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Infrequent and unidirectional colonization of hyperdiverse *Papuadytes* diving beetles in New Caledonia and New Guinea

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Abstract

We present a molecular phylogenetic analysis of 2808 aligned bp of *rrnL*, *cox1*, *cob*, H3 and 18S rRNA of all major morphological groups of *Papuadytes* diving beetles (Coleoptera: Dytiscidae) which are diverse in running water habitats throughout the Australian region. We focus on the origin of the fauna of the megadiverse islands of New Guinea and New Caledonia. Parsimony as well as Bayesian analyses suggest a basal position of Australian species in a paraphyletic series, with more recent nested radiations in New Caledonia and New Guinea. According to molecular clock analyses, both landmasses were colonized during the Miocene, which matches geological data and corroborates similar findings in other taxonomic groups. Our analyses suggest that dispersal played an important role in the formation of these large insular faunas, although successful colonization appears to be a rare event, and, in this case, is unidirectional. Whether or not a lineage is present on an island is due to chance: *Papuadytes* are absent from Fiji, where related *Copelatus* have radiated extensively in the same habitats occupied by *Papuadytes* in New Caledonia and New Guinea, while *Copelatus* are absent from New Caledonia. Lineages of *Papuadytes* apparently colonized New Caledonia twice, around 14 and 9 MYA according to the molecular calibration, and both lineages are derived from an Australian ancestor. The older clade is represented only by two apparently relictual mountain species (one morphologically strongly adapted to highly ephemeral habitats), while the younger clade contains at least 18 species exhibiting a great morphological diversity. The 150+ species in New Guinea are monophyletic, apparently derived from an Australian ancestor, and constitute a morphologically rather homogenous group. The tree backbone remains insufficiently supported under parsimony and Bayesian analyses, where shorter branches suggest a rapid sequence of major branching events.
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1. Introduction

The historical biogeography of Southern land masses, including New Guinea, New Caledonia, Australia, and New Zealand, has received considerable attention in the context of vicariance models related to ancient Gondwanian biotic origins (Brundin, 1966; Cracraft, 2001; Wanntorp and Wanntorp, 2003; Sanmartín and Ronquist, 2004). Recent studies estimating the age of insect radiations in the region (Arensburger et al., 2004: New Zealand cicadas; Murienne et al., 2005: New Caledonian cockroaches; De Jong, 2003: Australian butterflies) suggest that many groups are fairly recent colonizers, or constitute older lineages which have diversified only recently (but see Heads, 2005, for a critique). An analysis of biogeographical patterns based on molecular phylogenetic data in Pacific monarchs revealed an even more recent, rapid radiation across most of the archipelagoes in the region (Filardi and Moyle, 2005). Hence molecular phylogenetic

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analyses may change hypotheses of biogeographic evolution in the Australo-Oceanian region, but large-scale studies remain scarce (Austin et al., 2004).

Here, we investigate the diversity and evolution of aquatic beetles (genus *Papuadytes*) in two particularly species rich areas, New Guinea and New Caledonia. The former is the second largest island on the planet and ranked as one of only three remaining major tropical wilderness areas (Mittermeier et al., 1998, 2003). New Caledonia is one of 34 global biodiversity hotspots (Myers et al., 2000) and constitutes an ancient fragment of Gondwana, assumed to contain spectacular examples of relict flora (e.g. Lowry, 1998). However, such relicts might have dispersed into the area more recently (e.g. Swenson et al., 2001), explaining their presence in New Caledonia despite suggested complete submersion of the island during the Paleocene (see Murienne et al., 2005). New Guinea is a composite of 32 geological terranes (Pigram and Davies, 1987) and although megadiverse biologically (Gressitt, 1982), land in the region emerged only recently, with large land areas arising only over the past 10 MY (Hall, 1998).

The genus *Papuadytes* is an ecologically and taxonomically highly diverse group of predatory diving beetles (Dytiscidae) which are common in stream ecosystems throughout the region. There are about 20 known species in Australia, but by 1998 only three species had been described from New Guinea. Extensive recent fieldwork and taxonomic investigation revealed the existence of more than 150 species. Similarly, 15 new species were discovered during a single expedition to New Caledonia, raising the new total there to approximately 30 species. Hence, this group appears highly species rich and lends itself to investigations of species diversification in this region, in particular in the light of radiations into different habitats, including lowland pools, mountain streams, groundwater, interstitial, and highly ephemeral first order streams on mountain tops.

The molecular systematic analysis presented here includes these different ecological types, as well as all major morphological groups known so far (Balke, 1998; Shaverdo et al., 2005). This comprehensive taxonomic and ecological coverage of the genus now provides the most extensive molecular phylogenetic study of genus-level relationships of invertebrates in the Australo-Oceanian region. We use these data to assess geographical patterns in the diversity hotspots of New Guinea and New Caledonia, for inferences about the origin and colonization history of their aquatic insect fauna. Finally, relative ages based on molecular clock calibrations are used to estimate the dates of local lineages in the context of island ages.

2. Materials and methods

2.1. DNA extraction, PCR and sequencing

Three mitochondrial and two nuclear gene regions were chosen to provide information at different hierarchical levels (Otto et al., 1996; Barraclough et al., 1999; Ribera et al., 2001). Mitochondrial sequences included the 3′ ends of the 16S rRNA (*rrnL*) and cytochrome *c* oxidase subunit I (*cox1*) genes, and a central fragment of cytochrome *b* (*cob*); and nuclear markers were the 5′ end of 18S rRNA and a fragment of histone 3 (H3). Laboratory procedures were described by Balke et al. (2004). We used data from Balke et al. (2004), as well as new sequences which have been submitted to GenBank (Accession Nos. AM292106-197, AM296116-186, AM 396308-356 and 396771-839).

2.2. Taxon sampling and selection of outgroups

Papuadytes was established as a well-supported clade by Balke et al. (2004) in an analysis of relationships within Copelatinae, in which a sister group relationship of *Papuadytes* + all other Copelatinae (parsimony) or *Papuadytes* + all other Copelatinae excluding the two European species of *Liopterus* (Bayesian analysis) was suggested. Here, the following outgroups were selected to represent lineages outside of *Papuadytes*: *Aglymbus* cf *formosulus* Guignot, 1956, *Aglymbus elongatus* (Kolbe, 1883), *Liopterus atriceps* Sharp, 1882 and *Liopterus haemorrhoidalis* (F., 1787), plus *Hydrodytes opalinus* (Zimmermann, 1921) (Hydrodytinae), which has been separated from Copelatinae recently (Miller, 2001). As the phylogenetic placement of Copelatinae within Dytiscidae remains uncertain, all trees were rooted in the related family Amphizoidae (Miller, 2001; Ribera et al., 2002a,b).

Many species are represented by extraction numbers only: they are either undescribed (most New Guinean and New Caledonian, few Australian species) or still await taxonomic revision (e.g. Shaverdo et al., 2005). For example, Australian species were redescribed by Watts (1978), but remain in need of further research to delineate species boundaries, as their species diversity appears to be underestimated (Watts personal communication, 2001).

2.3. Vouchers

After the non-destructive extraction specimens were kept as vouchers, dry mounted along with the dissected male genitals, locality-labelled and an additional, dark green label stating the DNA extraction number as given in Table 1. Vouchers will be deposited in the Natural History Museum under Coleoptera collection accession number BMNH{E} 2006-92.

2.4. Data analysis

The 18S ingroup sequences were not length variable, and the most deviating outgroup sequence was only 4 bp shorter (*Aglymbus* cf *formosulus*) and could be aligned to the ingroup sequences by eye. Length of *rrnL* sequences ranged from 482 (e.g. *Papuadytes* sp. 26) to 491 bp (*Papuadytes* sp. 28). These sequences were also aligned by eye (Balke et al., 2004) but since gaps were ambiguous, nucleotide homologies were also assessed using Clustal W (Higgins et al., 1996) employing different multiple gap opening penalties (20, 10, 6, 4, 2, and 1) (Wheeler, 1995).

