Molecular phylogeny and biogeography of the dung beetle genus *Temnoplectron* Westwood (Scarabaeidae: Scarabaeinae) from Australia's wet tropics

K.L. Bell,[a,b,*] D.K. Yeates,[a,c] C. Moritz,[a,b,d] and G.B. Monteith[e]

[a] *Department of Zoology and Entomology, School of Life Sciences, University of Queensland, St Lucia, Queensland 4072, Australia*
[b] *Rainforest CRC, School of Life Sciences, University of Queensland, St Lucia, Queensland 4072, Australia*
[c] *Australian National Insect Collection, CSIRO Entomology, PO Box 1700, Canberra, ACT 2601, Australia*
[d] *Museum of Vertebrate Zoology, Department of Integrative Biology, 3101 Valley Life Sciences Building #3160, UC Berkeley, CA 94720-3160, USA*
[e] *Queensland Museum, Brisbane, Queensland 4000, Australia*

Abstract

The landscape of the Australian Wet Tropics can be described as "islands" of montane rainforest surrounded by warmer or more xeric habitats. Historical glaciation cycles have caused expansion and contraction of these rainforest "islands" leading to consistent patterns of genetic divergence within species of vertebrates. To explore whether this dynamic history has promoted speciation in endemic and diverse groups of insects, we used a combination of mtDNA sequencing and morphological characters to estimate relationships and the tempo of divergence among Australian representatives of the dung beetle genus *Temnoplectron*. This phylogenetic hypothesis shares a number of well-supported clades with a previously published phylogenetic hypothesis based on morphological data, though statistical support for several nodes is weak. Sister species relationships well-supported in both tree topologies, and a tree obtained by combining the two data sets, suggest that speciation has mostly been allopatric. We identify a number of speciation barriers, which coincide with phylogeographic breaks found in vertebrate species. Large sequence divergences between species emphasize that speciation events are ancient (pre-Pleistocene). The flightless, rainforest species appear to have speciated rapidly, but also in the distant past.
© 2003 Elsevier Inc. All rights reserved.

Keywords: Biogeography; Speciation; Molecular phylogeny; Tropical rainforest; Dung beetle

1. Introduction

The biodiversity of tropical rainforests has fascinated biologists since at least the 19th century. The species richness of these rainforests is higher than that of any other ecosystem (Fischer, 1960). The high level of biodiversity in tropical rainforest has been explained with both ecological and historical hypotheses. Ecological factors that could promote species richness include habitat productivity (e.g., Givnish, 1999), habitat heterogeneity (e.g., Kikkawa et al., 1981), habitat "favourableness" (e.g., Brown, 1988, although definitions

of this concept may be circular) and niche exploitation (e.g., Farrell, 1998). Historical processes that could lead to higher species richness in the tropics include rainforest contraction and isolation during more arid periods, and habitat persistence within rainforest refugia at these times (e.g., Avise et al., 1998; Bush, 1994).

Pliocene–Pleistocene global climatic cycles (for the last 2.8MY) are thought to have caused the fragmentation of montane habitats, with the potential to drive allopatric speciation. For rainforest species the most obvious types of disruption are gaps of dry habitat between rainforest, although these may not be obvious in the current landscape because the rainforest is more extensive and continuous than in previous, drier periods. While palynological evidence for rainforest contraction in most tropical regions is ambiguous (Colinvaux, 1987),

* Corresponding author. Fax: +61-7-33651655.
E-mail address: kbell@zen.uq.edu.au (K.L. Bell).

pollen cores from the Australian Wet Tropics (hereafter, the "Wet Tropics"; the rainforest region of northeast Queensland between Townsville and Cooktown) reveal a clear history of severe rainforest contraction during Pleistocene glacial periods (Kershaw, 1994). These data are consistent with palaeoclimate models, which predict that temperature and moisture regimes suitable for rainforest growth would have been fragmented and disjunct throughout the region (Nix, 1991).

Phylogeographic analyses of Wet Tropics vertebrate species strongly suggest refugial isolation and allopatric molecular divergence, but not complete speciation, across barriers (e.g. Schneider and Moritz, 1999; Schneider et al., 1999). The magnitude of molecular divergence varies among species, possibly reflecting more than one expansion and contraction, mostly pre-Pleistocene, across these boundaries (Joseph and Moritz, 1993; Joseph et al., 1995; Schneider et al., 1998). There have, however, been very few studies on the biogeography of endemic radiations in the Wet Tropics with which to explore the effects of rainforest contractions on speciation.

By studying species-rich and taxonomically well-known groups in rainforests, we can gain insight into the origins of the high level of biodiversity, and the causes of speciation. Dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) provide an ideal system for studying endemic radiations in the Wet Tropics. The Australian Scarabaeinae has been comprehensively reviewed, so that the majority of species are described, with special focus on the fauna of northeastern Queensland (Matthews, 1972; Matthews, 1974; Matthews, 1976). Since they occupy smaller ranges, invertebrates tend to be informative at a finer scale than are vertebrates (Moritz et al., 2001), and studies of invertebrates have shown evidence for vicariant speciation between Wet Tropics rainforest blocks, rather than just molecular divergence (e.g., Baehr, 1995; Bouchard et al., in press; Reid and Storey, 2000). Dung beetles can be collected efficiently in baited traps, and continuous and extensive collecting means that the distributions are known for most described species. Many species of Australian dung beetle are recorded as being habitat specific (Matthews, 1972; Matthews, 1974; Matthews, 1976). There is a distinct rainforest and open forest fauna, (Hill, 1996).

The genus *Temnoplectron* has recently been revised taxonomically (Reid and Storey, 2000). Its species are predominantly Wet Tropics endemics, and it has radiated within the Wet Tropics. The genus contains 19 species, 11 of which are restricted to a portion of the Wet Tropics (Reid and Storey, 2000, Fig. 1). These narrow endemics are commonly allopatric or parapatric with respect to their sister taxa according to the cladogram based on morphological characters (Reid and Storey, 2000). Many of these sister taxa have strongly divergent genitalia, suggesting reproductive isolation, with very little divergence in external morphology and

may be useful for studying the effects of rainforest fragmentation on speciation. The genus also has the potential to answer questions relating to the effect of flight loss on speciation, as it contains both volant and non-volant species (Reid and Storey, 2000).

Flightlessness leads to a decrease in mobility and, hence, it would be expected to increase the potential for allopatric or parapatric speciation (Roff, 1994). Several lines of evidence support this hypothesis; in general, flightless species are more concentrated in refugia, are found at higher elevations and have higher levels of endemism. The ranges of flightless carabid beetles are significantly smaller than those of volant species, and gene flow among flightless populations is less than among populations of volant species (Liebherr, 1986). For the Wet Tropics, Yeates et al. (2002) found high levels of endemism in flightless insects, although this was not directly compared to volant insect species. There have, however, been no studies of speciation rates or studies of gene flow within populations of volant and non-volant insects in this geographic region. The phylogenetic hypothesis of Reid and Storey (2000) suggests that flightlessness in *Temnoplectron* has led to a relatively high level of speciation within a small geographic range. Thus it would be expected that these flightless *Temnoplectron* species have speciated more rapidly than the fully winged species in this genus. A rapid speciation rate at some time in the past would lead to closely spaced cladogenesis events among flightless taxa in a molecular phylogeny.

2. Materials and methods

2.1. Sampling

All specimens included in this study are shown in Table 1. For each species at least two individuals, where possible from geographically distant sites, were used. Where multiple isolated populations of a species occurred, individuals from all populations were included. Species from New Guinea were not included, as the specimens could not be obtained. The species *Temnoplectron diversicolle* Blackburn, found at only two localities, in lowland rainforest, approximately 40 km north of Cooktown, was also omitted as the most recently collected specimens were from 1976, and no readable nucleotide sequence was obtained.

