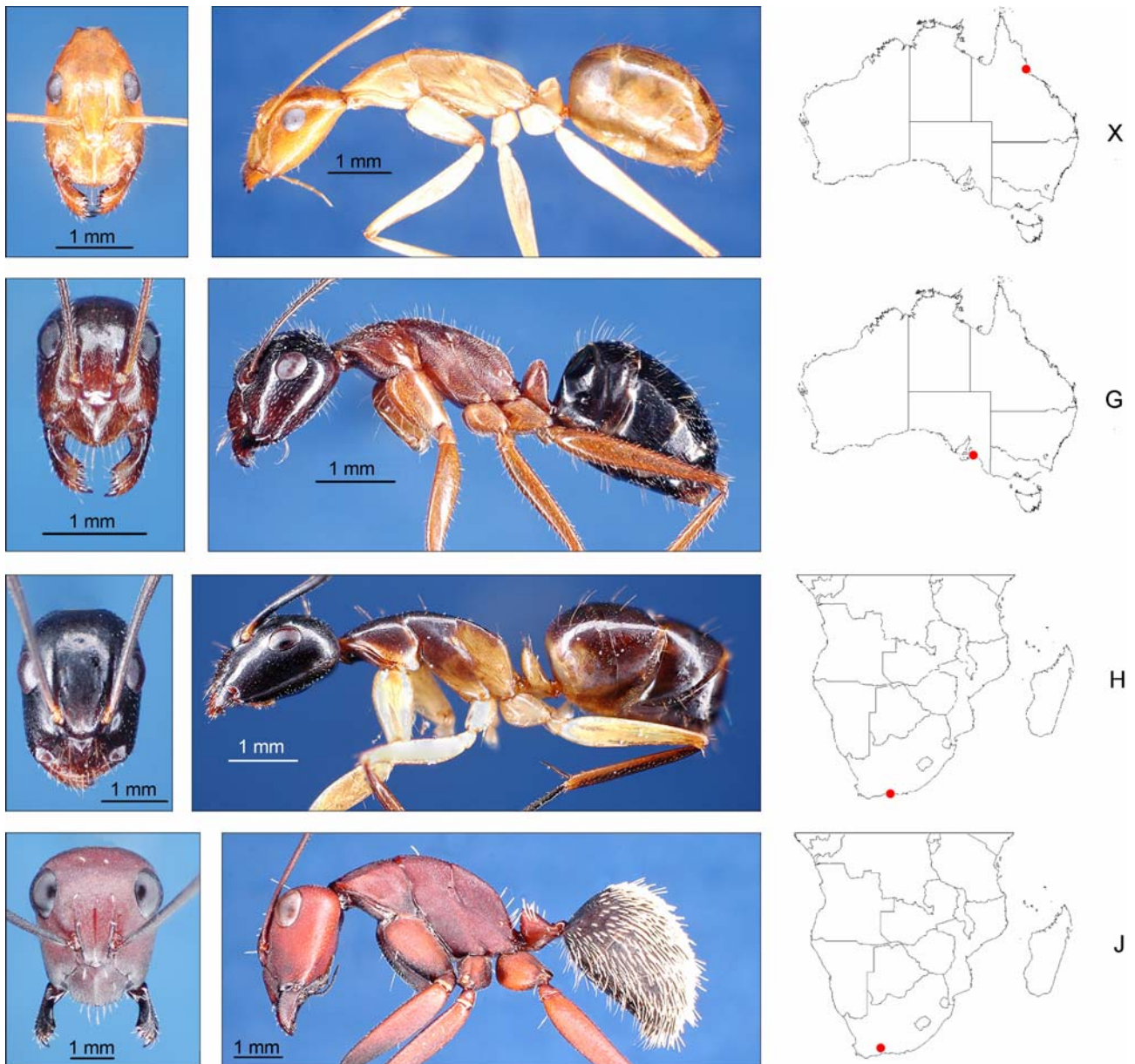


Fig. 6: Minor workers, lateral views and fronts of heads, in clades X, G, H, J and their collection sites.