Table 1
Collecting data for sequenced ingroup specimens

Taxon ID	Genus	Species	Country	Locality	Collector	Elev (m)	Date
MB 1	*Papuadytes*	*perfectus*	New Caledonia	South Prov., Dumbea, near road to Mt. Koghis (NC 1)	Wewalka & Balke	50	3.xi.2001
MB 2	*Papuadytes*	*aubei*	New Caledonia	South Prov., Dumbea, near road to Mt. Koghis (NC 1)	Wewalka & Balke	50	3.xi.2001
MB 4	*Papuadytes*	*perfectus*	New Caledonia	North Prov., 10km E Pouembout (NC 7)	Wewalka & Balke	50	6.xi.2001
MB 5	*Papuadytes*	*aubei*	New Caledonia	North Prov., 13km N Koumac (NC 12)	Wewalka & Balke	50	7.xi.2001
MB 18	*Papuadytes*	*bimaculatus*	New Caledonia	South Prov., Mt. Canala, 15–20km S Canala (NC 37)	Wewalka & Balke	600	15.xi.2001
MB 19	*Papuadytes*	sp. 26	New Caledonia	South Prov., Mt. Canala, 15–20km S Canala (NC 37)	Wewalka & Balke	600	15.xi.2001
MB 20	*Papuadytes*	sp. 29	New Caledonia	South Prov., Mt. Canala, 15–20km S Canala (NC 37)	Wewalka & Balke	600	15.xi.2001
MB 35	*Papuadytes*	sp. 23	New Caledonia	North Prov., Mt. Panié (NC 15)	Wewalka & Balke	1200	9.xi.2001
MB 38	*Papuadytes*	*bimaculatus*	New Caledonia	North Prov., Aoupinié, 15km SW Ponérihouen (NC 33)	Wewalka & Balke	500–700	14.xi.2001
MB 39	*Papuadytes*	sp. 28	New Caledonia	North Prov., Aoupinié, 15km SW Ponérihouen (NC 33)	Wewalka & Balke	500–700	14.xi.2001
MB 40	*Papuadytes*	sp. 28	New Caledonia	North Prov., Aoupinié, 15km SW Ponérihouen (NC 33)	Wewalka & Balke	500–700	14.xi.2001
MB 50	*Papuadytes*	*shizong*	China	Yunnan, 2 KM S Shizong	Bergsten		16.ix.2000
MB 56	*Papuadytes*	sp. 13	Indonesia	Papua, Wandammen, Wasior	Riedel		4–5.I.2001
MB 59	*Papuadytes*	sp. 10	Indonesia	Japen Isl., Serui, Mantembu	Riedel	100–500	16.XII.2000
MB 66	*Papuadytes*	sp. 4	Indonesia	Papua, N Wamena	Cerny		
MB 80	*Papuadytes*	*melanarius*	Australia	NSW, Bendolba (ABTC 9337)	Watts		
MB 83	*Papuadytes*	*australiae*	Australia	SA, Chain of Ponds (ABTC 9215)	Watts		
MB 84	*Papuadytes*	*punctipennis*	Australia	SA, 6 KM N Forreston (ABTC 9219)	Watts		
MB 86	*Papuadytes*	*rasilis*	Australia	QLD, Cunninghans Gap (ABTC 9324)	Watts		
MB 87	*Papuadytes*	*glyptus*	Australia	QLD, Wallaman Falls (ABTC 9285)	Watts		
MB 89	*Papuadytes*	*abditus*	Australia	NT, Newhaven Stn., Camel Bore (BES 7296)	Humphreys & Russ		15.vi.2001
MB 90	*Papuadytes*	*commatifer*	New Caledonia	North Prov., Mont Panié, camp below summit (NC 16)	Balke & Wewalka	1350	8–9.xi.2001
MB 104	*Papuadytes*	*ferrugineus* s.I.	Australia	SA, Adelaide, Watts Gully	Balke & Watts	<300	28.x.2001
MB 105	*Papuadytes*	*simplex* (1)	Australia	SA, Adelaide, Watts Gully	Balke & Watts	<300	28.x.2001
MB 106	*Papuadytes*	*simplex* (1)	Australia	SA, 10km E Penola	Balke & Watts	<300	30.x.2001
MB 107	*Papuadytes*	*simplex* (2)	Australia	SA, 10km E Penola	Balke & Watts	<300	30.x.2001
MB 121	*Papuadytes*	sp. 21	New Caledonia	North Prov., Aoupinié, 25km SW Ponérihouen (NC 34)	Wewalka & Balke	700	14.xi.2001
MB 122	*Papuadytes*	sp. 21	New Caledonia	North Prov., Aoupinié, 25km SW Ponérihouen (NC 34)	Wewalka & Balke	700	14.xi.2001
MB 128	*Papuadytes*	sp. 30	New Caledonia	South Prov., PN Rivière Bleue, trail 7C (NC 49/50)	Wewalka & Balke	500–600	20.xi.2001
MB 130	*Papuadytes*	*bimaculatus*	New Caledonia	South Prov., Mt. Mou, near Sanatorium (NC 52)	Wewalka & Balke	400	23.xi.2001
MB 131	*Papuadytes*	*aubei*	New Caledonia	South Prov., Mt. Mou, near Sanatorium (NC 52)	Wewalka & Balke	400	23.xi.2001
MB 133	*Papuadytes*	sp. 18a	New Caledonia	South Prov., Mt. Mou, near Sanatorium (NC 52)	Wewalka & Balke	400	23.xi.2001
MB 135	*Papuadytes*	sp. 24	New Caledonia	South Prov., 6km S Thio (NC 42)	Wewalka & Balke	50	17.xi.2001
MB 136	*Papuadytes*	*bimaculatus*	New Caledonia	South Prov., Mt. Koghis (NC 44)	Wewalka & Balke	500	19.xi.2001
MB 137	*Papuadytes*	sp. 30	New Caledonia	South Prov., Mt. Humboldt (NC 51)	Wewalka & Balke	800–900	22.xi.2001
MB 138	*Papuadytes*	sp. 27	New Caledonia	South Prov., PN Rivière Bleue, trail 7C (NC 49/50)	Wewalka & Balke	500–600	20.xi.2001
MB 140	*Papuadytes*	*brownei*	New Caledonia	South Prov., 6km S Thio (NC 42) check locality	Wewalka & Balke	50	17.xi.2001
MB 146	*Papuadytes*	*ferrugineus* s.I.	Australia	WA, Pinjarra (Watts 66)	Watts		
MB 163	*Papuadytes*	sp. 22	New Caledonia	North Prov., 10km SE Ouégoa, road to Mandjélia (NC 26)	Wewalka & Balke	560	11.xi.2001
MB 165	*Papuadytes*	sp. 26	New Caledonia	North Prov., 10km SE Ouégoa, road to Mandjélia (NC 26)	Wewalka & Balke		
MB 166	*Papuadytes*	sp. 20	New Caledonia	South Prov., Dumbea, near road to Mt. Koghis (NC 1)	Wewalka & Balke	50	3.xi.2001
MB 168	*Papuadytes*	*perfectus*	New Caledonia	North Prov., 1km SW Camp Minier (NC 10)	Wewalka & Balke	20	7.xi.2001
MB 170	*Papuadytes*	sp. 19	New Caledonia	North Prov., 9km SSW Ouégoa, nr crossing road Bondé, 50 (NC 23)	Wewalka & Balke	50	11.xi.2001
MB 253	*Papuadytes*	*interruptus*	New Caledonia	South Prov., Mt. Mou, near Sanatorium (NC 52)	Wewalka & Balke	400	23.xi.2001
MB 254	*Papuadytes*	sp. 25	New Caledonia	South Prov., Mt. Koghis (NC 44)	Wewalka & Balke	500	19.xi.2001
MB 255	*Papuadytes*	*munaso*	Papua New Guinea	Simbu / EHPr., Crater Mountain, Wara Sera Station (PNG 10)	Sagata	800	14.ix.2002
MB 256	*Papuadytes*	*hintelmannae*	Papua New Guinea	Simbu / EHPr., Crater Mountain, Wara Sera Station (PNG 10)	Sagata	800	14.ix.2002

(*continued on next page*)

Table 1 (*continued*)