2.2. Mitochondrial DNA sequencing

Up to 1330 bp of the mitochondrial gene cytochrome oxidase I (COI) was sequenced using the primers shown in Table 2 (GenBank Accession Nos. AY144730–AY144795). For fresh specimens, stored in 100% ethanol, the PCR product was obtained in one fragment.
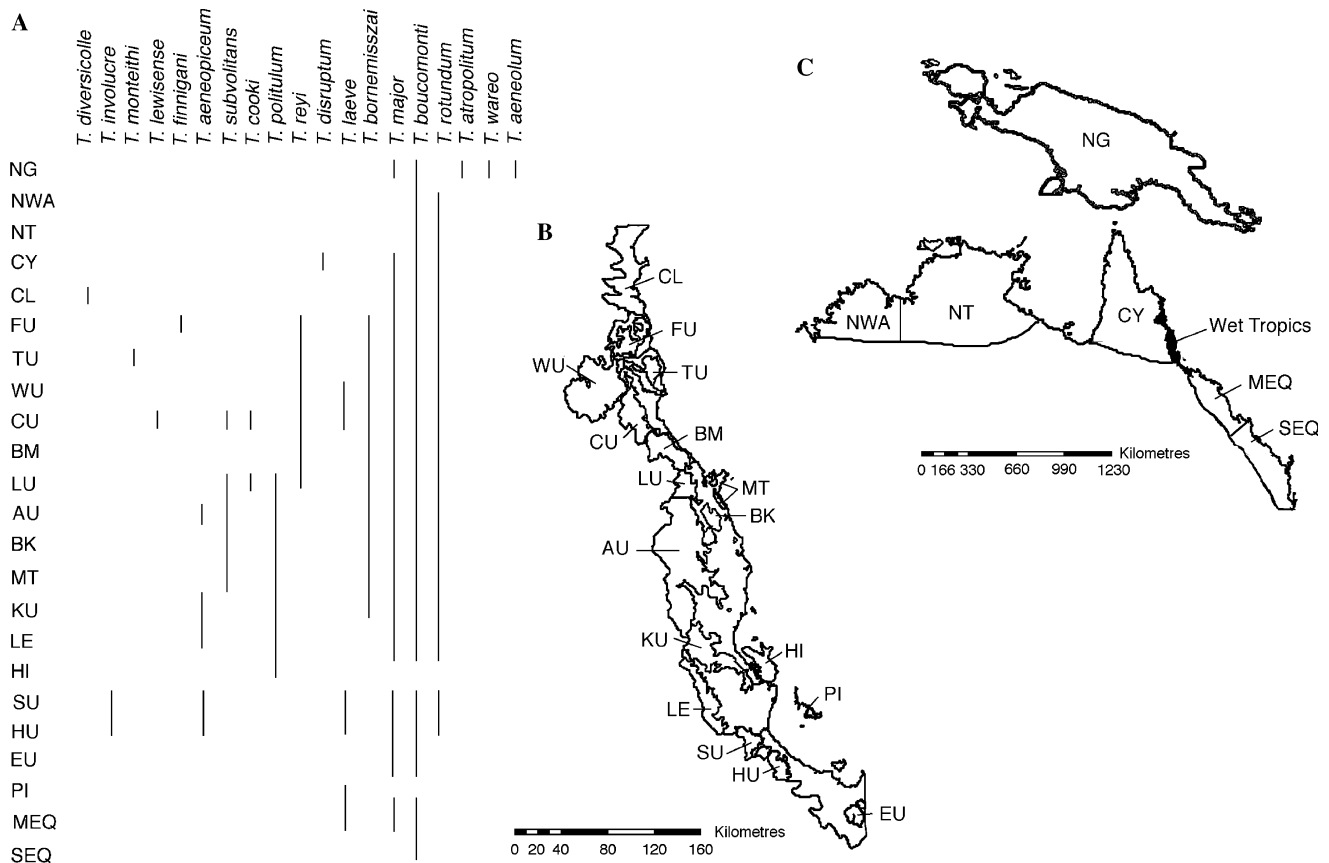
Fig. 1. Distribution data for *Temnoplectron* species. (A) Listing of subregions in which each species is present. (B) Map showing subregions within the Wet Tropics. After Williams et al. (1996). CL, Cooktown Lowlands; FU, Mount Finnigan Uplands; TU, Thornton Uplands; WU, Windsor Uplands; CU, Carbine Uplands; BM, Black Mountain Corridor; LU, Lamb Uplands; AU, Atherton Uplands; BK, Bellenden-Ker/Bartle-Frere; MT, Malbon-Thompson Uplands; KU, Kirrama Uplands; LE, Lee Uplands; HI, Hinchinbrook Island; SU, Spec Uplands; HU, Halifax Uplands; EU, Elliot Uplands; PI, Palm Islands (C) map showing subregions outside the Wet Tropics. NG, New Guinea; NWA, North Western Australia; NT, Northern Territory; CY, Cape York; MEQ, Mid-East Queensland; SEQ, South-East Queensland.

For older or poorly preserved museum specimens, internal primers were used to obtain 150, 300 or 500 bp products. For all species, multiple individuals (up to 5) were sequenced. The first 436 bp were consistently obtained, but longer sequence of COI (>800 bp) was usually obtained for only one individual. All amplifications used standard PCR conditions followed by automated sequencing using Applied Biosystems Big-Dye terminator sequencing chemistry. Sequences were edited in Sequence Navigator, and aligned using ClustalX.

2.3. Phylogenetic analysis

Aligned sequences were analysed using PAUP* version 4.0b10. Firstly, a maximum likelihood tree was obtained using the first 436 bp of COI sequence obtained for all specimens in Table 1, using *Monoplistes* Lansberge, *Diorygopyx* Matthews, and *Lepanus* Balthasar as outgroups. The purpose of this was to test the monophyly of all species, as defined by Reid and Storey (2000). Parameters for maximum likelihood analysis

using a model of general time reversible plus gamma distribution (GTR + Γ) were estimated based on preliminary topologies obtained by neighbour joining. A maximum likelihood tree was obtained by a heuristic search using a branch-swapping algorithm of subtree-pruning-regrafting (SPR). Bayesian support values for nodes on this tree were also obtained using MrBayes 2.01. If any species were not monophyletic in the tree topology, the analysis was repeated constraining those species to be monophyletic, and the likelihood scores of the constrained and unconstrained trees were compared with a SH-test (Shimodaira and Hasegawa, 1999).

Once monophyly of the species was confirmed by the above analysis, the longest sequence obtained for each species (408–994 bp, mean 933) was used in maximum likelihood analysis. The maximum likelihood tree was obtained by a heuristic search and Bayesian support values were also obtained using MrBayes 2.01 as described above. This phylogenetic hypothesis was tested for congruence with the cladogram based on morphology of Reid and Storey (2000) by producing a ML tree

Table 1
Listing of all specimens for which COI sequence was obtained, and information on their collection

