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A relict in New Caledonia: phylogenetic relationships of the family Troglosironidae (Opiliones: Cyphophthalmi)

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Abstract

The species richness and endemism of New Caledonia are traditionally held to result from the main island's Gondwanan origin and progressive diversification subsequent to extended isolation. Recent studies have challenged this hypothesis, promoting a scenario of recent origins and diversifications of New Caledonian arthropod groups. In the present study, the phylogeny of the endemic harvestman family Troglosironidae (Opiliones: Cyphophthalmi) is investigated using DNA sequence data from two nuclear ribosomal genes (18S rRNA and 28S rRNA) and two mitochondrial genes (the protein-coding cytochrome *c* oxidase subunit I and the ribosomal 16S rRNA). Phylogenetic analyses support the monophyly of Troglosironidae and a scenario of an ancient (> 200 Ma) origin of the family, with subsequent diversification of extant lineages in the Eocene. These results corroborate the relictual nature of taxa among New Caledonia's biota while being consistent with diversification in accordance with geological events in the Eocene.

A number of evolutionary studies have been directed at the southwestern Pacific islands, which by virtue of their heterogeneity have greatly contributed to the development of island biogeography theory (Wagner and Funk, 1995; Fleischer et al., 1998; Gillespie and Roderick, 2002). Within this region, New Caledonia has received comparatively less attention than its neighbours, such as Australia and New Zealand (Wagner and Funk, 1995; Fleischer et al., 1998). New Caledonia is a biodiversity hotspot, harbouring extraordinary levels of species richness and endemism and renowned for its unique biota, which comprise ecosystems found almost nowhere else in the world (Myers et al., 2000; Bouchet et al., 2002; Najt and Grandcolas, 2002), including the most basal lineage of flowering plants, the monotypic genus *Amborella* (Mathews and Donoghue, 1999; Qiu et al., 1999; Soltis et al., 2000).

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Situated on the Norfolk Ridge together with New Zealand, the New Caledonian "mainland" (Grande Terre) is a fragment of continental crust (Zealandia) that rifted away from the former supercontinent Gondwana about 65 Ma and drifted to its present position by 50 Ma, prior to prolonged isolation from Australia, Vanuatu and New Zealand (Raven, 1979; Schellart et al., 2006; Grandcolas et al., 2008; Neall and Trewick, 2008). Subsequent collision with the Loyalty Arc about 40 Ma led to the current geological configuration of the island (Schellart et al., 2006). A number of studies have proposed a total submersion of the mainland in the Paleocene, ophiolithic obduction in the Eocene, and orogenesis in the Pliocene (Paris, 1981; Brothers and Lillie, 1988; Cluzel et al., 2001; Lee et al., 2001; Schellart et al., 2006). Others have invoked adaptation to ultramafic substrata brought on by Eocene ophiolithic obduction as explanations for the uniqueness of its flora (Morat et al., 1986; Lowry, 1998). These reconstructions, in particular the hypothesis of complete submersion, are at odds with the supposed relictual

nature of some of its endemic biodiversity, and do not account for the incidence of poorly dispersing taxa on the Grande Terre. New Caledonia is known to have undergone extensive volcanism in the Cenozoic (Paris, 1981), and while it is theoretically possible that metapopulations of relictual taxa may have persisted on small, ephemeral volcanic islands, the paleogeography of these transient islands will probably never be known, making this hypothesis difficult to test (Heads, 2005).

Murienne et al. (2005) proposed that New Caledonia's biodiversity was of recent origin, demonstrating diversification of the endemic cockroach genus *Angustonicus* in the Pliocene subsequent to dispersal to the mainland from neighboring volcanic islands. The authors presented the evolutionary history of *Angustonicus* as a general model for New Caledonian biodiversity, concluding that "far from being a relict Gondwanan territory", New Caledonian biodiversity is result of recent dispersal events followed by diversification.