Taxon ID	Genus	Species	Country	Locality	Collector	Elev (m)	Date
MB 257	*Papuadytes*	sp. 14	Papua New Guinea	Simbu / EHPr., Crater Mountain, Wara Sera Station (PNG 10)	Sagata	800	14.ix.2002
MB 258	*Papuadytes*	sp. 16	Papua New Guinea	Simbu / EHPr., Crater Mountain (PNG 1)	Sagata	700	11.ix.2002
MB 259	*Papuadytes*	sp. 16	Papua New Guinea	Simbu / EHPr., Crater Mountain (PNG 1)	Sagata	700	11.ix.2002
MB 261	*Papuadytes*	sp. 15	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, upper Oh River (PNG 12)	Sagata	1200	15.ix.2002
MB 262	*Papuadytes*	*munaso*	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, Wara Hulene (PNG 17)	Sagata	1000	15.ix.2002
MB 263	*Papuadytes*	*rivulus* s.l.	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, Wara Hulene (PNG 17)	Sagata	1000	16.ix.2002
MB 264	*Papuadytes*	*hintelmannae*	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, Wara Hulene (PNG 17)	Sagata	1000	16.ix.2002
MB 265	*Papuadytes*	sp. 16	Papua New Guinea	Simbu / EHPr., Crater Mtain, Sera–Herowana, Wara Hulene (PNG 17)	Sagata	1000	16.ix.2002
MB 267	*Papuadytes*	*atowaso*	Papua New Guinea	Madang Pr., below Bundi (PNG 23)	Balke	500	26.ix.2002
MB 268	*Papuadytes*	*rivulus s.l.*	Papua New Guinea	Madang Pr., below Bundi (PNG 23)	Balke	500	26.ix.2002
MB 269	*Papuadytes*	*larsoni*	Papua New Guinea	Madang Pr., below Bundi (PNG 23)	Balke	500	26.ix.2002
MB 273	*Papuadytes*	*astrophallus*	Papua New Guinea	Madang Pr., Brahmin (PNG 24)	Balke	150	26.ix.2002
MB 279	*Papuadytes*	*ater*	Australia	WA, Perth/Ellenbrook, Mellbrooks Speedway (32/196)	Hendrich		10.-12.ix.2002
MB 292	*Papuadytes*	sp. 31	New Caledonia	North Prov., Mont Panié, camp below summit (NC 16)	Balke & Wewalka	1350	8-9.xi.2001
MB 295	*Papuadytes*	?*australiae*	Australia	Tasmania, Terraleah	Watts		2002
MB 296	*Papuadytes*	*australis*	Australia	Flinders Range	Leys		2002
MB 297	*Papuadytes*	*abditus*	Australia	NT, Newhaven Stn., Camel Bore	Humphreys & Read		19.viii.2002
MB 385	*Papuadytes*	*miriae*	Papua New Guinea	EHL, Yoginofi-Kainantu	Sagata	1825	ii.2003
MB 389	*Papuadytes*	*ullrichi*	Papua New Guinea	EHL, Aiyura	Sagata	1680	ii.2003
MB 390	*Papuadytes*	*miriae*	Papua New Guinea	EHL, Onerunka - Kainantu	Sagata	1735	ii.2003
MB 441	*Papuadytes*	*ferrugineus* s.I.	Australia	WA, 40 km E Perenjori, Perenjori-Wanarra Road (25/189)	Hendrich		7.ix.2002
MB 458	*Papuadytes*	*australiae*	Australia	Tasmania, 5km S Tarraleah	Watts		4.x.2002
MB 459	*Papuadytes*	*boulevardi*	Australia	Tasmania	Watts		
MB 481	*Papuadytes*	sp. 33	Australia	QLD, Brisbane Forest Park	Balke & Monteith		1.xi.2003
MB 483	*Papuadytes*	sp. 33	Australia	QLD, Brisbane Forest Park	Balke & Monteith		1.xi.2003
MB 485	*Papuadytes*	sp. 32	Australia	QLD, Brisbane Forest Park	Balke & Monteith		1.xi.2003
MB 487	*Papuadytes*	sp. 32	Australia	QLD, Brisbane Forest Park	Balke & Monteith		1.xi.2003
MB 608	*Papuadytes*	*ater*	Australia	WA, Ellen Brook Nature Reserve	Watts		1.x.2003
MB 611	*Papuadytes*	*ferrugineus* s.I.	Australia	WA, Bushy swamp	Watts		5.x.2003
MB 656	*Papuadytes*	*rivulus* s.l.	Papua New Guinea	Sandaun Pr., Faklows (WB87)	Sagata	720	24.x.2003
MB 657	*Papuadytes*	sp. 5 (nr. *messeri*)	Papua New Guinea	Sandaun Pr., Sokamin (WB97)	Sagata	1200	9.x.2003
MB 658	*Papuadytes*	sp. 5 (nr. *messeri*)	Papua New Guinea	Sandaun Pr., Mianmin 2 (WB70)	Sagata	1100	20.x.2003
MB 659	*Papuadytes*	sp. 12	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 660	*Papuadytes*	sp. 5 (nr. *messeri*)	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 662	*Papuadytes*	sp. 1	Papua New Guinea	Sandaun Pr., May River (WB47)	Sagata	2600	15.x.2003
MB 664	*Papuadytes*	sp. 1	Papua New Guinea	Sandaun Pr., May River (WB47)	Sagata	2600	15.x.2003
MB 666	*Papuadytes*	sp. 9	Papua New Guinea	Sandaun Pr., Sokamin - Mekil T Plot (WB102)	Sagata	1200–1700	11./19.x.2003
MB 667	*Papuadytes*	sp. 18	Papua New Guinea	Sandaun Pr., Mianmin 1 (WB75)	Sagata	800	9.x.2003
MB 670	*Papuadytes*	sp. 3	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 671	*Papuadytes*	sp. 2	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 672	*Papuadytes*	sp. 9	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 679	*Papuadytes*	sp. 1	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 680	*Papuadytes*	sp. 3	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 681	*Papuadytes*	sp. 9	Papua New Guinea	Sandaun Pr., Mekil K (WB106)	Sagata	1700	14.x.2003
MB 683	*Papuadytes*	sp. 5 (nr. *messeri*)	Papua New Guinea	Sandaun Pr., Mekil WX 25 (WB100)	Sagata	1700	13.x.2003
MB 685	*Papuadytes*	*rivulus* s.l.	Papua New Guinea	Sandaun Pr., Fak River (WB24)	Sagata	775	23.x.2003
MB 686	*Papuadytes*	sp. 6	Papua New Guinea	Sandaun Pr., Mekil W100 (WB19)	Sagata	1700	13.x.2003
MB 688	*Papuadytes*	sp. 17	Papua New Guinea	Sandaun Pr., May River (WB43)	Sagata	970	9./17.x.2003
MB 693	*Papuadytes*	*aubei*	New Caledonia	Ile des Pines, Kwanyi (NC 54)	Wewalka & Balke	50	24.xi.2000
IRPa	*Papuadytes*	sp. 11	Indonesia	Papua, Nabire, Kali Cemara	Balke	250	vi.1998

Outgroup data were taken from Balke et al. (2004).

Alignment was straightforward in protein coding sequences (*cox1*, *cob*, and H3) which did not show length variation. The 18S ingroup sequences were not length variable, and the most deviating outgroup sequence was only 4 bp shorter (*Aglymbus* cf *formosulus*) and could be aligned to the ingroup sequences by eye. Length of *rrnL* sequences ranged from 482 (e.g. *Papuadytes* sp. 26) to 491 bp (*Papuadytes* sp. 28). These sequences were aligned by eye (Balke et al., 2004) but since gaps were ambiguous, nucleotide homologies were also assessed using Clustal W (Higgins et al., 1996) employing different multiple gap opening penalties (20, 10, 6, 4, 2, and 1) (Wheeler, 1995). *rrnL* alignments were assessed based on two criteria: (i) retention index (RI) for the *rrnL* partition estimated on the shortest tree topology found in the simultaneous analysis of the three protein coding genes, and (ii) character congruence between the *rrnL* partition and the protein coding genes based on the incongruence length difference test (ILD; Mickevich and Farris, 1981; Farris et al., 1994). The best *rrnL* alignment would be those with highest RI in the simultaneous analysis, and lowest ILD.

Bayesian analyses were conducted on the combined data set with MrBayes 3.04 (Huelsenbeck and Ronquist, 2001), using a GTR + I + Γ model as selected with Modeltest (Posada and Crandall, 1998). We used the default priors starting with random trees, and ran three heated and one cold Markov chains for 3,000,000 generations, sampled at intervals of 1000 generations. To determine the point at which the Markov chains reached stationarity, the log-likelihood scores were plotted against generation time, to determine when the log-likelihood values stabilize. After burn-in samples were discarded, trees were combined in a single majority consensus topology, and the percentage of the nodes were taken as *a posteriori* probabilities (Huelsenbeck and Ronquist, 2001).

Parsimony searches were conducted using PAUP* version 4.0b10 (Swofford, 2002) performing 500 TBR heuristic searches with random addition sequences, keeping 50 trees per replicate, gaps coded as 5th character state and all characters weighted equally. Bootstrap resampling was performed with 1000 pseudoreplicates and 100 random addition TBR searches each (Felsenstein, 1985). Partitioned Bremer Support (PBS) (Baker and DeSalle, 1997) was established searching on constrained trees generated with TreeRot (Sorenson, 1996) as a measure of support provided by different gene partitions to the combined analysis tree. PBS values for each data partition were summed across all nodes of the combined analysis tree and standardized by the minimum possible number of steps for each partition. Positive PBS values support the node in question, negative values suggest that a shorter tree for this data partition can be found and hence incongruence between partitions (Baker and DeSalle, 1997; Gatesy et al., 1999; Remsen and O'Grady, 2002). Partitioned hidden branch support (PHBS; Gatesy et al., 1999) was calculated to examine phylogenetic signal that emerges in the combined analysis only.