Canonical discriminant analysis of morphological characters was performed on 80 specimens (Tab. 1). We used the plain character measurements as well as ratios of several characters to avoid high correlations due to body size variation. Ratio of characters highlight differences in body shape. The ratios used were head length vs. head width, frontal carinae width and pronotum width; tibia length vs. head width, frontal carinae width and pronotum width. The result of this analysis is depicted in Fig. 7. The separate position of clade U is clearly supported by the morphological analysis. The remaining clades are more or less overlapping. Clades P and R are completely overlapping, but hardly overlap with clade T. Specimens of clade Q are completely overlapped by clade T, but appear quite distant in the combined phylogram (Fig. 3). Characters that contribute most to the separation of the clades are the ratios of pronotum width / tibia length, pronotum width / head length, head width / head length and tibia length.

Discussion

Relationship of Australian and African taxa in the *C. maculatus* group

The molecular results showed no support for the assumption that the African species, which are morphologically similar to the Australian species, belong to the same group. Pairwise distances are large (12.1 - 18.4 %) and relationships among these groups are unresolved with the inclusion of some species that clearly do not have "*maculatus*" morphological features. The very deep divergences among the unresolved clades (Fig. 3) indicate that these clades would be better assigned to different species groups. In particular, specimens of *C. maculatus humilior* from Australia, clade (U) (Fig. 3), are shown to be unrelated to other African species in the *C. maculatus* group. We therefore raise it to species rank as *Camponotus humilior* FOREL, 1902 stat.n. (see Systematics).

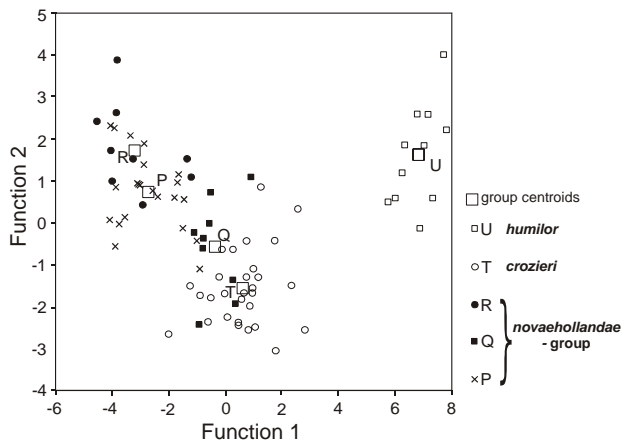


Fig. 7: Plot of Canonical discriminant analysis.

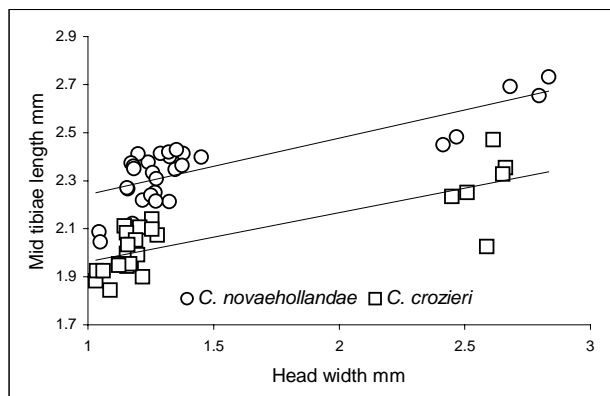


Fig. 8: Regression line for *C. novaehollandiae* ($y = 0.2353x + 2.0061$, $n = 30$, $R^2 = 0.6491$) derived from lumping measurements of specimens from WA and QLD from the same series as sampled for clade (P R) Fig. 3, regression line for *C. crozieri* sp.n. ($y = 0.2043x + 1.759$, $n = 30$, $R^2 = 0.6473$) derived from lumping measurements of specimens from WA and QLD from the same series as sampled for clade (T) in Figure 3.

Relationship between *C. novaehollandiae* and related taxa

The sequence data (Fig. 3) shows that within the Australian taxa studied, *C. tricoloratus* is a distinct clade (X) while the other taxa form a monophyletic group (clades P Q R S T U). This result was expected, as *C. tricoloratus* is clearly morphologically distinct in having an elongated node even though it possesses characters 1 to 4 above. It appears to belong to a group that is widespread across Australia and New Guinea and includes *C. rufus* CRAWLEY, 1925 and *C. subnitidus* MAYR, 1876.