Species	Collection locality	Altitude	Collector(s)	Date	Specimen code no.
T. aeneopiceum Matthews	Tully R. causeway (17°54′S 145°39′E)	750 m	D.J. Cook	2 Dec 1998	2002-1
	Kjellberg Rd (17°32′S 145°35′E)	920 m	G.B. Monteith	6–9 Feb 1999	2154-1
	Millaa Millaa Falls (17°29′48.7″S 145°36′34.0″E)	825 m	K.L. Bell	8–10 Dec 2000	AT-1
	Tully Falls Rd (17°43′49″S 145°32′18″E)		K.L. Bell	9–10 Jul 2000	WH-1
	Birthday Creek Falls (18°58′42.1″S 146°09′34.8″E)	880 m	K.L. Bell	1–2 Dec 2000	PR-1
T. bornemisszai Matthews	Bartle Frere, W. base (17°23′S 145°46′E)	750 m	G.B. Monteith & D.J. Cook	28–30 Nov 1998	1962-1
	Tully R. causeway (17°54′S 145°39′E)	750 m	D.J. Cook	3–5 Dec 1998	2015-1
	Mt Hugh Nelson, S. base (17°31′S 145°33′E)	1080 m	G.B. Monteith & D.J. Cook	7–9 Feb 1999	2167-1
T. boucomonti Paulian	Station Creek (16°37′S 145°14′E)	350 m	G.B. Monteith	23–25 Nov 1998	1938-1
	Gregory NP (16°07′S 130°25′E)		P. Bouchard	Jun 2001	NT-1
T. cooki Reid and Storey	Mt Spurgeon (16°27′S 145°11′E)		G.B. Monteith, D.J. Cook & C. Burwell	19–23 Nov 1997	1632-1
	Mt Haig (17°07′S 145°33′E)	1070 m	G.B. Monteith & D.J. Cook	6–10 Feb 1998	1798-1
					1798-2
T. disruptum Matthews	Iron Range (12°46′S 143°17′E)		J. Skevington	20–24 Jul 1999	UQ-1
					UQ-2
T. finnigani Reid and Storey	Mt Finnigan (15°49′S 145°17′E)	1100 m	G.B. Monteith	20–21 Nov 1998	1929-1
					1929-2
					1929-3
T. involucre Matthews	Birthday Creek Falls (18°58′42.1″S 146°09′34.8″E)	880 m	K.L. Bell	1–2 Dec 2000	PR-1
	Paluma Dam Rd (19°00.716′S 146°10.461′E)	660 m	K.L. Bell	2–5 Dec 2000	PR-2
					PR-4
T. laeve Laporte	Cameron Ck, via Koumala (21°35′S 149°14′E)		G.B. Monteith	1–2 Oct 1999	7799-1
					7799-2
	Birthday Creek Falls (18°58′42.1″S 146°09′34.8″E)	880 m	K.L. Bell	1–2 Dec 2000	PR-1
	Mt Spurgeon (16°27′S 145°11′E)		G.B. Monteith, D.J. Cook & C. Burwell	19–23 Nov 1997	T58245 (QM)
					T58246 (QM)
T. lewisense Reid and Storey	Mt Lewis, site 3 (16°33′04.2″S 145°16′45.6″E)	980 m	K.L. Bell	13–14 Dec 2000	CT-1
	Mt Lewis barracks (16°35′40″S 145°16′27″E)	990 m		19 Apr 1997	QM-1
T. major Paulian	Taroom (25°36′S 149°46′E)	200 m	D.J. Cook & G.B. Monteith	15–16 Dec 2000	9853-1
	Ravenshoe, 2km SW (17°37′S 145°25′E)	880 m	G.B. Monteith	6–8 Feb 1999	T72896 (QM)
T. monteithi Reid and Storey	Cape Tribulation (16°05′S 145°26′E)		G.B. Monteith	17 Nov 1998	1913-1
					1913-2
T. politulum Macleay	Bartle Frere, W. base (17°23′S 145°46′E)	750 m	G.B. Monteith	28 Nov 1998	1970-1
	Vine Creek (17°41′S 145°32′E)	1060 m	G.B. Monteith	4–6 Feb 1999	2094-1
	Wallaman Falls (18°35′26.6″S 145°48′16.3″E)	300 m	K.L. Bell	2 Dec 2000	SR-1
T. reyi Paulian	Cape Tribulation (16°05′S 145°26′E)		G.B. Monteith	17 Nov 1998	1913-1
	Mt Lewis, site 1 (16°35′11.9″S 145°17′19.9″E)	860 m	K.L. Bell	13–14 Dec 2000	CT-2
	2km ENE Kuranda (16°48′S 145°38′E)	360 m	C. Reid	19–21 Apr 1999	ANIC-2
	Cedar Pocket (16°56′S 145°36′E)	440 m	I. Reid & C. Reid	17–19 Apr 1999	ANIC-3

	Location	Elevation	Collector	Date	Catalog
T. rotundum Westwood	Andoom, nr Weipa (12°32'S 141°50'E)		G.B. Monteith	5–8 Feb 1975	T50617 (QM), T50618 (QM)
T. subvolitans Matthews	Bartle Frere, W. base (17°23'S 145°46'E)	750 m	G.B. Monteith	28 Nov 1998	1970-1
	Mt Murray Prior (16°56'S 145°51'E)		G.B. Monteith	7–8 Dec 1998	2035-1
	Millaa Millaa Falls (17°29'48.7'S 145°36'34.0''E)	825 m	K.L. Bell	8–10 Dec 2000	AT-1
	Mt Lewis (16°35'14'S 145°18'07''E)		P. Bouchard & A. McDowell	11 Jul 2000	CT-2
Diorygopyx tibialis Macleay	Sunday Ck barracks, 6km NE (26°40'S 152°35'E)	728 m	K.L. Bell & G.B. Monteith	17–18 Mar 2001	SQ-1
Lepanus monteithi Matthews	Iron Range (12°46'S 143°17'E)		J. Skevington	20–24 Jul 1999	UQ-1, UQ-2
Lepanus ustulatus Lansberge	Bulburin barracks (24°32'S 151°28'E)	580 m	G.B. Monteith & D.J. Cook	7–9 Nov 1999	7819-1, 7819-2
Monoplistes occidentalis Macleay	Macleay Nipping Gully (25°20'S 151°26'E)		Monteith & Gough	18 Dec 1998–25 Jan 1999	7579-1, 7579-2, 7579-3, 7579-4

constrained to be consistent with this cladogram. The likelihoods of the two phylogenetic hypotheses were compared with a SH-test (Shimodaira and Hasegawa, 1999).

To increase support for the tree, the molecular data were combined with the data set of Reid and Storey (2000). The combined data set was analysed by parsimony. Molecular and morphological characters were unordered and equally weighted. Heuristic search settings were as previously used for maximum likelihood analysis. Partitioned Bremer support (Baker and DeSalle, 1997) was applied using TreeRot version 2.0, to determine if there was any conflict between the two data sets, and in which regions of the tree conflict occurred.

3. Results

3.1. Confirmation of current taxonomy

To test for monophyly of species according to the current definitions (Reid and Storey, 2000), a maximum likelihood tree was produced with the first 436 bp of each sequence (Fig. 2). This phylogenetic hypothesis has a likelihood score of 4653.19535, and confirms the monophyly of all species, with bootstrap values >80%, except *Temnoplectron politulum* Macleay, which for mtDNA appears paraphyletic without including *Temnoplectron reyi* Paulian, its hypothesised sister taxon (Reid and Storey, 2000). The location of the root within this clade is uncertain and the analysis was repeated with *T. politulum* constrained to be monophyletic. The tree topology obtained had a −log-likelihood of 4655.15683. A SH-test of the two tree topologies gave $P = 0.257$, which is not significant. In addition, DNA sequencing of 96 individuals of *T. politulum* and *T. reyi* suggests monophyly of both species (KLB, CM, A. Moussalli & DKY, in prep). Therefore, we treat all species as monophyletic.

3.2. Sequence divergence within and between species

For specimens taken from up to 100 km apart, within-species sequence divergence (using ML model) in the winged species ranged from 0 to 9.4% (mean 3.9%). Sequence divergence within the wingless species is higher, ranging from 0.23 to 13.2% (mean 6.7%). Estimates of sequence divergence within the wingless species can be substantial, even for specimens collected from the same location.