Incongruence between partitions was further estimated using the incongruence length difference test (ILD), and the associated partition homogeneity test (Mickevich and Farris, 1981; Farris et al., 1994) in PAUP* with 100 replicates. To assess if the topology of a parsimony tree significantly differs from those obtained in the Bayesian analysis we used a Shimodaira-Hasegawa test using the RELL approximation (Shimodaira and Hasegawa, 1999), with 1000 bootstrap replicates, as implemented in PAUP*.

The temporal pattern of the *Papuadytes* radiation was explored using the topology and branch lengths obtained in the Bayesian analysis. A likelihood ratio test (Felsenstein, 1981) was used to test for compliance with a molecular clock, which was rejected ($p < 0.0001$). Hence, clock-like branch lengths were fitted by using penalized likelihood (PL) using r8s software (Sanderson, 2002), with an optimal smoothing parameter estimated by cross-validation of four smoothing values (1, 10, 100, and 1000). Absolute ages of nodes were calibrated by setting the split of the stygobiont *Papuadytes abditus* and its sister clade to 10.28 MY. This date has been estimated for a clade of stygobiont diving beetles in the tribe Bidessini occurring in the same Central Australian locality as *P. abditus* (Leys et al., 2003), providing a time frame for the invasion of underground waters due to the desertification of the area. The analysis with smoothing cost = 1000 retrieved the lowest χ^2 error value (3439.41) and hence it was chosen as optimal. To take stochastic variation into account (due to a finite number of characters), and hence to estimate the confidence of node ages we applied a resampling scheme (Baldwin and Sanderson, 1998). One hundred bootstrap replicates of the data were generated in PAUP*, calculating branch lengths on each of these new data sets given the original tree topology and parameters estimated in the Bayesian analysis. Finally, branch lengths were fitted to a clock using PL and the optimal smoothing value 1000 in r8s.

3. Results

3.1. Molecular phylogenetics

Sequences from 98 ingroup and 6 outgroup individuals were included in the phylogenetic analysis. MtDNA was A + T rich (average 74%), whereas the nuclear DNA was slightly G + C rich (average 55%), with greater biases in informative positions (83% A + T vs. 64% G + C). Nucleotide composition across species was homogeneous according to the conservative statistics implemented in PAUP when all the nucleotides positions were included in the test, although investigation of various data partitions revealed significant heterogeneity for informative sites of *cob*. Mitochondrial data provided twice as many positions in the aligned matrix than the nuclear markers (1883 vs. 925; Table 2) but nearly seven times more informative sites (732 vs. 114).

Alignment of *rrnL* sequences revealed several ambiguously placed indels, and hence we generated several Clustal

Table 2
Tree statistics for partitioned and combined data sets

Partition	miss	NChars	cons	inf	Trees	lgth	ci	ri	con nodes	l const	% incr lgth	shared nodes	sum PBS	PBS/length
cox1	4	736	412	285	626	2029	0.2587	0.6825	65	2096	3.2	54	560.4	1.0672725
cob	5	353	167	164	723	1447	0.2108	0.6709	62	1511	4.2	46	230.2	0.7548581
rrnL	2	794	434	283	19637	1423	0.3921	0.7514	73	1593	10.7	48	278.1	0.4982434
H3	5	321	213	92	11700	404	0.4307	0.8327	47	439	8	33	211.5	1.2159768
18S	49	604	567	22	6668	66	0.697	0.8425	10	81	18.5	5	26.7	0.5800955
mtDNA	0	1883	1013	732	527	5176	0.2682	0.6759	81	5200	0.5	65	1068.7	0.7694407
nDNA	3	925	780	114	2050	483	0.4555	0.8249	53	520	7.1	31	238.2	1.0829528
Combined	0	2808	1793	846	36	5720	0.2811	0.6882	96	n/a	n/a	n/a	1306.9	0.8120335

miss, number of terminals without data; con nodes, resolved nodes in strict consensus of most parsimonious trees; shared nodes, number of nodes shared by the consensus tree of a partition and the consensus tree of the combined analysis; l const, length of a particular tree when constrained to the combined analysis topology; % incr lgth, percentage increase in number of steps for a partition when constrained to the combined analysis topology; in all cases the trees were constrained to a particular tree arbitrarily selected from the 36 equally parsimonious trees obtained in the combined analysis; sum PBS, sum of PBS values for a partition across all nodes on the combined analysis tree; PBS/length, sum PBS normalized by the minimum possible number of steps for each partition (i.e. the numerator of the CI; Baker et al., 2001).

alignments, plus a manual alignment, to explore alignment space and select the optimal one (see Section 2). All *rrnL* alignments had very similar RI when being analyzed separately (RI = 0.75–0.76), when combined with the protein coding genes (RI = 0.68–0.69), or estimating the values of the *rrnL* partition on the tree topology based on the protein coding genes (*cox1*, *cob*, and H3) alone (RI = 0.67–0.68). Specifically, higher gap penalties usually showed slightly higher RI values. In contrast, when incongruence was assessed based on the ILD of *rrnL* and the protein coding partitions, lower gap penalties generally led to slightly lower values (ILD 219–243, and 0.039–0.043 if they are normalized by the number of steps in the simultaneous analysis tree). The manual alignment of the *rrnL* sequences had RI values estimated on the protein coding genes tree topology more similar to the Clustal alignments with higher gap penalties (0.6715), but ILD values (228 and 0.040) were more similar to the alignments obtained under lower gap penalties. Because differences between alignments, based on RI and ILD, were relatively small, criteria suggested opposite choices, and since *rrnL* topologies were very similar we selected our manual alignment (see Simmons, 2004).

The parsimony analysis of the final data matrix (2808 aligned characters, 846 parsimony informative) resulted in 36 trees of 5720 steps (CI = 0.28, RI = 0.69). The data were partitioned according to genetic loci to test for their phylogenetic signal and potential conflict. Incongruence between mitochondrial partitions was highest for *rrnL* with the two protein coding sequences (e.g. an ILD normalized for the number of steps in the simultaneous analysis tree of 0.051 for *rrnL* and *cox1* vs. 0.020 for *cob* and *cox1*; Table 3). The incongruence between nuclear and mitochondrial sequences was lower than between the three mitochondrial partitions alone (ILD = 0.011 vs. 0.054) but incongruence was significant in the partition homogeneity test (Table 3).

Phylogenetic signal was mostly provided by the mitochondrial data, resolving more nodes (81 vs. 53), and showing a larger sum PBS than the nuclear partitions (1068.7 vs. 238.2). The partition from all mtDNA loci was in closer agreement with the tree topology obtained in the combined analysis. It required a smaller number of extra steps (0.5%) to fit to, and shared more nodes (65) with, the combined analysis tree than the nuclear partition (7.1% increased length, 31 shared nodes). Within the mitochondrial partition, *cox1* had more phylogenetic signal than *cob* and *rrnL*, and required a smaller number of extra steps to fit to, and shared more nodes with, the simultaneous analysis tree (Table 2).

Table 3
Incongruence between data sets measured by the ILD and the partition homogeneity test of Farris et al. (1994)

	ILD	ILD/combined tree length	*p* value
cox1/*cob*	70	0.020	0.01
cox1/*rrnL*	185	0.051	0.01
cob/*rrnL*	137	0.046	0.01
cox1/*cob*/*rrnL*	277	0.054	0.01
H3/28S	13	0.027	0.49
cox1/remaining markers	108	0.019	0.01
cob/remaining markers	82	0.014	0.01
rrnL/remaining markers	230	0.040	0.01
H3/remaining markers	46	0.008	0.13
18S/remaining markers	15	0.003	0.99
Mt/Nuclear	61	0.011	0.01
cox1/*cob*/*rrnL*/H3/18S	351	0.061	0.01

Despite the fact that the nuclear partitions combined showed a sum PBS six times lower than the mtDNA (Table 2), their PBS value was greater per number of steps (1.082 vs. 0.769). A similar trend can be observed in the number of nodes resolved. The nuclear markers resolved only ca. 2/3 of the nodes resolved by mtDNA when estimated in absolute numbers, but resolution is identical when normalized by the number of steps. PHBS was calculated for *cox1*, *cob*, H3 and *rrnL*. Agreement or conflict were identified by a mixture of positive and negative PHBS values for nodes supported by each particular partition. The sum PHBS for *cox1* was negative (−22.3), and positive for each of the other partitions. Net PHBS for H3 was higher than for *cob* and *rrnL* (81.5 vs. 16.6 and 13.4, respectively), in particular when normalized for the PBS (PHBS:PBS = 0.385) vs 0.072 for cob and 0.048 for *rrnL*.