Within the monophyletic group (clades P Q R S T U), there is evidence for three distinct species and possibly more.

1. Specimens in clade U (Fig. 3) are clearly distinct from the other clades (P Q R S T) on both morphological (Fig. 7) and sequence grounds (Fig. 3). This corresponds to *C. humilior* st.n. (see Systematics) and can be separated by its frontal carinae width which can be seen by comparing Figure 4P and Figure 6U.

2. Specimens in Clade T separate from Clades P R on both molecular (Fig. 3) and morphological grounds (Figs. 7,

8) and we consider it represents a separate species, *C. crozieri* sp.n., (see Systematics), which is brown in colour.

3. Specimens in the remaining clades (P Q R S) are either distinctly yellow (clades P R) and closely resemble the type of *C. novaehollandiae* or are brownish (clades Q S). Other than colour we could find no useful character to separate them.

However only a more extensive morphological and molecular study will confirm the boundaries of *C. novaehollandiae*, including whether it is only yellow as represented by clades (P R) or whether it includes brownish specimens represented by clades (Q S) (Fig. 4). In the interim we leave the status of specimens in clades (Q S) unresolved an example of a cryptic species complex and propose that *C. novaehollandiae* includes both yellow and brownish specimens (Fig. 4).

Systematics

Camponotus crozieri sp.n. (Fig. 12)

Type material examined: Holotype: minor worker, pinned, labelled "Qld, Townsville James Cook Uni 19.33775°S 146.7592° E 26/9/05 Simpson SAM Exp 2005" in SAMA. Paratypes: workers, with same label, 3 in SAMA, 3 in ANIC, 3 in QM.

Other material examined (in SAMA): Northern Territory: Newcastle Waters (17° 28' S, 133° 35' E), 16.VI.1981, leg. B.B. Lowery; Berrimah (12° 26' S, 130° 55' E), 1.XII.1992, leg. S. Morrison; Cape Crawford, 106 km S (17° 36' S, 135° 47' E), 4.IV.2003, leg. R. Foster, A. Skinner; Darwin (12° 27' S, 130° 50' E), 1.VI.1991, leg. S. Morrison; Darwin (12° 27' S, 130° 50' E), leg. W.K. Hunt; Jabiru (12° 40' S, 132° 54' E), 22.II.1992, leg. S. Morrison.

Queensland: Doonan (26° 28' S, 153° 01' E), 16.II.1987, leg. "D.A.I."; Bli Bli (26° 37' S, 153° 02' E), 23.IV.1996, leg. R. Eastwood; Brisbane (27° 28' S, 153° 02' E), 1.II.1988, leg. E. Cartley; Calliope Crossing (23° 58' S, 151° 09' E), 31.X.1995, leg. R. Eastwood; Emerald (23° 31' S, 148° 10' E), leg. K. Schneider; Esk (27° 14' S, 152° 25' E), 1.IX.1954, leg. "RD"; Highvale (27° 23' S, 152° 48' E), 28.V.1958, leg. F.A. Perkins; Kennedy (18° 12' S, 145° 57' E), 2.IV.1990, leg. R. Piper; Mareeba (17° 00' S, 145° 26' E), 23.IX.1960, leg. G.W. Saunders; Mareeba (17° 00' S, 145° 26' E), 7.I.1970, leg. J. H. Barrett; Maryborough (25° 32' S, 152° 42' E), 29.I.2000, leg. R. Eastwood, D. Lohman; Mornington Island (16° 31' S, 139° 39' E), 3.IV.1960, leg. P. Aitken, N. B. Tindale; Townsville (19° 16' S, 146° 49' E), 2.II.1997, leg. C.H. Watts; Cairns (16° 55' S, 145° 46' E), 14.IX.1992, leg. B. Hoffman; Dunwich (27° 30' S, 153° 24' E), 15.IV.1960, leg. E.M. Exley; Iron Range (12° 44' S, 143° 17' E), 18.VI.1999, leg. L. Hunt. **Western Australia:** Broome (7° 58' S, 122° 14' E), 16.IX.2005, leg. A.J. McArthur, A.A. Simpson; Cape Bertholet (17° 14' S, 122° 11' E), 22.IV.1977, leg. D.H. Colless; Cape Leveque (16° 24' S, 122° 55' E), 4.VI.1998, leg. D. Hirst; Derby (17° 19' S, 123° 38' E), 4.X.1913, leg. W.D. Dodd.