The merit of the Murienne et al. (2005) study lies in its demonstration of dispersal preceding speciation and endemism. Few would argue that such processes have not occurred in New Caledonia. Diversification on New Caledonia subsequent to recent dispersal has similarly been demonstrated in other arthropod groups (Balke et al., 2007a,b; Buckley et al., 2008; Murienne et al., 2008). However, the generalization of the dispersalist model (with associated diversification events in the Pliocene or later) to the entirety of New Caledonian biota is implausible. An unambiguous counterargument to this generalization would be best provided by a taxon endemic to New Caledonia with demonstrably poor dispersal ability, whose origin and diversification were both comparatively ancient. This taxon has already been reported in the literature, though its significance might have been overlooked.

Cyphophthalmi, an ancient group of harvestmen, have been frequently utilized for biogeographical studies (de Bivort and Giribet, 2004; Boyer et al., 2005, 2007a,b; Clouse and Giribet, 2007; Murienne and Giribet, 2009), due to their distribution across all major landmasses, with the exception of Antarctica, and their inability to disperse across oceanic barriers (Boyer et al., 2007b). A recent comprehensive study of cyphophthalmid phylogeny and biogeography elucidated the relationships within the suborder with some success (Boyer et al., 2007b). A salient outcome of this study was the supported relationship between the Tropical Gondwanan family Neogoveidae Shear, 1980 (currently distributed in Tropical South America, Tropical West Africa, and south-eastern USA) and Troglosironidae Shear, 1993, endemic to New Caledonia. Cladogenesis between these two families (and hence the origin of the New Caledonian group) was estimated from molecular dating methods to have taken place between 124 and 221 Myr, preceding the breakup of Gondwana as a whole, and of New Caledonia in particular. Diversification of Troglosironidae (four exemplars) was dated at only 28–49 Myr.

The limited sampling of Troglosironidae in the previous study precluded both assessment of systematics and biogeography on the Grande Terre, and testing of hypothesized relictual evolutionary history, largely because it was unknown whether the four exemplars comprised a derived clade. To investigate the diversity and distribution of Troglosironidae, as well as to estimate dates of cladogenetic events on the New Caledonian mainland, a molecular phylogenetic study of the monotypic family Troglosironidae was undertaken. The study included 40 specimens from 11 of 13 known species within Troglosironidae, representing the full geographical range of the family.

Materials and methods

Species sampling

Specimens were collected by G. Monteith over three collecting expeditions (2000–2005), and by J. Murienne and P.S. (2007), from leaf litter from sites throughout New Caledonia using different sampling strategies. Of 13 described species of Troglosironidae (Juberthie, 1979; Shear, 1993; Sharma and Giribet, 2005, 2009), 11 species are represented in our analyses by 40 specimens. The two excluded species (*Troglosiro tillierorum* and *Troglosiro platnicki*) were unsuitable for molecular methods due to the age of available specimens. All specimens included in the study and their locality data are given in Appendix 1. Outgroup molecular sequence data representing four other Cyphophthalmi families were obtained from GenBank from specimens sequenced in our laboratory.

Molecular methods

Molecular markers consisted of two nuclear ribosomal genes—the complete 18S rRNA and a 2.2-kb fragment of 28S rRNA—and two mitochondrial genes—a 0.45-kb fragment of 16S rRNA and a 0.65-kb fragment of cytochrome *c* oxidase subunit I (henceforth COI). Total DNA was extracted from single legs or whole animals using Qiagen's DNEasy tissue kit (Qiagen, Valencia, CA, USA) by incubating in lysis buffer overnight, as described by Boyer et al. (2005).

Purified genomic DNA was used as a template for PCR amplification. The complete 18S rRNA (*ca.* 1.8 kb) was amplified in overlapping fragments, using primer pairs 1F–5R, 3F–18Sbi, and 18Sa2.0–9R (Giribet et al., 1996; Whiting et al., 1997). The fragment of 28S rRNA was amplified using primer sets 28S

D1F–28Srd4b (Schwendinger and Giribet, 2005; Whiting et al., 1997), 28Sa–28Srd5b (Schwendinger and Giribet, 2005; Whiting et al., 1997) and 28Srd4.8a–28Srd7b1 (Schwendinger and Giribet, 2005). The fragments of 16S rRNA and COI were amplified using primers sets 16Sa–16Sb (Xiong and Kocher, 1991; Edgecombe et al., 2002) and LCO–HCOoutout (Folmer et al., 1994; Prendini et al., 2005), respectively.