Sequence divergences between species are shown in Fig. 3. The level of divergence between sister-species for 994 bp of sequence is highly variable, ranging from 6.4% between *T. politulum* and *T. reyi*, to 21.5% between *Temnoplectron involucre* Matthews and *Temnoplectron monteithi* Reid and Storey. The level of net divergence (i.e., corrected for variation within species) between

Table 2
Primers used for sequencing of COI

Designation	Alias	Sequence	Reference
C1-J-1706	CO1-FA	CCA ATT TTA ATT GGR GGR TTT GG	Custom primer
C1-J-1718	Geoff	GGA GGA TTT GGA AAT TGA TTA GTT CC	(Simon et al., 1994)
C1-J-1751	Ron	GGA TCA CCT GAT ATA GCA TTC CC	(Simon et al., 1994)
C1-J-1752	CO1-F5-D	GAG CCC CTG ATA TAG CCT TTC C	Custom primer
C1-J-1754	Johnny	GCT CCT GAT ATA GCA TTC CCA CG	Custom primer
C1-J-1844	CO1-150-F	GTT GAA AGA GGA GCT GGA ACA GG	Custom primer
C1-N-1841	CO1-150-R	TGA TAA AGG RGG ATA AAC TGT TC	Custom primer
C1-N-1970	MiniCO1	TAT TCC TGT TGA TCG TAT ATT RA	Custom primer
C1-J-1994	CO1-300-F	CAA TAT ACG ATC AAC AGG AAT AAC	Custom primer
C1-J-2166	CO1-F3	GGA GAC CCA ATT CTT TAY CAA C	Custom primer
C1-J-2166	C01-F3-D	GGA AAC CCG ATT TTA TAT CAA C	Custom primer
C1-J-2183	Jerry	CAA CAT TTA TTT TGA TTT TTT GG	(Simon et al., 1994)
C1-N-2191	Nancy	CCC GGT AAA ATT AAA ATA TAA ACT TC	(Simon et al., 1994)
C1-J-2195	CO1-RLR	TTG ATT TTT TGG TCA TCC AGA AGT	(Simon et al., 1994)
C1-N-2329	K525	ACT GTA AAT ATA TGA TGA GCT CA	(Simon et al., 1994)
C1-N-2454	CO1-800-R	GGG AAT AAT TTA ATT GAG ATC C	Custom primer
C1-J-2474	CO1-800-F	CTC TTC ATG GAT CTC AAT TAA ATT AYT C	Custom primer
C1-N-2652	CO1-R3	TCC TGT AAA TAA TGG AAA TCA ATG	Custom primer
C1-N-2751	Jeanette	CCA CTT AAT CCT AAA AAA TGY TG	Custom primer
C1-N-2751	Pauline	CCA CTT AAA CCA AAR AAA TGY TG	Custom primer
L2-N-3014	Pat	TCC AAT GCA CTA ATC TGC CAT ATT A	(Simon et al., 1994)

sister-species for 437 bp ranges from 4.0% between *T. politulum* and *T. reyi*, to 15.2% between *T. involucre* and *T. monteithi*.

3.3. Maximum likelihood analysis of full length COI sequence

While the tree topology obtained using 436 bp of COI (Fig. 2) supports many of Reid and Storey's (2000) hypotheses of sister-species relationships (Fig. 4B), the tree tends to have short internal branch lengths and correspondingly low levels of support. To obtain higher support levels, longer sequences of COI were used. For most species, we obtained at least a 994 bp sequence, the exceptions being *T. rotundum* Westwood (408 bp) and *Temnoplectron lewisense* Reid and Storey (781 bp). The resulting phylogenetic hypothesis (Fig. 4A) has a −log-likelihood of 5520.55169.

Support for nodes was highly variable, with essentially no support for those at the base, but strong support, especially with regard to Bayesian posterior probabilities, for several nodes involving species endemic to the Wet Tropics (Fig. 4A). A well-supported node separates a major clade of northeast Queensland and mostly rainforest-restricted species from a group of more ecologically generalized and widely distributed taxa, among which relationships are poorly defined. Considering the former clade, the Cape York rainforest endemic *Temnoplectron disruptum* Matthews appears to be outside of a moderately supported clade comprising nine Wet Tropics endemics, of which all but one (*Temnoplectron cooki* Reid and Storey, in wet sclerophyll forest) are restricted to rainforest. Within this

clade is a monophyletic group of wingless species. Each of these rainforest-restricted and wingless species is found in a single subregion, but area relationships cannot be deduced because of poor support for nodes within this clade. Among the four winged rainforest species from the Wet Tropics, two essentially parapatric and widely distributed pairs are identified with strong support; (*Temnoplectron aeneopiceum* Matthews, *Temnoplectron subvolitans* Matthews; 14.2% divergence) and (*T. politulum*, *T. reyi*; 6.4% divergence). The former is related to the wingless clade whereas the latter is sister group to the wingless wet-sclerophyll species, *T. cooki*.

Relatively few nodes on the morphology-based tree of Reid and Storey (2000, Fig. 4B) were strongly supported, but there are some areas of consistency with the mtDNA tree (Fig. 4A) and also some potential conflicts. In both analyses, the four wingless rainforest species are monophyletic, as are the winged clades of (*T. aeneopiceum*, *T. subvolitans*) and (*T. politulum*, *T. reyi*). The only potential conflict between strongly supported nodes of the mtDNA tree and the morphological cladogram concern the relationships of (*T. aeneopiceum*, *T. subvolitans*); this clade is monophyletic with the wingless clade in the mtDNA tree, but appears as a sister group to (*T. politulum*, *T. reyi*) and to the exclusion of *T. cooki* in the morphological analysis.

Constraining the maximum likelihood tree to have only clades consistent with the poorly resolved morphological tree of Reid and Storey (2000) produced a phylogenetic hypothesis with a −log-likelihood of 5536.79051, which is not significantly different to the unconstrained tree (−log-likelihood of 5520.55169), according to a SH-test (Shimodaira and Hasegawa, 1999).
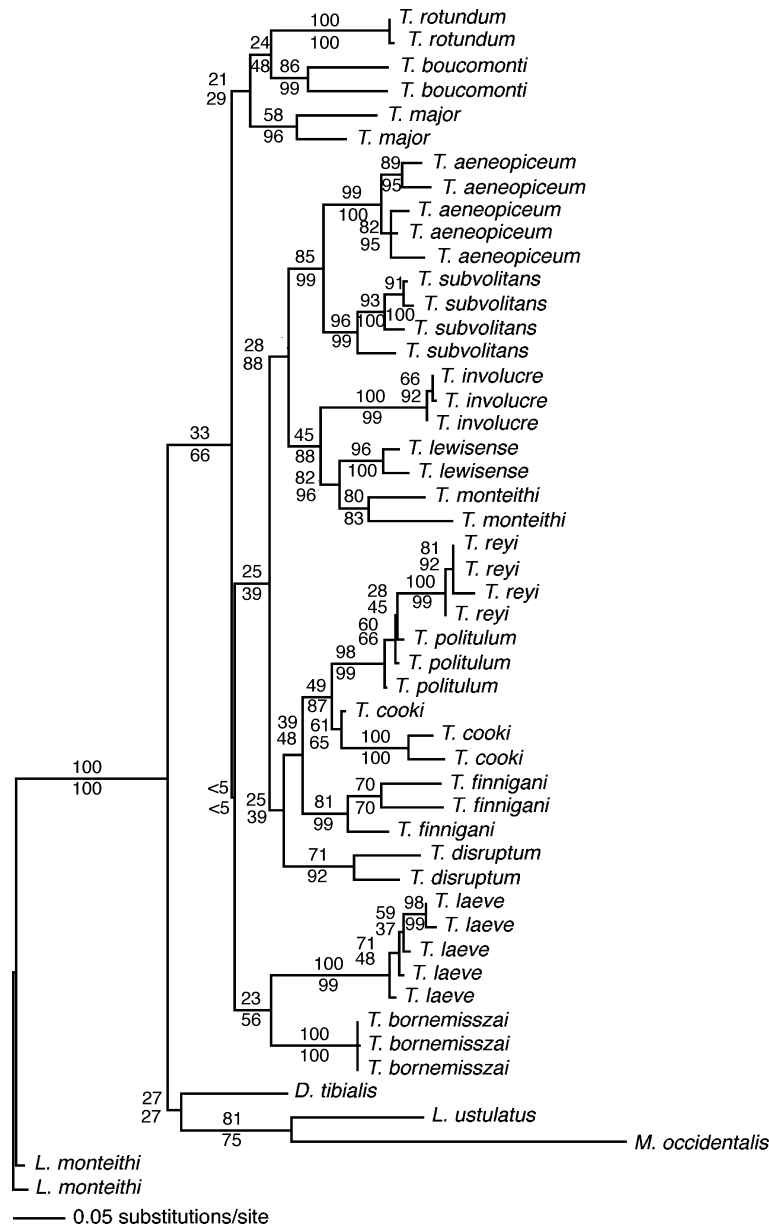
Fig. 2. Maximum likelihood tree based on 437 bp of COI, with bootstrap values shown above branches and Bayesian support values shown below branches.