The runs of MrBayes reached stationarity after ca. 130,000 generations, although we discarded (burn-in) 150,000 generations as a conservative estimate. We ran a second independent Bayesian analysis under the same model and conditions but starting from different random trees to investigate whether chains got trapped in suboptimals. The second analysis retrieved identical burn-in, topology and very similar *a posteriori* probability values (not shown). The Bayesian tree (Fig. 1) showed an overall similar topology to the parsimony tree, the major differences being the position of *Papuadytes shizong* (China) and *P. abditus* (Australian groundwater), both at the base of the New Guinea clade with parsimony, but at the base of the New Caledonian clade with MrBayes (although with very low support in both cases). Despite their overall similarity in topology, trees obtained with parsimony and Bayesian analyses were significantly different according to the Shimodaira-Hasegawa test (likelihood difference-Ln 58.09544, $p = 0.008$).

3.2. Papuadytes relationships

The monophyly of the genus *Papuadytes* (Fig. 1, node N, 100% posterior probability), and the monophyly of the New Guinea species (node C, 100%) were well-supported, as were most nodes near the tip of the tree. The backbone of the tree remained less strongly supported, in three cases with posterior probabilities of less than 90% (e.g. nodes H, I and K). The Australian *Papuadytes* were paraphyletic with respect to five major clades found in the other areas, confirming preliminary analyses of Balke et al. (2004). Two of these clades represent single, ecologically unusual species, the stygobiont *P. abditus* (F) and *Papuadytes australis* (H), found in pools, in the interstitial, and in the groundwater. The position of both species was not well-supported, with low bootstrap (<50%) and posterior probability (<90%) values (Fig. 1).

New Caledonian species formed a monophyletic group (node E, posterior probability 100%) with the exception of two mountain species (node I), which formed a separate clade included among Australian species. The isolated Chinese species *P. shizong* was found sister to a clade comprising the Australian *P. abditus* + most of the New Caledonian species (G), again with very low support. Among the New Guinean species (Fig. 1), the previously suggested *Papuadytes me* group (Balke, 1998) was confirmed as monophyletic (node B). The same is true for the undescribed '*Papuadytes ekari* group' (Balke, 2001 and unpublished) (node A). *Papuadytes miriae* and a species near *Papuadytes broschii*, previously not assigned to a species group, were here placed with individuals of *Papuadytes rivulus* s.l. of the *P. rivulus* group (Balke, 1998).

Morphospecies as delimited so far were not always monophyletic on the tree. *Papuadytes hintelmannae* and *P. miriae* were both included in a group of genotypes consisting mainly of individuals ascribed to *P. rivulus* sensu lato. The *P. rivulus*-group is a complex of several morphologically similar species (Balke, 1998) including some yet undescribed. The *P. rivulus* sensu lato in this study in fact refers to several new species. A cluster of very similar genotypes here referred to as "species 1" might also consist of three very similar morphospecies, differing only in size, surface sculpture (fine vs. dense punctation) and subtle differences in genital shape. In addition, there were several cases where a species represented by several exemplars was paraphyletic with respect to the sole representative of another species, e.g. *Papuadytes bimaculatus* was paraphyletic for *Papuadytes interruptus*, *P.* sp. 3 paraphyletic for *P.* sp. 2, *Papuadytes perfectus* for *P.*sp. 18a, and *P.*sp. 22 for *P.*sp.21. Paraphyly may not be entirely unexpected in an island radiation where speciation might be the result of colonization of new mountain ranges or unoccupied habitats. However, these cases require a re-examination of currently perceived morphospecies boundaries and a survey of additional individuals and populations to investigate patterns of mitochondrial and nuclear DNA variation.

3.3. Divergence time estimation

Node F, including *P. abditus* and the New Caledonian species, was set to 10.28 MY as described above. Using the GTR + I + Γ model, sequence divergence between these two clades was $23.44 \pm 4.56\%$ (for mtDNA). Assuming their split at 10.28 MY would result in a substitution rate close to the 2.3% divergence per MY frequently cited as the rate of mtDNA clocks in insects (Brower, 1994) which is in agreement with estimates in another group of adephagan beetles (Barraclough and Vogler, 2002). Age estimate confidence for node C (New Guinean species) was 7.30 ± 0.59 MY; node E (most New Caledonian species) 9.34 ± 1.59 MY, node I (for the first colonization of New Caledonia) 14.52 ± 1.02 MY, and for node N (origin of all *Papuadytes*) 23.34 ± 2.54 MY.

4. Discussion

4.1. Molecular systematics and clock calibrations

Although the gene partitions were incongruent, our analyses show that all partitions provided support on various hierarchical levels. PBS and HPBS calculations suggested that despite incongruence between partitions, data interaction is complex and a combined analysis was warranted. Nuclear partitions contributed only half as many characters as mtDNA, and had seven times fewer informative positions, but support (as measured by PBS) per character change on the tree was twice that of mtDNA. Similarly, node recovery was similar between mtDNA and nuclear partitions when normalized for the number of steps. Finally, hidden support as a proportion of the total support (HPBS/PBS), was much higher in the nuclear H3 partition compared to mtDNA. This is in agreement with general findings in insects which indicate that mtDNA is affected by patterns of substitution that produce greater