Worker diagnosis: Workers. HW 1.0 - 2.7; HL 1.6 - 2.9; PW 1.0 - 1.6; ($n = 10$). Mesosoma elongate with a distinct but well rounded propodeal angle; brown; tibiae and scapes with distinct setae raised up to 30°, a few scattered long setae overall including under head. Major worker. Head sides posterior half parallel, anterior half tapering to front; anterior margin of clypeus, strongly projecting with square corners, feebly concave between; node summit blunt.

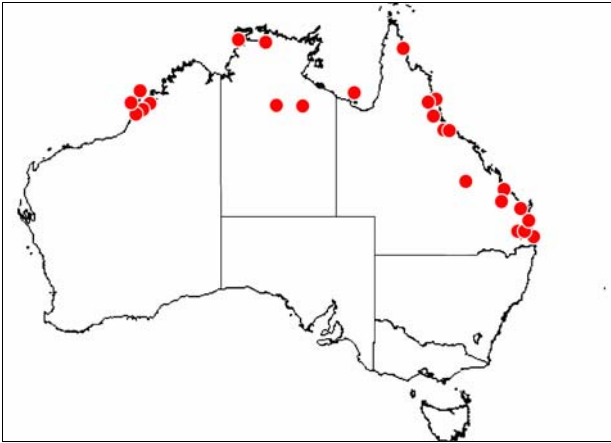


Fig. 9: Distribution map of specimens of *C. crozieri* sp.n. in the South Australian Museum.

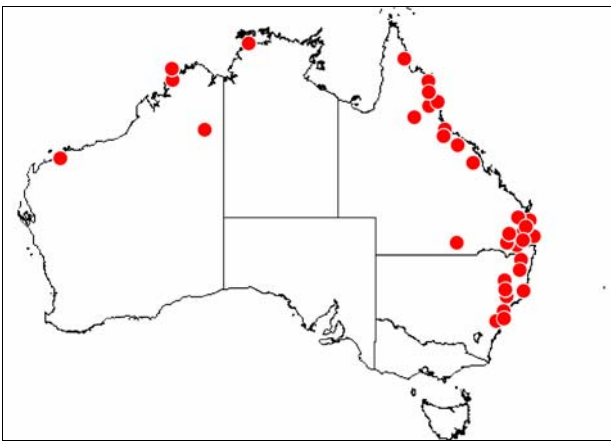


Fig. 10: Distribution map of specimens of *C. humilior* st.n. in the South Australian Museum.

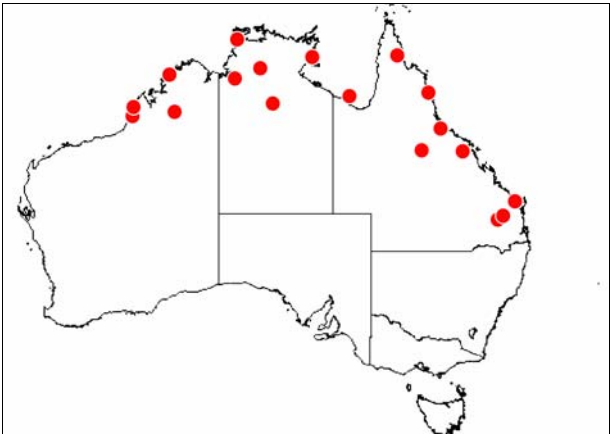


Fig. 11: Distribution map of specimens of *C. novaehollandiae* in the South Australian Museum.

Minor worker. Head sides tapering to the rear, occipital carinae distinct; anterior margin of clypeus, strongly projecting with square corners, wide, straight; ratio propodeal dorsum / declivity about 3.