Polymerase chain reactions (PCR) (50 μL) consisted of 4 μL of template DNA, 1 μM of each primer, 200 μM of deoxynucleotide triphosphates (dNTPs; Invitrogen, Carlsbad, CA, USA), 1 × PCR buffer containing 1.5 mM $MgCl_2$ (Applied Biosystems, Branchburg, NJ, USA) and 1.25 units of AmpliTaq DNA polymerase (Applied Biosystems). The PCR reactions were carried out using a GeneAmp PCR System 9700 thermal cycler, and were comprised of an initial denaturation step (5 min at 95 °C), followed by 35 cycles including denaturation at 95 °C for 30 s, annealing at 49 °C for 30 s, and extension at 72 °C for 60 s, with a final extension step at 72 °C for 10 min.

Double-stranded PCR products were visualized by agarose gel electrophoresis (1% agarose) and purified using Qiagen QiaQuick spin columns. The purified PCR products were sequenced directly; each sequence reaction contained a total volume of 10 μL consisting of 3 μL of PCR product, 1 μM of one of the PCR primer pairs, 0.5 μL of ABI BigDye 55 sequencing buffer, and 1 μL of ABI BigDye Terminator ver. 3.0 (Applied Biosystems). The sequence reactions, performed using the thermal cycler described above, involved an initial denaturation step for 3 min at 95 °C, and 25 cycles (95 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min). The BigDye-labelled PCR products were cleaned with AGTC gel filtration cartridges or plates (Edge BioSystems, Gaithersburg, MD, USA). The sequence reaction products were then analysed using an ABI Prism 3730 genetic analyser.

Chromatograms obtained from the automatic sequencer were read and assembled sequences assembled using the sequence editing software Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequence data were edited in MAC GDE 2.2 (Linton, 2005). All new sequences have been deposited in GenBank under accession numbers EU887036–EU887130.

Due to the lack of within-species variability in the nuclear ribosomal genes, these markers were sequenced for one to four exemplars per species in those represented by multiple individuals.

Phylogenetic analysis

Data analyses were based on a direct optimization approach (dynamic homology) using parsimony as the optimality criterion (Wheeler, 1996). DNA sequence data were analysed in the computer package POY ver. 4, build 2635 (Varón et al., 2007). Sequence data of the coding gene COI were treated as pre-aligned (static homology); other data partitions were not. Each gene was analysed independently and in combination with all other molecular data. Tree searches were conducted by a combination of random addition sequences with subtree pruning and grafting and tree bisection and reconnection branch swapping, followed by multiple rounds of tree fusing (Goloboff, 1999, 2002) on 24 CPUs of a cluster at Harvard University (portal.cgr.harvard.edu). A sample script is provided in Appendix 2.

A parameter space of two variables (indel/transversion ratio and transversion/transition ratio) was explored, for a total of 12 parameter sets analysed per partition. We undertook a sensitivity analysis of the 12 parameter sets, varying the relative contributions of insertion–deletion and base substitution events (Wheeler, 1995), and used the incongruence length difference (ILD) test for selecting a favoured parameter set, designated 121 (gap/change = 1; transversion/transition = 2), that minimized the overall ILD value for all data partitions.

Nodal support was estimated via jackknifing (250 replicates) with a probability of deletion of e^{-1} (Farris et al., 1996; Farris, 1997). The data were analysed for the optimal parameter set (121), in combination and for each independent partition (Giribet, 2003).

Estimation of divergence times

The optimal parameter set (121) was used for generating an implied alignment (Wheeler, 2003; Giribet, 2005), and the ages of several clades were estimated assuming a molecular clock, as implemented in the program r8s ver. 1.71 (Sanderson, 2006). This method requires at least one node of fixed age within the tree; other ages assigned to clades may be entered as constraints on maximum and/or minimum ages.