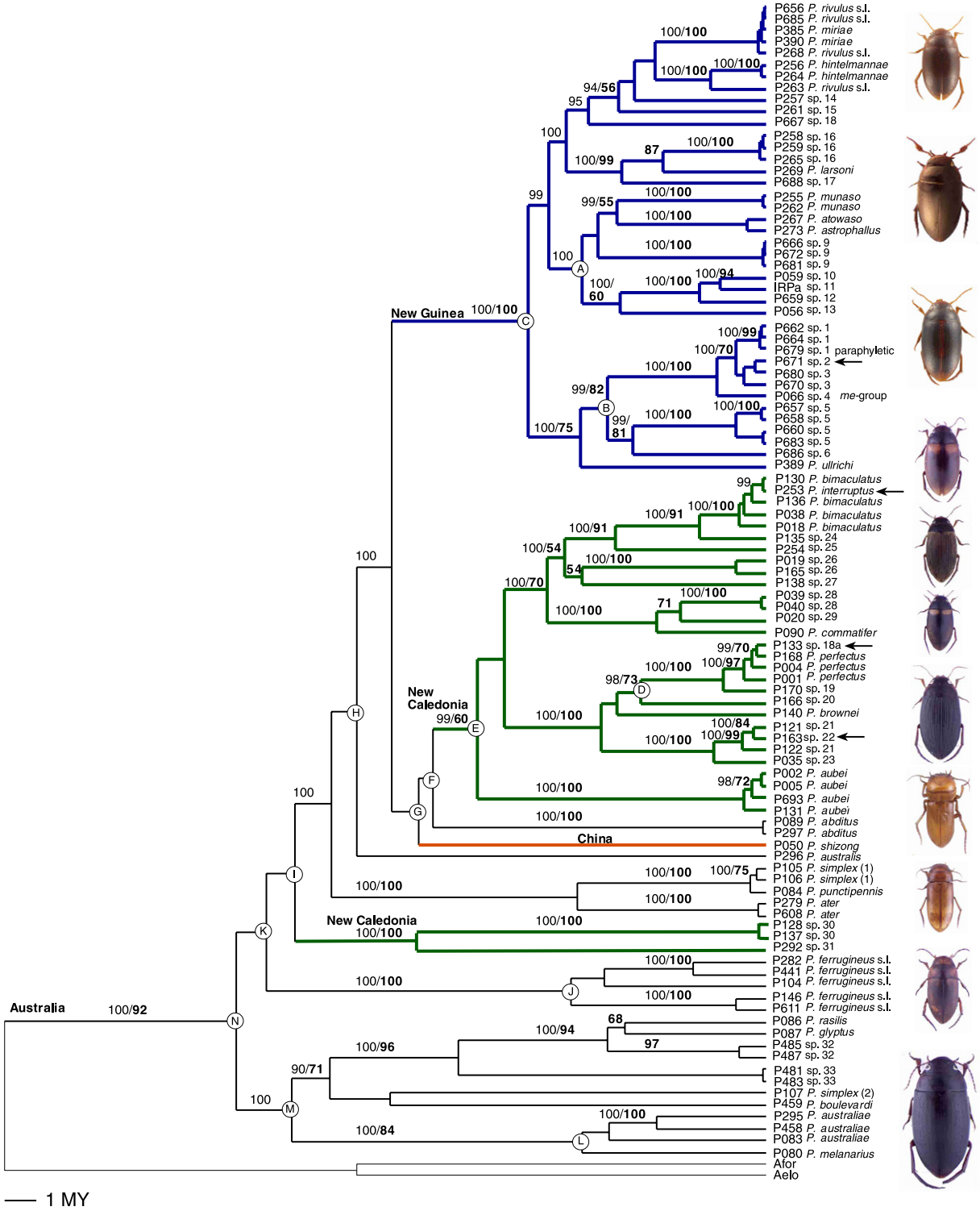

Fig. 1. Topology of phylogenetic relationships of *Papuadytes* species inferred from Bayesian analysis of mtDNA and nuclear gene regions. Bayesian branch lengths were fitted to a clock using penalized likelihood in r8s. Numbers above nodes are posterior probabilities. Arrows indicate cases of species paraphyly. Habitus illustrations are, from top to bottom: *P. marinae*, *P. bagus*, *P. vladimiri* (not included in the analyses, but shown here to illustrate representatives of closely related New Guinean species), *P. bimaculatus*, *P. perfectus*, *P.* sp. 30, *P.* aubei, *P. abditus*. *P. australis*, *P.* sp. 31, *P. commatifer*. Outgroups pruned except for *Aglymbus* cf *formosulus* ("Afor"), *Aglymbus elongatus* ("Aelo").

levels of homoplasy and hence are less useful for resolving deeper nodes (Danforth et al., 2005; Lin and Danforth, 2004) Our findings suggest that nuclear markers will be pivotal to solve the problem of the deeper nodes which remained poorly supported. Questions about the topology especially concerned the placement of a few species showing distinct characteristics, which are divergent from all others either in their morphology, ecology, geographic dis-

tribution or a combination of them (*P. abditus, P. australis, P. shizong*). Despite these uncertainties, we have been able to identify major lineages within *Papuadytes*, allowing for a more focused future sampling effort.

Our time estimate for the diversification of the genus is in contrast to Balke et al. (2004), where the origin of *Papuadytes* was estimated as at least 60 MYA. We have reanalyzed our data and found that our estimates were erroneously multiplied by a factor of 2, so that the actual age of *Papuadytes* should have been given as 30 MY. Differences between that and our current estimate of c. 24 MY may be due to several factors such as species sampling, estimation of parameters of the GTR + I + Γ model, the method used to produce clock-like branch lengths, or the absolute age used to calibrate the tree (invasion of *Papuadytes* into Australian groundwater). Since the sampling of the ingroup is here more dense (99 terminals vs. 24), with fewer and more closely related outgroups, and we use a more accurate method (PL vs. NPRS, which is known to introduce some deformations, Barraclough and Vogler, 2002), we believe our new estimate to be more reliable.

The finding of paraphyletic morphospecies indicates that current morphological species delineation might need to be revised in some cases for a re-evaluation of species limits. Incongruence between DNA based and morphologically defined species is a well-documented phenomenon (Funk and Omland, 2003), and has already been shown to be irreconcilable in a detailed study of the closely related (Balke et al., 2004) genus *Copelatus* in Fiji (Monaghan et al., 2006). This radiation of some 30 morphologically recognized species showed broad incongruence of mtDNA and morphological species characters presumably due to gene flow between partially separated populations, mainly within the larger islands of the archipelago but occasionally between islands (Monaghan et al., 2006). The current findings suggest that recent speciation events and a complex history of population separation and confluence, as is often seen in island radiations such as the Canaries and Hawaii (Emerson and Oromi, 2005; Gillespie and Roderick, 2002), could equally have affected the radiation of *Papuadytes* in New Guinea. It will be of great interest to test species limits in *Papuadytes* in greater detail, examining the mode of speciation in New Guinea's mountain and foothill ranges which are densely packed with locally endemic species (Balke, 1998). Their aggregate altitudinal range is from ca. 100 to 2600 m, whereby local endemics have apparently evolved in situ and separation by mountain ranges could act in a similar way as the island environment of Fijian *Copelatus*. The group therefore provides an exciting model system for detailed investigations of the factors leading to lineage diversification in New Guinea generally.

4.2. Evolution of Papuadytes

The present analysis includes *Papuadytes* species from all major morphological species groups and geographical regions with the only exception of Hawaii (Balke, 1998; Shaverdo et al., 2005). Basal lineages were found in Australia and paraphyletic for all others, indicating that dispersal and successful colonization proceeded unidirectionally out of Australia into Oceania and in one case to China (Fig. 2A). The monophyly of deep clades confined to New Guinea and New Caledonia further suggests that these colonization events were rare and have occurred early in the clade's history. Their distributional pattern and clade age is in agreement with the assumed geological ages of the areas (Hall, 1998, 2001): the basal groups occur in the oldest landmass, Australia, followed by New Caledonian species, and finally by the lineages on an even younger New Guinea. While this suggests that these areas were colonized early in their existence, this is different for New Zealand where the Australian *P. australis* has also been recorded. This group may represent a complex of unrecognised species, but in any case the separation from Australian lineages is much more recent.

Papuadytes is absent from Fiji (Fig. 2D) where genus *Copelatus* has radiated extensively (Monaghan et al., 2006; Wewalka and Balke, unpublished) (Fig. 2C), partly occupying the same habitats as *Papuadytes* in New Guinea or New Caledonia. *Copelatus* is absent in New Caledonia, and only a few species are found in New Guinea and Australia where they typically occur in small ponds, unlike *Papuadytes* which are confined mainly to running water. These observations agree with one of Gressitt's (1982) major themes in Oceanian biogeography, i.e. that rare arrivers gave rise to faunas unique to particular island groups.

The New Caledonian fauna is composed of two independent clades with sister groups in Australia in each case. The only two representatives of the deeper clade occur on high altitudes from c. 700 to 1400 m, and one of them (*P.* sp.31, individual 292) represents one of the morphologically and ecologically most derived species of Copelatinae (Fig. 1). The head is relatively large with small eyes, and the non-functional wings are strongly reduced in size. These beetles were only collected on Mt. Panie, hidden under stones in otherwise dry beds of first order streams, a highly ephemeral habitat where small puddles only form after extended periods of rainfall. They were absent from a nearby stream pool where *Papuadytes commatifer* was abundant, and were also absent from small waterholes on tracks. This could be interpreted as a relictual species pushed to marginal habitats by subsequent arrivers, in agreement with the "taxon cycle" hypothesis of Wilson (1961).

We obtained DNA sequence data for at least 20 out of a total of about 30 morphospecies currently identified in New Caledonia. The total number of species could still increase, as *P. bimaculatus*, *P. perfectus* and morphospecies *P.* sp. 21 and *P.* sp. 22 may represent species complexes (Wewalka and Balke, unpublished). This considerable diversity might be explained by the combination of isolated mountain ranges (with strong altitudinal gradients), diversity of climates (seasonal and arid to tropical), and the relative stability of the species' habitats, thus decreasing the need for frequent dispersal. Murienne et al. (2005) have shown for a