Distribution: A distribution map of specimens in the South Australian Museum is shown in Figure 9.

Etymology: This ant is named in recognition of Ross Crozier's contribution to Myrmecology.

Camponotus humilior FOREL, 1902, stat.n. (Fig. 5: U)

Camponotus maculatus var. *humilior* FOREL, 1902.

Camponotus maculatus humilior FOREL, 1902: TAYLOR & BROWN (1985).

Type material examined: Syntypes: 1 major and 1 minor on 1 pin labelled "C Novae Hollandiae v humilior Cairns, Queensland, Nugent Cotypus", in ANIC.

Other material examined (in SAMA): **New South**

Wales: Gordon (33° 45' S, 151° 09' E), 7.XI.1974, leg.

P.S. Ward; Harrington, 7 km NE (31° 49' S, 152° 45' E),

14.X.1998, leg. G. Williams; Ku-ring-gai (33° 44' S, 151°

09' E), 22.VII.1974, leg. P.S. Ward; Barrington (32° 02' S,

151° 24' E), 27.V.1963, leg. G. Monteith; Coal And Candle

Creek (33° 38' S, 151° 13' E), 11.VII.1976, leg. B.B. Lowery;

Dorrigo (30° 20' S, 152° 43' E), 1914, leg. W.M. Wheeler;

Eatonville (29° 38' S, 152° 50' E), 28.XI.1997, leg. R. Eastwood;

Eccleston (32° 16' S, 151° 30' E), 4.X.1975, leg. P.S. Ward;

Heathcote (31° 14' S, 151° 27' E), 1914, leg. W.M. Wheeler;

Mount Ku-ring-gai (33° 39' S, 151° 08' E), 11.V.1963, leg. B.B. Lowery;

Mount Nebo (34° 25' S, 150° 50' E), 1.VIII.1956, leg. D. Bostock;

Royal National Park (34° 04' S, 151° 04' E), 1914, leg. W.M. Wheeler.

Northern Territory: T. Howard Springs (12° 27' S,

131° 03' E), 20.IX.2002, leg. A. Salvarani. **Queensland:**

Mount Greville (28° 05' S, 152° 30' E), leg. C. Hembrow;

Mount Surprise (18° 07' S, 144° 07' E), 12.VIII.1975, leg. B.B. Lowery;

Atherton (17° 16' S, 145° 29' E), leg. B.B. Lowery;

Bahrs Scrub (27° 45' S, 153° 10' E), leg. J. Birrell;

Bribie Island (26° 57' S, 153° 07' E), 24.IX.1995, leg. R. Eastwood;

Collinsville, 4 km N (20° 32' S, 147° 51' E), 24.V.1981, leg. B.B. Lowery;

Finch Bay (15° 28' S, 145° 16' E), 14.V.1990, leg. B.B. Lowery;

Gunalda Ra (26° 00' S, 152° 34' E), 28.I.2000, leg. R. Eastwood;

Home Rule Falls (15° 44' S, 145° 18' E), 1.X.1974, leg. V.E. Davies;

Leyburn (28° 01' S, 151° 35' E), 3.XII.1995, leg. R. Eastwood;

Leyburn (28° 01' S, 151° 35' E), 21.I.1999, leg. R. Eastwood;

North Stradbroke Island (27° 36' S, 153° 27' E), 10.I.2002, leg. Queensland Museum;

Picnic Bay Magnetic Island (19° 11' S, 146° 51' E), 30.XI.1981, leg. B.B. Lowery;

Toowoomba (27° 34' S, 151° 57' E), 19.X.1989, leg. B.B. Lowery;

Cunninghams Gap (28° 03' S, 152° 24' E), 17.IV.1962, leg. E.M. Exley;

Daintree, 10 km N (16° 10' S, 145° 19' E), 17.V.1990, leg. B.B. Lowery;

Daintree, 10 km N (16° 10' S, 145° 19' E), 17.V.1990, leg. B.B. Lowery;

Dunwich (27° 30' S, 153° 24' E), 15.IV.1960, leg. E.M. Exley;