Combining the molecular and morphological characters in a "total evidence," unweighted parsimony analysis (Fig. 5) returned the congruent relationships identified above and provided additional support within the wingless clade, with 87% bootstrap support for (*T. lewisense, T. monteithi*), species restricted to the adjacent Carbine and Thornton uplands, respectively. Aside from these and the congruent relationships (above), bootstrap support for individual nodes remained low (<80%). Partitioned Bremer support shows some conflict between the two datasets. This is predominantly localised to the basal lineages which also have low support in the separate analyses of the two data sets.

4. Discussion

4.1. Speciation patterns within the Wet Tropics

Overall, the phylogenetic hypothesis produced for *Temnoplectron* had variable support. For the tree produced using mtDNA, support was low at the base, possibly due to site saturation, but within the Wet Tropics clade support was higher, except among the wingless species. In addition, there was some conflict between the molecular and morphological data sets in the basal part of the tree produced with the combined data. Where the phylogenetic hypothesis is well
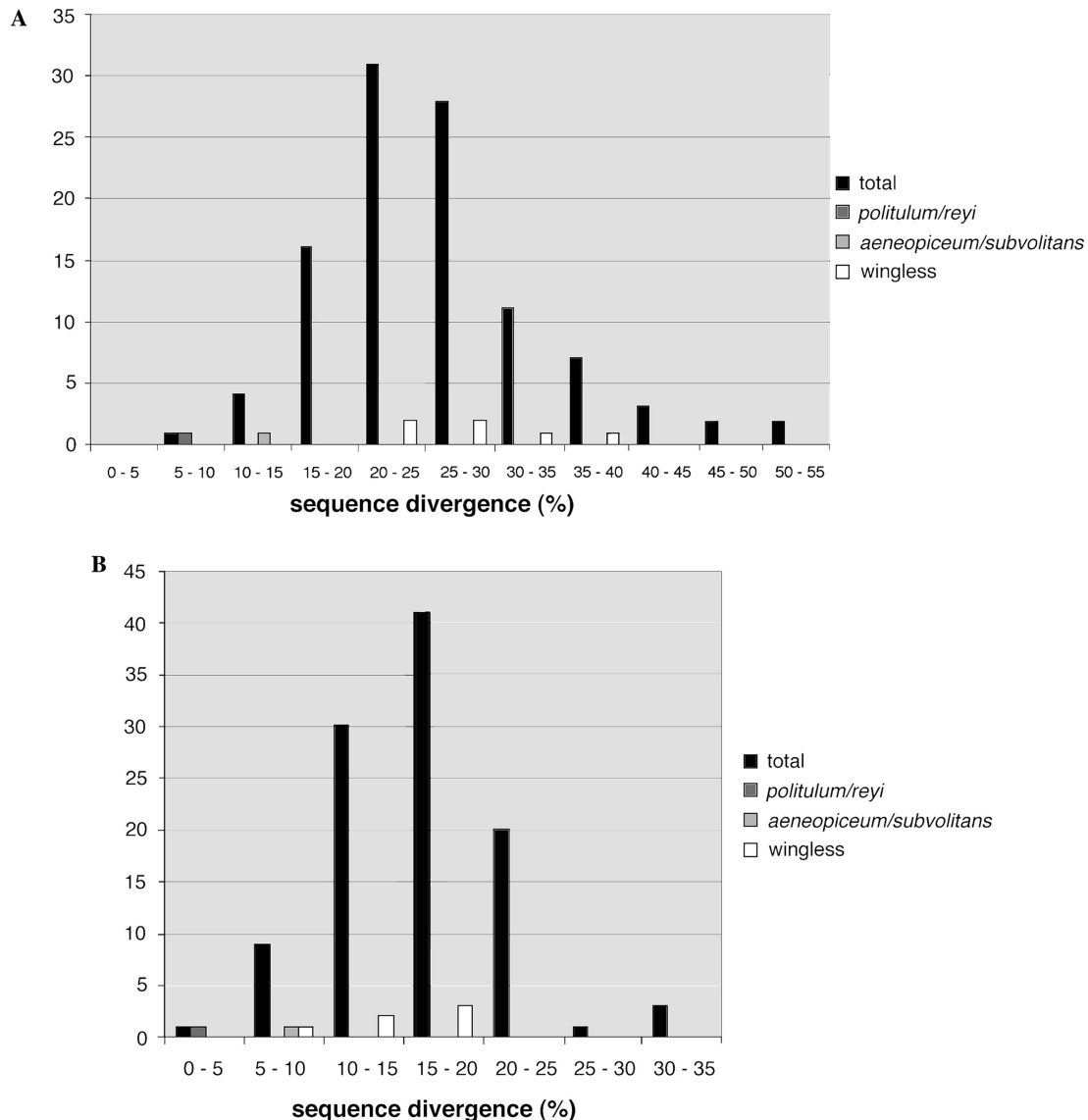
Fig. 3. (A) Histogram of total interspecific sequence divergences for 994 bp of COI based on a maximum likelihood model. (B) Histogram of net interspecific sequence divergences (ie. corrected for variation within species) for 437 bp of COI based on a maximum likelihood model.

supported we can make some inferences about speciation processes.

There are a remarkable number of sister species with adjacent ranges according to the tree topology produced with the combined data sets (see Figs. 6–8 for distributions of these species). The distributions of 47% of species are non-overlapping with their sister taxa. Vicariance barriers coincide with those observed in other species (Bouchard et al., in press; Joseph et al., 1995; Pope et al., 2000; Schneider and Moritz, 1999; Schneider et al., 1998) and predicted breaks in rainforest during Pleistocene, and earlier, glaciations (Nix, 1991). There is evidence for allopatric speciation being a major cause of speciation in this genus.

Temnoplectron subvolitans is known from two populations—the northern population is centered on the