To improve the estimation of the date of Troglosironidae diversification following Boyer et al. (2007b), we used the early rifting of Gondwana, assigned to the clade containing *Paragovia* and *Metasiro* (165 Ma), as the fixed constraint (extant *Paragovia* and *Metasiro* are endemic to tropical west Africa and south-eastern USA, respectively). The open constraints, derived from the analyses of Boyer et al. (2007b), consisted of (i) the split between Troglosironidae and Neogoveidae (124–221 Ma); (ii) the diversification of Pettalidae (120–215 Ma), and (iii) the diversification of Neogoveidae (109–179 Ma). The diversification of Troglosironidae was set to maximum 49 Ma, in accordance with both geological data and the result of the previous study (Boyer et al., 2007b), therefore we did not aim at estimating this age, instead focusing on the diversification within the family. Using these dates, we ran a

series of analyses employing different combinations of constraints (Table 2).

Results

Overall incongruence among partitions was minimized by parameter set 121, the "optimal" parameter set for these data (Table 1). After 100 iterations of tree fusing, the analyses found 18 shortest trees of 6078 weighted steps. The strict consensus of the 18 trees found under the optimal parameter set is shown in Fig. 1. A similar analysis with twice the initial random addition sequences was conducted with a subset of these taxa (excluding terminals lacking nuclear ribosomal sequence data). The strict consensus of the two shortest trees of 6031 weighted steps is shown in Fig. 2. Both phylogenies show monophyly of Troglosironidae. The tree obtained from the reduced data set (Fig. 2) shows some geographical structure within New Caledonia, with distinct northern, central, and southern clades, but nodes are poorly supported within the southern clade. Phylogenies derived from the individual data sets 16S rRNA, COI, and nuclear ribosomal (18S rRNA + 28S rRNA) genes are shown in Figs 3–5, respectively.

Results of estimated divergence times are shown in Table 2. These indicate that the diversification of Troglosironidae lies at the geologically older end of the 28–49-Ma range derived by Boyer et al. (2007b). The split between the families Neogoveidae and Troglosironidae is consistently dated to *ca*. 221 Ma, which suggests a more ancient relationship than previously estimated. Four clearly defined clades within Troglosironidae were assessed for estimated age of diversification (Fig. 6). The estimated diversification of the northern clade (*T. aelleni* + *T. sheari*) is 27.48–28.12 Ma. The sister clade, comprising all other troglosironid lineages, was dated to 35.90–36.27 Ma. Within these lineages, the central lineage (*T. monteithi* + *T. oscitatio* + *T. ninqua*) was dated to 25.85–25.86 Ma. One of the southern lineages (*T. longifossa* + *T. urbanus* + *T. raveni* + *T. wilsoni*) was dated to 22.53 Ma. One of the more derived lineages (*T. longifossa* + *T. urbanus*), found in the southernmost part of the mainland, was dated to 6.70 Ma.

Table 1
Tree lengths for different data partitions analysed and congruence values (ILD) for the combined analysis of the four molecular loci

	18S rRNA	28S rRNA	16S rRNA	COI	Molecular	ILD
111	150	781	1112	1803	3921	0.01912
121	211	1132	1875	2757	6078	0.01694
141	332	1811	3344	4560	10239	0.01875
181	568	3151	6268	8151	18532	0.02126
211	153	885	1274	1831	4226	0.01964
221	217	1318	2160	2784	6603	0.01877
241	344	2159	3884	4640	11262	0.02086
281	592	3835	7325	8312	20564	0.02431
411	159	1054	1465	1863	4647	0.02281
421	229	1638	2524	2858	7407	0.02133
441	368	2794	4587	4768	12821	0.02371
481	640	5071	8705	8568	23610	0.02651

Discussion

Systematics of Troglosironidae

A principal objective of this study was to investigate the phylogeny of Troglosironidae, which have been poorly represented in previous data sets. Of 13 described species of Troglosironidae (Juberthie, 1979; Shear, 1993; Sharma and Giribet, 2005, 2009), 11, plus an additional putative new species, were represented in this study.