Fordsdale (27° 42' S, 152° 08' E), 2.VI.1959, leg. F. McDonald;

Leo Creek (13° 33' S, 143° 28' E), 15.IX.1958, leg. "TLW";

Moggill (27° 34' S, 152° 53' E), 19.IX.1961, leg. R. Shephine;

Mooloolah (26° 46' S, 152° 58' E), 26.I.1986, leg. "D.A.I";

Murray Prior Range (16° 56' S, 145° 51' E), 2.VIII.1977, leg. R.W. Taylor;

Nebo (21° 42' S, 148° 42' E), leg. Hock;

Noosa Heads (26° 23' S, 153° 06' E), 24.XI.1983, leg. Queensland Dept. of Primary Industry;

Warwick, 11 km SE (28° 17' S, 152° 07' E), 30.XI.1997, leg. R. Eastwood. **Western Australia:**

Careening Bay (15° 06' S, 125° 00' E), 10.VIII.1999, leg. Riot Kimberly Exp., South Australian Museum;

Wolf Creek Crater (19° 11' S, 127° 48' E), 23.VIII.1998, leg. J.B. Stuckey;

Boomerang Bay (14° 32' S, 125° 07' E), 2.VIII.1999, leg. Riot Kimberly Exp., South Australian Museum;

Pannawonica (21° 39' S, 116° 20' E), leg. J.D. Majer.



Fig. 12: Major and minor workers of *C. crozieri* sp.n.

Worker diagnosis: Workers. HW 1.0 - 2.1; HL 1.45 - 2.3; PW 0.85 - 1.2; (n = 10). Mesosoma elongate with a distinct but well rounded propodeal angle; yellowish, head slightly darker than mesosoma; tibiae and scapes with distinct setae raised up to 30°, a few scattered long setae overall including under head; frontal carinae widely spaced. Major worker. Head sides posterior half parallel, anterior tapering to front; anterior margin of clypeus, strongly projecting with 90° corners, feebly concave between; node summit blunt; eye centres at or just anterior to line joining posterior tips of frontal carinae. Minor worker. Head sides mostly parallel, occipital carinae distinct; posterior rounded; anterior margin of clypeus, strongly projecting with square corners, wide, straight; ratio propodeal dorsum/ declivity about 3.

Distribution: A distribution map of specimens in the South Australian Museum is shown in Figure 10.

Notes: One may wonder, whether Forel mistakenly named this taxon "*Camponotus maculatus* v *humilior*" because he wrote on the label "*C. Novae Hollandiae* v *humilior*" and the concluding sentence of his description translates as "otherwise like the type *Novae Hollandiae*".

FOREL (1902: 497) relegated the former species *Camponotus novaehollandiae* MAYR, 1870 to subspecific rank under *C. maculatus* FABRICIUS, 1781, in the combination "*Camponotus maculatus* F. r. *Novae Hollandiae* MAYR". Immediately thereafter he described "*Camponotus macu-*

latus F. var *humilior* n. var.", concluding its diagnosis with the words "Du reste comme le *Novae Hollandiae* typique". That sentence and his use of the terms "r." (= race) and "var." (= variety) might imply that Forel intended *humilior* to have infrasubspecific rank following the trinomen *Camponotus maculatus novaehollandiae*. Any such intention by Forel is however not unambiguously demonstrated, so *humilior* can be considered a valid name of the species group with authorship "FOREL, 1902" under the terms of the International Code of Zoological Nomenclature, Art. 45.6.4. (R.W. Taylor, pers. coms. 2006).

***Camponotus novaehollandiae* MAYR, 1870** (Fig. 4: P, R)

Camponotus novaehollandiae MAYR, 1870.

Camponotus villosus CRAWLEY, 1915, **syn.n.**

Type material examined: Syntypes of *Camponotus novaehollandiae*: 5 major workers and 4 minor workers on 8 pins labelled "Cape York coll. G Mayr" "*C. novaehollandiae* det. G Mayr" in NHMW. – Syntypes of *Camponotus villosus*: 1 major worker labelled "*Camponotus villosus* BM type 11-915 Batchelor Northern Territory G F Hill" (head is missing) on one pin and 1 minor worker labelled "*Camponotus villosus* det W C Crawley Batchelor Northern Territory G F Hill", on one pin in BMNH.