Carbine Uplands, while the southern population occurs on the Atherton Uplands and Lamb Uplands between Millaa Millaa Falls and Cairns. *T. aeneopiceum*, sister of *T. subvolitans*, is found in rainforest from the Halifax Uplands north to the southern end of the Atherton Uplands. The two species are in contact at the southern end of the Atherton Uplands (Fig. 6). Brachyptery (reduced wings) occurs in *T. subvolitans* in this region of overlap. No morphological traits expected in hybrids have been observed (Reid and Storey, 2000). A study by Bouchard et al. (in press) of arthropod macroevolution in the Wet Tropics using secondary Brooks Parsimony Analysis (an approach to historical biogeography which uses area cladograms, duplicating areas with reticulate histories, Brooks et al., 2001) included the genus *Temnoplectron*, using the cladogram based on morpho-
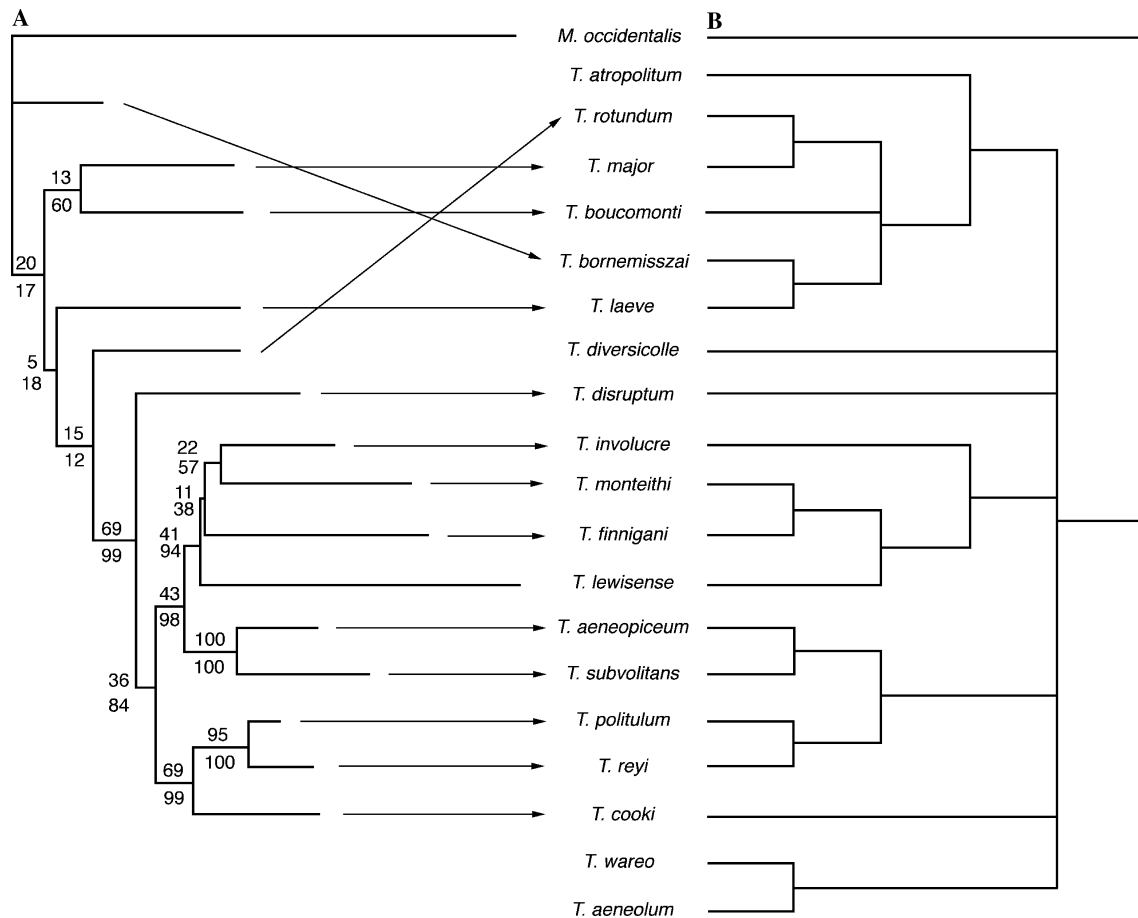
Fig. 4. (A) Maximum likelihood tree based on 994 bp of COI with bootstrap values shown above branches and Bayesian support values shown below branches. (B) Consensus of most parsimonious trees based on morphological data from Reid and Storey (2000).

logical characters (Reid and Storey, 2000). Consistent with our results, they concluded that these two species were probably involved in a vicariant event, with *T. subvolitans* speciating in Bellenden–Ker/Bartle–Frere, and *T. aeneopiceum* speciating in the Atherton Uplands, followed by extensive post speciation dispersal in both species and probably ecological differentiation. A detailed analysis of the population genetics of *T. aeneopiceum* and *T. subvolitans* is underway to further investigate the speciation processes involved. This hypothesised vicariant speciation event at the southern end of the Atherton Uplands is coincident with secondary contacts of phylogroups in widespread vertebrates, including the frogs *Litoria rheocola* and *Litoria nannotis* (Schneider et al., 1998), and in a widespread snail *Gnarosophia bellendenkerensis* (Hugall et al., 2002).

Temnoplectron reyi is known from the Mount Finnigan Uplands area, south to the northern slopes of the Lamb Uplands and shows little divergence across its range. It's sister species, *T. politulum* is found from the Lamb Uplands south to the Lee Uplands (Fig. 7). Collection records show the two species to have adjacent,

non-overlapping ranges, within 6 km of each other in the Lamb Uplands. Secondary Brooks Parsimony Analysis by Bouchard et al. (in press) suggested that these species evolved by peripheral isolation with an unknown ancestor. However, they also concluded that the distribution pattern could possibly be explained by a vicariant event with a post-speciation dispersal of *T. reyi* into the Lamb Uplands. Our evidence favours the latter hypothesis in that the geographic break across the Lamb Uplands coincides with secondary contacts of phylogroups in widespread vertebrates, including the frog *Litoria genimaculata* (Schneider et al., 1998), the skink *Carlia rubrigularis* (B. Phillips, unpublished) and the small marsupial *Bettongia tropica* (Pope et al., 2000) and in a widespread snail *G. bellendenkerensis* (Hugall et al., 2002).

Although the molecular, morphological, and combined tree topologies differ in the arrangement of the clade of wingless, montane, rainforest species, branch lengths on the molecular tree imply almost simultaneous speciation of these four species. *T. monteithi* (found on Thornton Uplands) and *T. finnigani* Reid and Storey
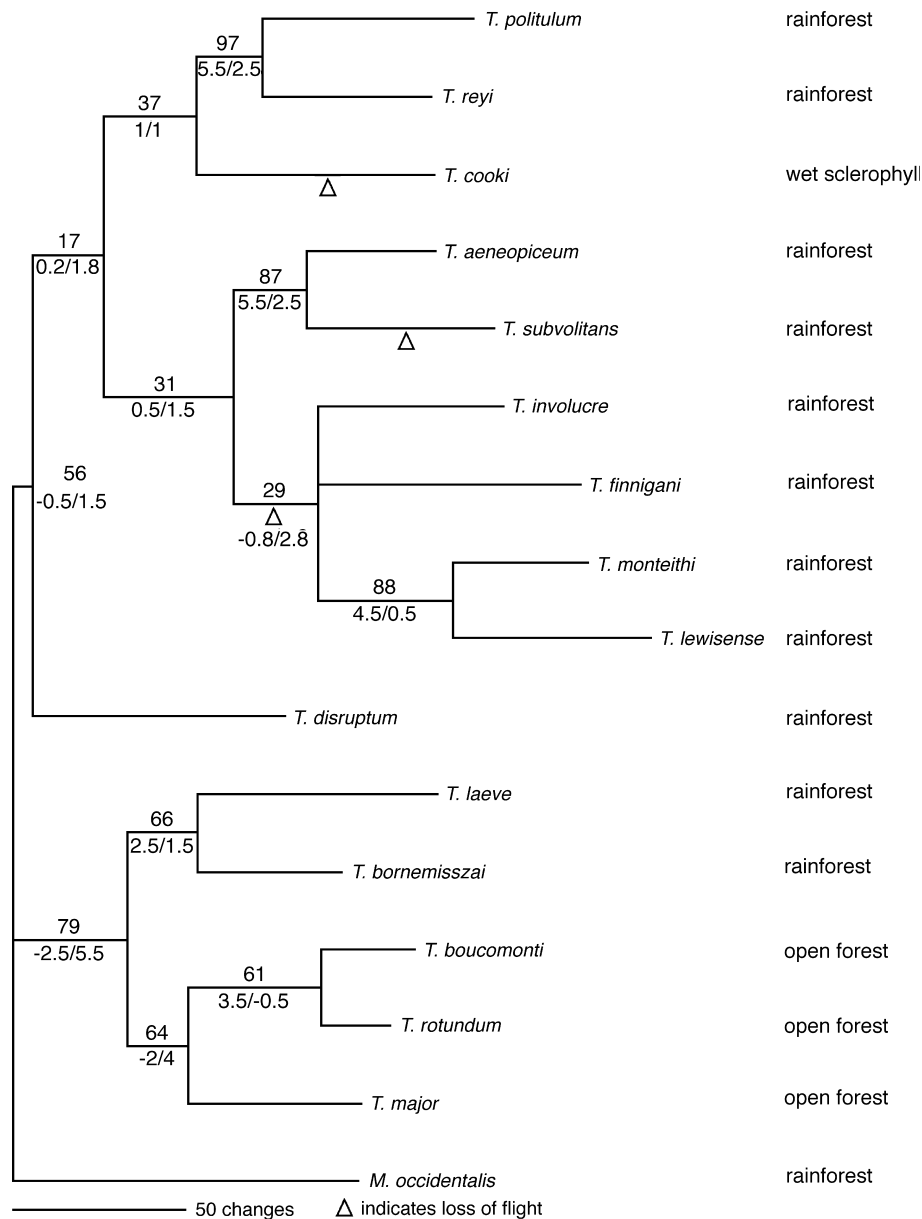
Fig. 5. Consensus of two most parsimonious trees of combined molecular and morphological data with bootstrap values shown above branches, and partitioned Bremer support values (molecular/morphological) shown below branches.