Results of this study largely coincide with different analyses of a larger data set (Boyer et al., 2007b). The family Troglosironidae is a stable, strongly supported monophyletic group with a similarly supported relationship to Neogoveidae (Figs 1 and 2). Relationships within Troglosironidae suggest cladogenesis in accordance with island geography (Fig. 6): a northern lineage [(*T. aelleni* + *T. sheari*), highlighted in red] was found sister to the central (highlighted in orange) and southern (highlighted in green and blue) lineages. One of the two southern lineages (highlighted in green) has a greater range than the other three lineages (Fig. 6), probably indicative of significant passage of time. This is corroborated by the estimated age of divergence of the two most derived species (*T. longifossa* and *T. urbanus*) within this lineage (*ca*. 6.70 Myr; Table 2).

The phylogeny accords with the morphology of Troglosironidae; males of southern species are distinguishable from northern counterparts by depressions of the opisthosomal sternites (Fig. 2). For this reason, it is expected that *T. tillierorum* (a northern species, not included in this study) would fall within the northern clade. *Troglosiro platnicki* (a southern species with a small sternal depression, not included in this study) would probably cluster with the southern lineage containing *T. juberthiei* and *T. brevifossa*. It is a curious observation that species in the southern lineage with the larger range (highlighted in green; Fig. 6) tend to have longer sternal depressions than other troglosironids (Fig. 2), though the function of these modifications is not known (Shear, 1993; Sharma and Giribet, 2005, 2009).

Cryptic speciation

A number of specimens resembling *T. juberthiei* were included in this study (labelled *T.* cf. *juberthiei*) to test