Other material examined (in SAMA): **Northern Territory:** Groote Eylandt (13° 56' S, 136° 36' E), 1.I.1982, leg. J.D. Majer; Katherine (14° 28' S, 132° 16' E), 1.V.1991,

leg. S. Morrison; Elliott, 2 km S (17° 34' S, 133° 32' E), 9.VII.1997, leg. R. Foster; Timber Creek (15° 37' S, 130° 26' E), 1.V.1991, leg. S. Morrison. **Queensland:** Chinchilla (26° 44' S, 151° 06' E), 4.VI.1959, leg. E.M. Exley; Coen Aerodrome (13° 46' S, 143° 07' E), 11.IV.1994, leg. R. Eastwood; Bentinck Island (16° 59' S, 139° 30' E), 13.VI.1963, leg. P. Aitken; Eungella (21° 01' S, 148° 28' E), 17.XII.1972, leg. B.B. Lowery; Tingoorra (26° 22' S, 151° 49' E), 4.VI.1959, leg. E.M. Exley; Torrens Creek, 24 km S (21° 00' S, 145° 01' E), 20.V.2000, leg. R. Foster; Elliott River (24° 56' S, 152° 28' E), 14.X.1986, leg. R.R. Snelling. **Western Australia:** Barn Hill (18° 25' S, 122° 07' E), 15.VII.1998, leg. D. Hirst; Careening Bay (15° 06' S, 125° 00' E), 20.VII.1999, leg. Riot Kimberly Exp., South Australian Museum; Geike Gorge (18° 04' S, 125° 45' E), 4.VII.1967, leg. G. Campbell.

Worker diagnosis: Workers. HW 1.4 - 3.0; HL 2.1 - 3.2; PW 1.2 - 1.70; (n = 10). Yellow, sometimes brownish; glossy; scapes and tibiae with plentiful short setae raised to about 45°; mesosoma with about 20 erect setae about eye length mostly on pronotum and near angle, plentiful under head; propodeal dorsum slightly convex, angle well rounded; dimorphic. Major worker. Head much wider behind than in front; node summit blunt; anterior clypeal margin median section projecting, bounded by square corners with a wide concavity between. Minor worker. Head sides convex, parallel, tapering feebly to rear (more so in smallest workers); vertex convex, with distinct occipital carinae; anterior clypeal margin median section feebly convex, bounded by rounded corners; ratio propodeal dorsum/declivity about 3.

Distribution: A distribution map of specimens in the South Australian Museum is shown in Figure 11.

Notes: Crawley described *C. villosus* without any reference to Mayr's description of *C. novaehollandiae*. A.J.M. examined both types and could find no characters to separate them.

Acknowledgements

The generous financial contribution of A.A. Simpson and the facilities of the South Australian Museum made this study possible. Grateful thanks are due to C.H.S. Watts, H.R. Robertson, Nokuthula Mbanyana, H. Adie, A. & M. Cory, H. Landsburg, F. Koch, B. Merz and R.H. Crozier.

Zusammenfassung

Captain Cook sammelte als erster *Camponotus maculatus* (FABRICIUS, 1782) in Sierra Leone. Seither wurden viele Unterarten beschrieben, zumeist aus Afrika. Eine aber, *Camponotus maculatus humilior* FOREL, 1902, ist im Norden Australiens häufig. Wir präsentieren hier eine morphologische und molekulare Studie, die darauf abzielte, die Verwandtschaft von Arten aus der *C. maculatus*-Gruppe in Australien und Afrika zu klären. Wir konnten keine nahe Verwandtschaft zwischen den australischen und afrikanischen Arten feststellen. Wir heben *Camponotus maculatus humilior* in den Artrang, synonymisieren *Camponotus villosus* CRAWLEY, 1915 mit *Camponotus novaehollandiae* MAYR, 1870 und beschreiben *Camponotus crozieri* sp.n. Wir zeigen Forschungsbedarf zur Frage der Artabgrenzung von *Camponotus novaehollandiae* auf.

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