(found on Mount Finnigan Uplands) are separated by the Bloomfield River. Bouchard et al. (in press) hypothesised that these two species evolved through a vicariant event. The valley of the Daintree River separates *T. lewisense* (found on the Carbine Uplands) and *T. monteithi* (Thornton Uplands). The other species in this clade, *T. involucre*, is found in the Spec Uplands and Halifax Uplands, far to the south of the other species. Bouchard et al. (in press) suggest that both *T. lewisense* and *T. involucre* are the result of peripheral isolate speciation, from an ancestor common to all four of the above species. Reid and Storey (2000) hypothesized speciation by vicariance rather than dispersal. This explanation was supported by the variation of tibial

morphology, in which the currently geographically and sexually isolated species appear to preserve the longitudinal clinal range of variation of a widespread ancestor (Reid and Storey, 2000). Data from our study is inconclusive in determining mode of speciation for these species, as support values are low. However, it does show that speciation was rapid and ancient in this clade. It is noteworthy that there are no wingless *Temnoplectron* species present on the Atherton Uplands or Bellenden–Ker/Bartle–Frere. Interestingly, there are species of the wingless scarabaeine genus *Aptenocanthon* Matthews present in these areas, which may occupy a similar ecological niche to wingless *Temnoplectron* species (Storey and Monteith, 2000).
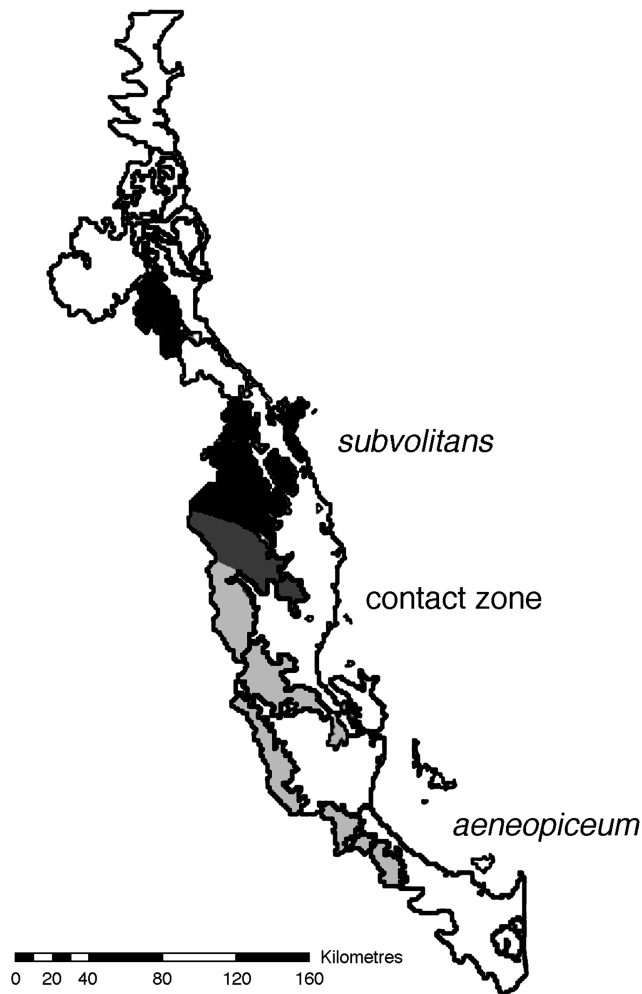
Fig. 6. Map of the Wet Tropics World Heritage Area, showing distribution of *T. subvolitans* (black) and *T. aeneopiceum* (light grey), and overlap between species (dark grey).

Three species isolated on either side of known vicariance barriers have failed to diverge morphologically in characters used to discriminate species, but have diverged in size and microsculpture (Reid and Storey, 2000). These species also have low levels of sequence divergence between the populations, relative to that found between species. The flightless *T. cooki* has isolated populations on Carbine Uplands and Lamb Uplands, separated by the Black Mountain Corridor, with a divergence between populations of 8%. Genetic divergence across the Black Mountain Corridor has been recorded in many Wet Tropics species (Joseph et al., 1995; Schneider et al., 1999). This break separates the two main rainforest refugia identified by Nix (1991). *T. subvolitans* has populations on the Carbine Uplands and (Atherton Uplands + Lamb Uplands), also separated by the Black Mountain Corridor, with 3% sequence divergence. Further studies of population genetics will more accurately determine this sequence divergence. *Temnoplectron laeve* Laporte has four iso-
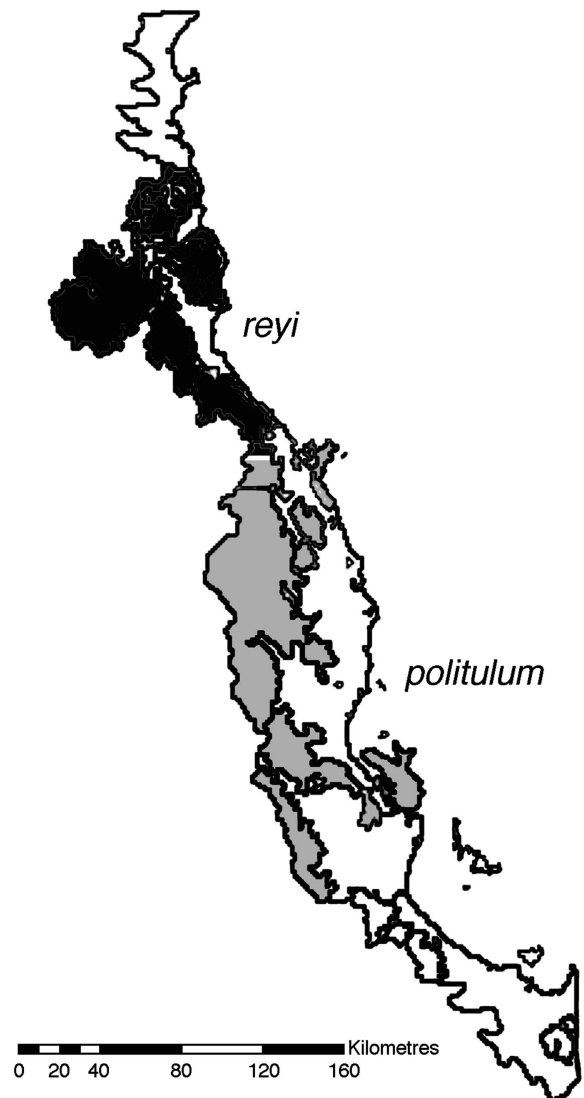

Fig. 7. Map of the Wet Tropics World Heritage Area, showing distribution of *T. politulum* (grey) and *T. reyi* (black).

lated populations—Carbine Uplands, (Spec Uplands + Halifax Uplands), Mid-East Queensland and Palm Islands—one (Carbine Uplands) of which is partially sympatric with its sister species, *T. bornemisszai* Matthews. Within species variation in this widely distributed and winged species is relatively low (max 2%).