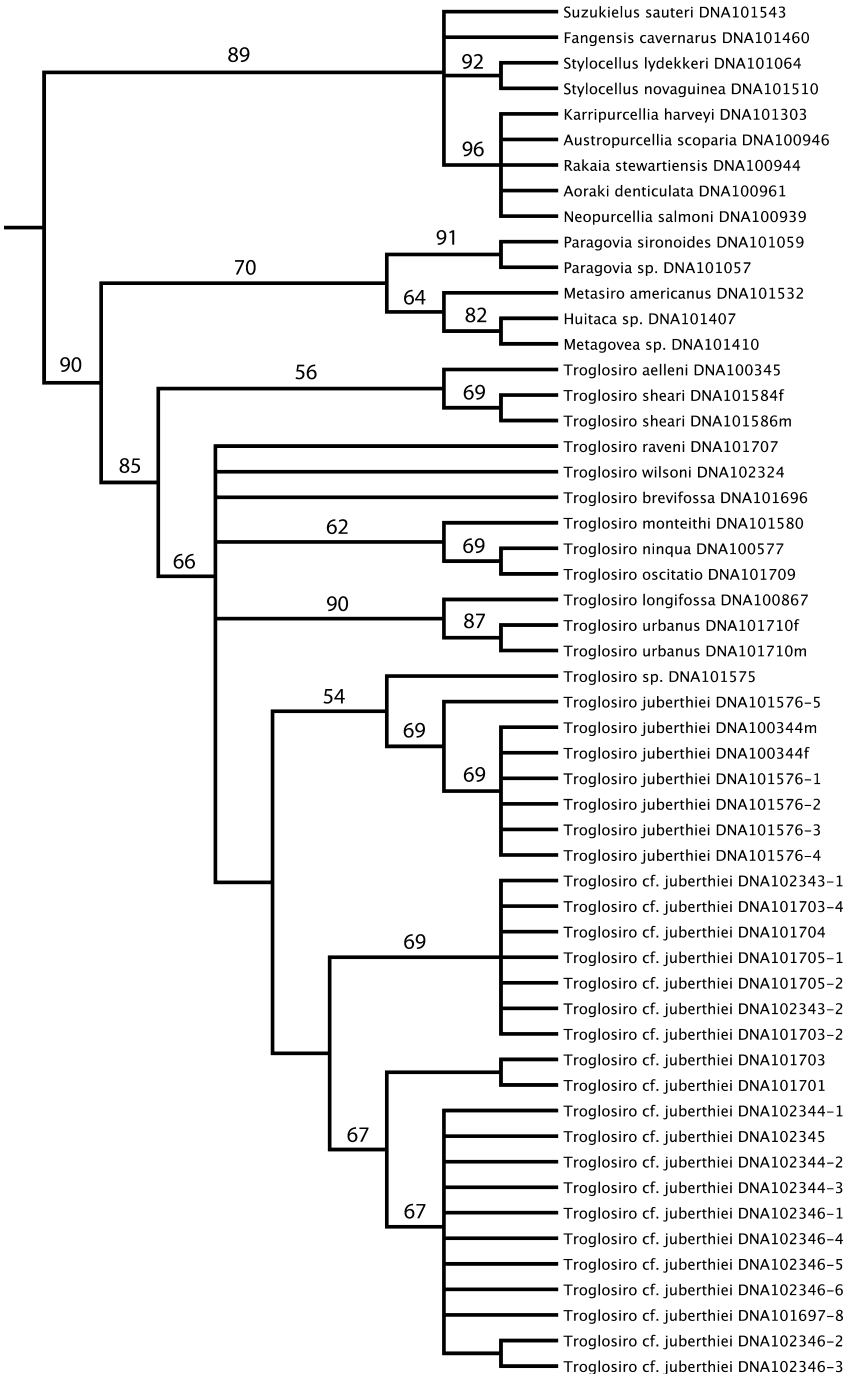

Fig. 1. Phylogenetic relationships of Troglosironidae based on the strict consensus of 18 most parsimonious trees (parameter set 121, 6078 weighted steps) of all data partitions. Numbers on branches indicate jackknife support values.

for the incidence of cryptic species. Phylogenetic placement of these specimens indicates that they comprise three closely related populations of a single lineage, found in Pic du Pin, Foret Nord, and Pic du Grand Kaori, in the south-eastern region of the Grande Terre. Moreover, molecular sequence data suggest that this lineage, while related to *T. juberthiei*, is distinct from that species, most notably by a three-bp insertion in the 16S rRNA sequence. Although without clear jackknife support, none of the markers analysed places *T.* cf. *juberthiei* with specimens from the type locality of *T. juberthiei*. For this reason, the lineages designated here as *T.* cf. *juberthiei* should probably be recognized as a new species. Detailed morphological analysis of

