

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