4.2. Effect of flight loss on genetic diversity and speciation rate

There are two origins of flightlessness in this genus—a monophyletic group of four wingless, montane, rainforest species, and an individual species, *T. cooki*, which is found in two isolated populations in montane wet sclerophyll forest. There is also a reduction of wings in specimens of *T. subvolitans* in the area where they are in contact with their sister species, *T. aeneopiceum*. There is no indication of recent speciation in the wingless
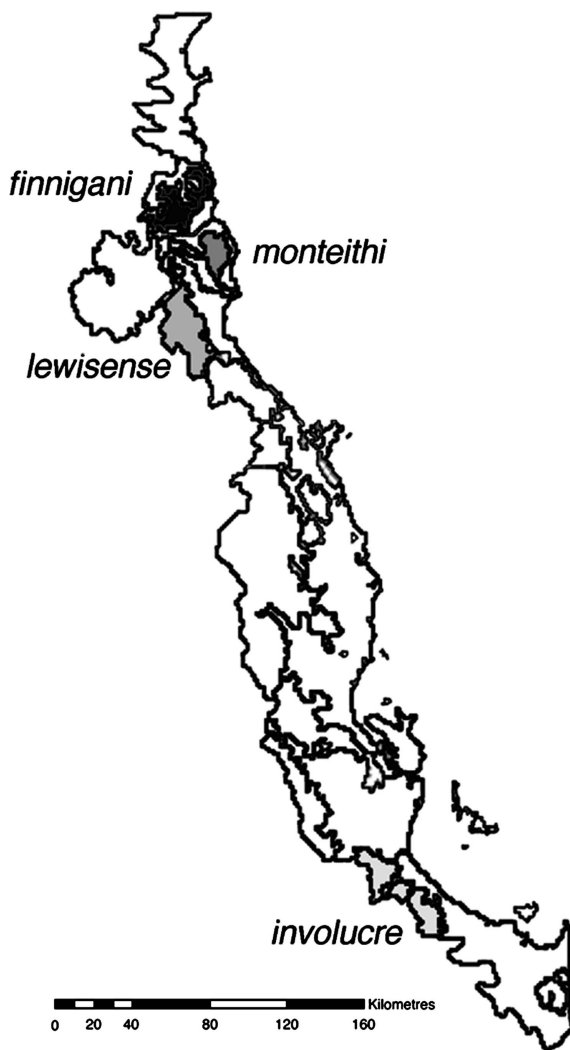
Fig. 8. Map of the Wet Tropics World Heritage Area, showing distributions of wingless rainforest species.

clade—winged as well as wingless sister taxa are highly divergent. While we do not have sufficient data to make precise comparisons between speciation rates in fully winged and flightless lineages, in the flightless lineage, short branch lengths of ancestral nodes suggest almost simultaneous speciation of these species.

Acknowledgments

References

Avise, J.C., Walker, D., Johns, G.C., 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc. R. Soc. Lond. B 265, 1707–1719.

Baehr, M., 1995. Revision of *Philipis* (Coleoptera: Carabidae: Bembidiinae), a genus of arboreal tachyine beetles from the rainforests of Eastern Australia: taxonomy, phylogeny and biogeography. Mem. Qld. Mus. 38, 315–381.

Baker, R.H., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaiian Drosophilids. Syst. Biol. 46, 654–673.

Bouchard, P., Brooks, D.R., Yeates, D.K., in press. J. Biogeogr. Mosaic Macroevolution in Australian Wet Tropics Arthropods: Community Assemblage by Taxon Pulses.

Brooks, D.R., van Veller, M.G.P., McLennan, D.A., 2001. How to do BPA, really. J. Biogeogr. 28, 345–358.

Brown, J.H., 1988. Species Diversity. In: Myers, A.A., Giller, P.S. (Eds.), Analytical Biogeography. Chapman & Hall, London, pp. 57–89.

Bush, M.B., 1994. Amazonian speciation: a necessarily complex model. J. Biogeogr. 21, 5–17.

Colinvaux, P.A., 1987. Amazon diversity in the light of the paleoecological record. Quat. Sci. Rev. 6, 93–114.

Farrell, B.D., 1998. Inordinate fondness explained: why are there so many beetles? Science 281, 555–559.

Fischer, A.G., 1960. Latitudinal variation in organic diversity. Evolution 14, 64–81.

Givnish, T.J., 1999. On the causes of gradients in tropical tree diversity. J. Ecol. 87, 193–210.

Hill, C.J., 1996. Habitat specificity and food preferences of an assemblage of tropical Australian dung beetles. J. Trop. Ecol. 12, 449–460.

Hugall, A., Moritz, C., Moussalli, A., Stanisic, J., 2002. Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). Proc. Natl. Acad. Sci. USA 99, 6112–6117.

Joseph, L., Moritz, C., 1993. Phylogeny and historical ecology of eastern Australian scrubwrens *Sericornis* spp.: evidence from mitochondrial DNA. Mol. Ecol. 2, 161–170.

Joseph, L., Moritz, C., Hugall, A., 1995. Molecular support for vicariance as a source of diversity in rainforest. Proc. R. Soc. Lond. B 260, 177–182.

Kershaw, A.P., 1994. Pleistocene vegetation of the humid tropics of northeastern Queensland, Australia. Palaeogeogr. Palaeoclimatol. Palaeoecol. 109, 399–412.

Kikkawa, J., Monteith, G.B., Ingram, G., 1981. Cape York Peninsula: major region of faunal interchange. In: Keast, A. (Ed.), Ecological Biogeography of Australia. W. Junk, The Hague, pp. 1695–1742.

Liebherr, J.K., 1986. Comparison of genetic variation in two carabid beetles (Coleoptera) of differing vagility. Ann. Ent. Soc. Am. 79, 424–433.

Matthews, E.G., 1972. A revision of the scarabaeine dung beetles of Australia I. Tribe Onthophagini. Aust. J. Zool., Suppl. Ser. 9, 3–330.

Matthews, E.G., 1974. A revision of the scarabaeine dung beetles of Australia. II. Tribe Scarabaeini. Aust. J. Zool., Suppl. Ser. 24, 1–211.

Matthews, E.G., 1976. A revision of the scarabaeine dung beetles of Australia III, Tribe Coprini. Aust. J. Zool., Suppl. Ser. 38, 1–52.

Moritz, C., Richardson, K.S., Ferrier, S., Monteith, G.B., Stanisic, J., Williams, S.E., Whiffin, T., 2001. Biogeographical concordance and efficiency of taxon indicators for establishing conservation priority in a tropical rainforest biota. Proc. R. Soc. Lond. B. Bio 268, 1875–1881.

Nix, H., 1991. Biogeography: pattern and process. In: Nix, H.A., Switzer, M.A. (Eds.), Rainforest Animals: Atlas of Vertebrates Endemic to Australia's Wet Tropics. Australian National Parks and Wildlife Service, Canberra, pp. 11–39.

Pope, L.C., Estoup, A., Moritz, C., 2000. Phylogeography and population structure of an ecotonal marsupial, *Bettongia tropica*, determined using mtDNA and microsatellites. Mol. Ecol. 9, 2041–2053.

Reid, C.A.M., Storey, R.I., 2000. Revision of the Dung Beetle Genus *Temnoplectron* Westwood (Coleoptera: Scarabaeidae: Scarabaeini). Mem. Qld. Mus. 46, 253–297.

Roff, D.A., 1994. The evolution of flightlessness: is history important? Evol. Ecol. 8, 639–657.

Schneider, C., Moritz, C., 1999. Rainforest refugia and evolution in Australia's Wet Tropics. Proc. R. Soc. Lond. B. 266, 191–196.

Schneider, C.J., Cunningham, M., Moritz, C., 1998. Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. Mol. Ecol. 7, 487–498.

Schneider, C.J., Smith, T.B., Larison, B., Moritz, C., 1999. A test of alternative models of diversification in tropical rainforests: ecological gradients vs. rainforest refugia. Proc. Natl. Acad. Sci. USA 96, 13869–13873.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Simon, C., Frati, F., Beckenback, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ent. Soc. Am. 87, 651–701.

Storey, R.I., Monteith, G.B., 2000. Five new species of *Aptenocanthon* Matthews (Coleoptera: Scarabaeidae: Scarabaienae) from tropical Australia, with notes on distribution. Mem. Qld. Mus. 46, 349–358.

Williams, S.E., Pearson, R.G., Walsh, P.J., 1996. Distributions and biodiversity of the terrestrial vertebrates of Australia's Wet Tropics: a review of current knowledge. Pacific Conserv. Biol. 2, 327–362.

Yeates, D.K., Bouchard, P., Monteith, G.B., 2002. Patterns and levels of endemism in the Australian Wet Tropics rainforest: evidence from flightless insects. Invert. Systematics 16, 605–619.