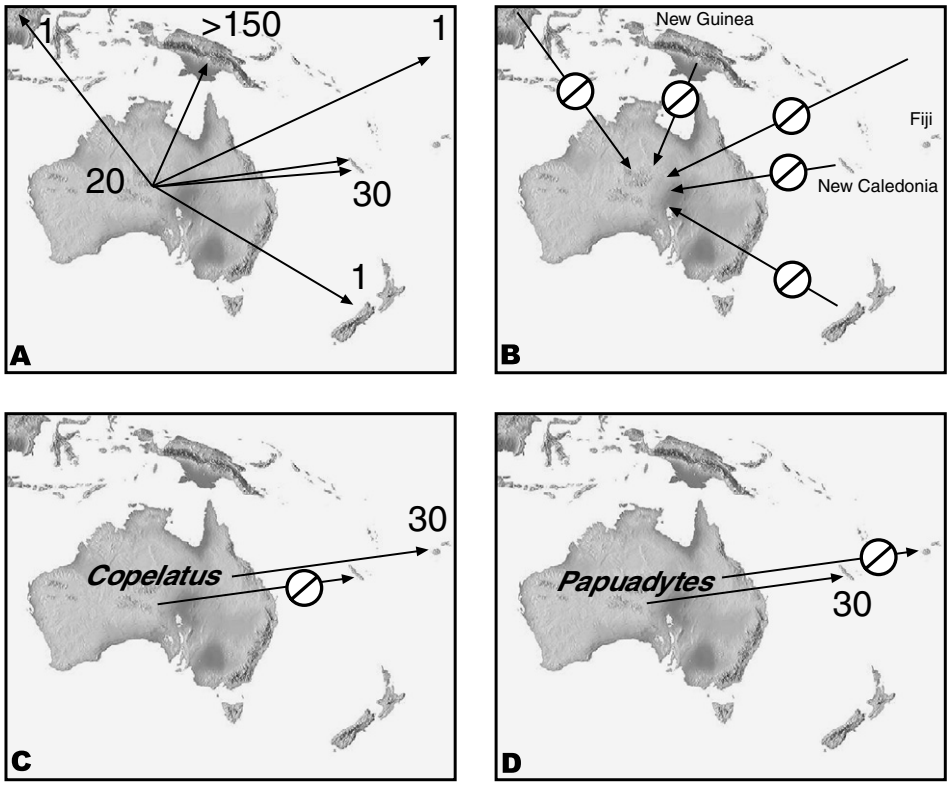

Fig. 2. Overall geographical patterns and processes in *Papuadytes* and Australo-Oceanian *Copelatus* (dispersal of *Copelatus* out of the Australian region simplified). Explanation of (A)–(D), refer to Section 4.

group of New Caledonian crickets that what used to be considered an ancient fauna is in fact a comparably recent radiation of less than 2 MY in age. This is similar to our findings, and while the New Caledonian *Papuadytes* are structurally and ecologically rather diverse, comprising 'relictual' species (at node I), they did possibly not occur before the mid Miocene.

The most remarkable island radiation of *Papuadytes* is in New Guinea, which resulted from a single colonization of an Australian lineage perhaps only c. 7 MYA, and led to a radiation of probably more than 150 species (Balke, 1998; unpublished). New Guinea and Australia are geographically close, and at times of lowered sea levels during the ice ages were connected by a broad land bridge (e.g. Gressitt, 1982; Balke, 1995). Yet, the phylogenetic evidence favors a single colonization event from Australia to New Guinea, and no dispersal back into Australia despite the great abundance of *Papuadytes* in New Guinea. Suitable habitat exists in Northern Queensland and along the Eastern Coast, but is occupied by the ancestrally Australian lineages of *Papuadytes* (Fig. 2B).

New Guinea is a jigsaw puzzle of geological elements (terranes) with a complex geological past (Gressitt, 1982; Pigram and Davies, 1987; Hall and Holloway, 1998). Uplift is recent, and highlands emerged only c. 5–10 MYA. Terrane accretion mainly occurred during the Oligocene and Miocene between 5 and 30 MYA (Michaux, 1994), but lowlands of present day New Guinea did not emerge until the late Pliocene (Hall, 1998, 2001). Biogeographers have mainly assumed that the fauna is a composite of lineages which had independent origins on drifting, isolated terranes (e.g. Heads, 2002). However, phylogenetic tests of this hypothesis to explain present-day distributional patterns and diversity in New Guinea remain scarce. Our work suggests that despite the high diversity of endemic *Papuadytes* in New Guinea, the group did not occur there prior to the late Miocene, i.e. too recent for major tectonic events to explain extant patterns (Hall, 1998, 2001). Local endemics have apparently evolved in situ and should not be older than the present-day landmasses, as confirmed by our present age estimation.

Similar patterns of recent diversification have been suggested for Australasian *Anopheles* mosquitoes (Foley et al., 1998; Beebe and Cooper, 2002). Climatic and geological change in a young, unstable area would have promoted rapid diversification, in what could be considered a "cradle of diversity" (Bermingham and Dick, 2001). Some of the basal *Papuadytes* (e.g. node L) inhabit pools and ditches. In contrast, some Australian and most New Caledonian *Papuadytes* occur in running water habitats. We interpret this habitat association as a prerequisite for a successful exploitation of the new opportunities forming with the emergence of New Guinea. New Guinean *Papuadytes* are a morphologically comparably homogenous group of dark brown beetles (Fig. 1), and major differences are in body length (3.4–6.4 mm) and elytral surface sculpture (punctation sparse and fine to dense and coarse), male genital structure, and secondary sexual characters of the male antennae and protarsi (Balke, 1998). Structural diversity

is larger in New Caledonian and Australian species, which exhibit stronger variation in body size and shape (3.4–10 mm), coloration (yellow, orange, brown with pale markings to black) and surface structure (smooth, with deep cuts, with longitudinal lines on elytron, various patterns of punctation) (Fig. 1). The Australian *P. australis* has reduced eyes and pigmentation, occurring in temporal ponds and in the groundwater, while the stygobiont *P. abditus* is blind and wingless, with strongly modified body contours (Fig. 1), providing unambiguous examples of how morphology reflects colonization of new habitats.

In conclusion, we show that colonization of New Guinea and New Caledonia was unidirectional and goes back no further than the mid or late Miocene. Lineages underwent rapid diversification in particular in New Guinea, possibly favoured by a wide range of habitats available and the complex topography of the island. Our results are in agreement with a growing body of phylogenetic literature (e.g. Fuller et al., 2005; de Queiroz, 2005; Waters and Craw, 2006) suggesting that, although rare, long-distance dispersal might play a prominent role in the formation of Southern hemisphere distribution patterns.

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References

Arensburger, P., Buckley, T.R., Simon, C., Moulds, M., Holsinger, K.E., 2004. Biogeography and phylogeny of the New Zealand cicada genera (Hemiptera: Cicadidae) based on nuclear and mitochondrial DNA data. J. Biogeogr. 31, 557–569.

Austin, A.D., Yeates, D.K., Cassis, G., Fletcher, M.F., La Salle, J., Lawrence, J.F., McQuillan, P.B., Mound, L.A., Bickel, D.J., Gullan, P.J., Hales, D.F., Taylor, G.S., 2004. Insects 'Down Under': diversity, endemism and evolution of the Australian insect fauna: examples from select orders. Aust. J. Ent. 43, 216–234.

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

Baker, R.H., Wilkinson, G.S., DeSalle, R., 2001. Phylogenetic utility of different types of molecular data used to infer evolutionary relationships among stalk-eyed flies (Diopsidae). Syst. Biol. 50, 87–105.

Baldwin, B.G., Sanderson, M.J., 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). Proc. Natl. Acad. Sci. USA 95, 9402–9406.

Balke, M., 1995. The Hydroporini (Coleoptera: Dytiscidae: Hydroporinae) of New Guinea: systematics, distribution and origin of the fauna. Invertebr. Taxon. 1995, 1009–1019.

Balke, M., 1998. Revision of New Guinea *Copelatus* Erichson, 1832 (Insecta: Coleoptera: Dytiscidae): the running water species, Part I. Ann. Naturhist. Mus. Wien 100B, 301–341.

Balke, M., 2001. Die Schwimmkäfer Neu Guineas. Artenreichtum, Phylogenie, Biogeographie und Lebensweise (Coleoptera: Dytiscidae). PhD Thesis Free University Berlin. Available at <www.Dissertation.de>.

Balke, M., Ribera, I., Vogler, A.P., 2004. MtDNA phylogeny and biogeography of Copelatinae, a highly diverse group of tropical diving beetles (Dytiscidae). Mol. Phylogenet. Evol 32, 866–880.

Barraclough, T.G., Hogan, J.E., Vogler, A.P., 1999. Testing whether ecological factors promote cladogenesis in a group of tiger beetles (Coleoptera: Cicindelidae). Proc. R. Soc. Lond., B, Biol. Sci. 266, 1061–1067.

Barraclough, T.G., Vogler, A.P., 2002. Recent diversification rates in North American tiger beetles estimated from a dated mtDNA phylogenetic tree. Mol Biol. Evol 19, 1706–1716.

Beebe, N.W., Cooper, R.D., 2002. Distribution and evolution of the *Anopheles punctulatus* group (Diptera: Culicidae) in Australian and Papua New Guinea. Int. J. Parasitol. 32, 563–574.

Bermingham, E., Dick, C., 2001. The *Inga*—newcomer or museum antiquity? Science 293, 2214–2216.

Brower, A.V.Z., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. USA 91, 6491–6495.

Brundin, L., 1966. Transantarctic relationships and their significance, as evidenced by chironomid midges with a monograph of the subfamilies Podonominae and Aphroteniinae and the austral Heptagyiae. Kungl. Svenska Vetenskapsakademiens Handlingar 11, 1–472.

Cracraft, J., 2001. Avian evolution, Gondwana biogeography and the Cretaceous-Tertiary mass extinction event. Proc. R. Soc. Lond., B, Biol. Sci. 268, 459–469.

Danforth, B.N., Lin, C.P., Fang, J., 2005. How do insect nuclear ribosomal genes compare to protein-coding genes in phylogenetic utility and DNA substitution patterns? Syst. Ent. 30, 549–562.

De Jong, R., 2003. Are there butterflies with Gondwanan ancestry in the Australian region? Inv. Syst. 17, 143–156.

de Queiroz, A., 2005. The resurrection of oceanic dispersal in historical biogeography. Trends Ecol. Evol. 20, 68–73.

Emerson, B.C., Oromi, P., 2005. Diversification of the forest beetle genus *Tarphius* in the Canary Islands, and the evolutionary origins of island endemics. Evol. Int. J. Org. Evol. 59, 586–598.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Filardi, C.E., Moyle, R.G., 2005. Single origin of a pan-Pacific bird group and upstream colonization of Australasia. Nature 438, 216–219.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Foley, D.H., Bryan, J.H., Yeates, D., Saul, A., 1998. Evolution and systematics of Anopheles: Insights from a molecular phylogeny of Australasian mosquitoes. Mol. Phylogenet. Evol. 9, 262–275.

Fuller, S., Schwarz, M., Tierney, S., 2005. Phylogenetics of the allodapine bee genus *Braunsapis*: historical biogeography and long-range dispersal over water. J. Biogeogr. 32, 2135–2144.

Funk, D.J., Omland, K.E., 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Syst. 34, 397–423.

Gatesy, J., O'Grady, P., Baker, R.H., 1999. Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl laxa. Cladistics 15, 271–313.

Gillespie, R.G., Roderick, G.K., 2002. Arthropods on islands: evolution and conservation. Annu. Rev. Entomol. 47, 595–632.

Gressitt, J.L., 1982 (ed). Biogeography and ecology of New Guinea. Vol. 1, 2.- Junk, The Hague, vii + 983 pp.

Hall, R., 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Hall, R., Holloway, J.D. (Eds.), Biogeography and geological evolution of SE Asia. Backhuys, Leiden, pp. 99–131.

Hall, R., 2001. Cenozoic reconstructions of SE Asia and the SW Pacific: changing patterns of land and sea. In: Metcalfe, I., Smith, J.M.B., Morwood, M., Davidson, I.D. (Eds.), Faunal and Floral Migrations and Evolution in SE Asia–Australasia. A.A. Balkema (Swets and Zeitlinger Publishers) Lisse, pp. 35–56.

Hall, R., Holloway, J.D. (Eds.), 1998. Biogeography and geological evolution of SE Asia. Backhuys, Leiden, p. 417.

Heads, M., 2002. Birds of paradise, vicariance biogeography and terrane tectonics in New Guinea. J. Biog. 29, 261–283.

Heads, M., 2005. Dating molecular phylogenies: a critique of molecular biogeography. Cladistics 21, 62–78.

Higgins, D.G., Thompson, J.D., Gibson, T.J., 1996. Using CLUSTAL for multiple sequence alignments. Method Enzymol. 266, 383–402.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Leys, R., Cooper, S.J.B., Watts, C.H.S., Humphreys, W.F., 2003. Evolution of subterranean diving beetles (Coleoptera, Dytiscidae, Hydroporini, Bidessini) in the arid zone of Australia. Evolution 57, 2819–2834.

Lin, C.P., Danforth, B.N., 2004. How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined data sets. Mol. Phylogenet. Evol. 30, 686–702.

Lowry, P.P. II, 1998, Diversity, Endemism, and Extinction in the Flora of New Caledonia: a Review. Pp. 181–206 In: Peng, C.-I., Lowry, P.P. II (eds), Rare, threatened, and endangered floras of the Pacific Rim. Institute of Botany, Academica Sinica, Monogr. Ser. No. 16, Taipei.

Michaux, B., 1994. Land movements and animal distributions in east Wallacea (eastern Indonesia, Papua New Guinea and Melanesia). Palaeogeogr. Palaeocl. 112, 323–343.

Mickevich, M.F., Farris, J.S., 1981. Methods for investigating taxonomic congruence and their application to the Leptopodomorpha. Syst. Zool. 30, 331–351.

Miller, K.B., 2001. On the phylogeny of the Dytiscidae (Insecta: Coleoptera) with emphasis on the morphology of the female reproductive system. Insect Syst. Evol. 32, 45–92.

Mittermeier, R.A., Myers, N., Thomsen, J.B., da Fonseca, G.A.B., Olivieri, S., 1998. Biodiversity hotspots and major tropical wilderness areas: approaches to setting conservation priorities. Cons. Biol. 12, 516–520.

Mittermeier, R.A., Mittermeier, C.G., Brooks, T.M., Pilgrim, J.D., Konstant, W.R., da Fonseca, G.A.B., Kormos, C., 2003. Wilderness and biodiversity conservation. Proc. Natl. Acad. Sci. USA 100, 10309–10313.

Monaghan, M.T., Balke, M., Pons, J., Vogler, A.P., 2006. Beyond barcodes. Complex DNA taxonomy of a South Pacific Island radiation. Proc. R. Soc. Lond., B Biol. Sci. 273, 887–893.

Murienne, J., Grandcolas, P., Piulachs, M.D., Bellés, X., D'Haese, C., Legendre, F., Pellens, R., Guilbert, E., 2005. Evolution on a shaky piece of Gondwana: is local endemism recent in New Caledonia? Cladistics 21, 2–7.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Otto, S., Cummings, M., Wakeley, J., 1996. Inferring phylogenies from DNA sequence data: the effects of sampling. In: Harvey, P.H. (Ed.), New Uses for New Phylogenies. Oxford University Press, pp. 103–115.

Pigram, C.J., Davies, P.J., 1987. Terranes and the accretion history in the New Guinea orogen. BMR J. Aust. Geol. Geop. 10, 193–212.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Remsen, J., O'Grady, P., 2002. Phylogeny of Drosophilinae (Diptera: Drosophilidae), with comments on combined analysis and character support. Mol. Phylogenet. Evol 24, 249–264.

Ribera, I., Hernando, C., Aguilera, P., 2001. *Agabus alexandrae* n.sp. from Morocco, with a molecular phylogeny of the western Mediterranean species of the *A. guttatus* group (Coleoptera: Dytiscidae). Ins. Syst. Evol. 32, 253–262.

Ribera, I., Hogan, J.H., Vogler, A.P., 2002a. Phylogeny of Hydradephagan water beetles Inferred from 18S rDNA sequences. Mol. Phylogenet. Evol 23, 43–62.

Ribera, I., Beutel, R.G., Balke, M., Vogler, A.P., 2002b. Discovery of Aspidytidae, a new family of aquatic Coleoptera. Proc. R. Soc. Lond., B Biol. Sci. 269, 2351–2356.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19, 101–109.

Sanmartín, I., Ronquist, F., 2004. Southern Hemisphere biogeography inferred by event-based models: plant vs. animal patterns. Syst. Biol. 53, 216–243.

Shaverdo, H., Sagata, K., Balke, M., 2005. Five new species of *Papuadtyes* Balke, 1998 from New Guinea. Aquat. Insects 27, 269–280.

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

Simmons, M.P., 2004. Independence of alignment and tree search. Mol. Phylogenet. Evol. 31, 874–879.

Sorenson, M.D., 1996. TreeRot. University of Michigan, Ann Arbor.

Swenson, U., Backlund, A., McLoughlin, S., Hill, R.S., 2001. *Nothofagus* biogeography revisited with special emphasis on the enigmatic distribution of subgenus Brassospora in New Caledonia. Cladistics 17, 28–47.

Swofford D.L., 2002. PAUP*. Phylogenetic Analysis using Parsimony (* and other methods), Version 4.0b10. Sinauer Associates, Sunderland, MA.

Wanntorp, L., Wanntorp, H.-E., 2003. The biogeography of *Gunnera* L.: vicariance and dispersal. J. Biogeogr. 30, 979–987.

Waters, J.M., Craw, D., 2006. Goodbye Gondwana? New Zealand biogeography, geology, and the problem of circularity. Syst. Biol. 55, 351–356.

Watts, C.H.S., 1978. A revision of Australian Dytiscidae (Coleoptera). Aust. J. Zool., Suppl. Ser. 57, 1–166.

Wheeler, W.C., 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. Syst. Biol. 44, 321–331.

Wilson, E.O., 1961. The nature of the taxon cycle in the Melanesian ant fauna. Am. Nat. 95 (882), 169–193.