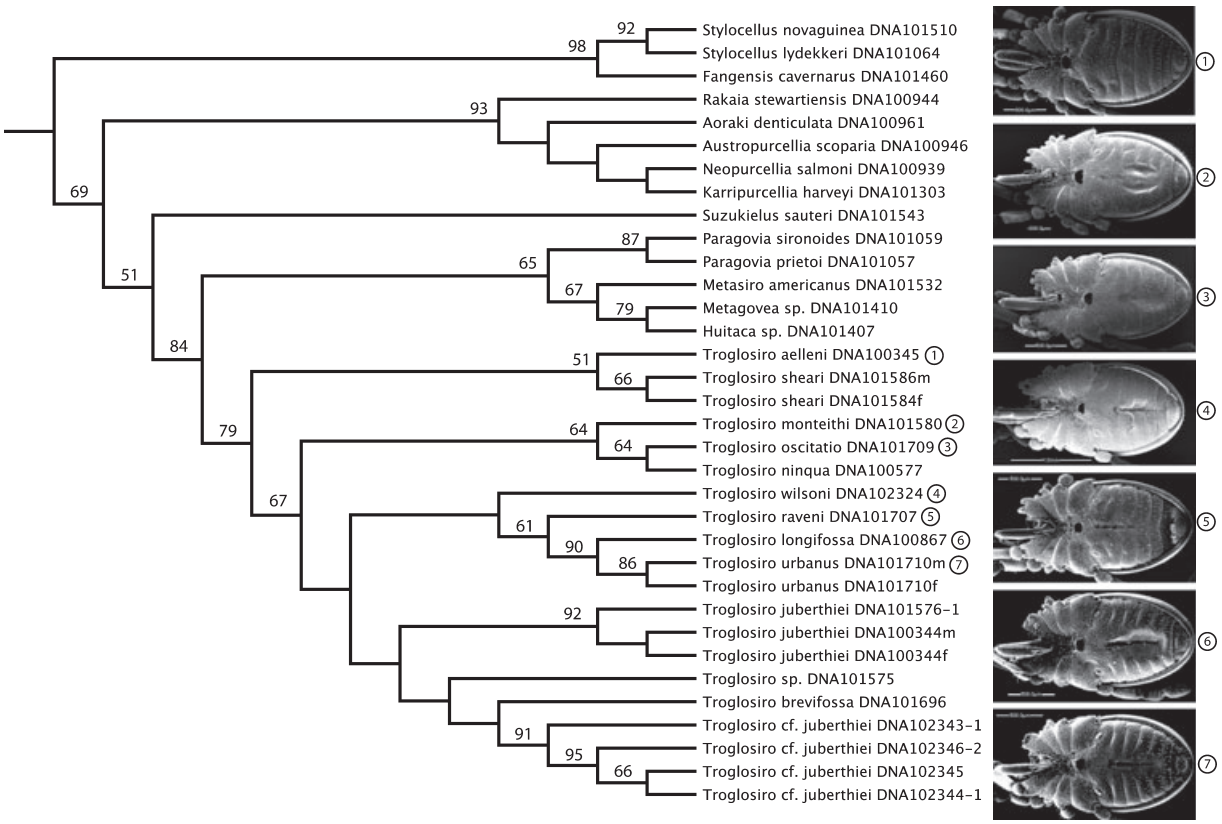

Fig. 2. Phylogenetic relationships of Troglosironidae with exclusion of specimens lacking nuclear ribosomal sequence data, based on the strict consensus of two most parsimonious trees (parameter set 121, 6031 weighted steps) of all data partitions. Numbers on branches indicate jackknife support values. Representative male specimens of particular clades are illustrated in ventral view.

multiple individuals from the different populations should be undertaken in the future.

Molecular dating

Previous estimates of both the origin (124–221 Myr) and diversification (28–49 Myr) dates of Troglosironidae obfuscated clear interpretation of troglosironid evolutionary history due to their breadth and scope (Boyer et al., 2007b). The estimated dates of diversification were particularly speculative because it was not known if the four Troglosironidae utilized in that study (*T. aelleni*, *T. juberthiei*, *T. longifossa*, *T. ninqua*) comprised a derived clade, thereby yielding an underestimate. The phylogenetic placement of *T. aelleni* in a clade sister to all other Troglosironidae (this study) validates the sampling and dating procedure utilized by Boyer et al. (2007b) for troglosironids.

Estimates of divergence times of several Cyphophthalmi lineages, namely Neogoveidae and Pettalidae, are concordant with the results of Boyer et al. (2007b), which utilized a greater sampling of Cyphophthalmi lineages outside of Troglosironidae. This suggests that the limited sampling of non-troglosironid Cyphophthalmi lineages in this study does not skew the dating analysis. Emphasis on improved sampling of Troglosironidae in the present study serves two purposes: (i) increasing the precision of estimated origin and diversification dates of Troglosironidae, and (ii) establishing a temporal dimension that complements geographical data in studying the diversification of Troglosironidae on the mainland. Moreover, the improved precision of the estimated dates confirms an expectation of the previous study; the split between Neogoveidae and Troglosironidae is ancient, occurring sometime in the Late Triassic (*ca.* 220 Ma). This result has significant implications for the biogeography of Troglosironidae, discussed below.

Biogeography

The evolutionary relationships within Troglosironidae comprise the fulcrum of the ongoing debate over the nature of New Caledonia's biota. Its origin, once thought to be of ancient Gondwanan origin, has been questioned repeatedly. A number of studies (Setoguchi et al., 1998; Murienne et al., 2005, 2008; Balke et al., 2007a,b) have demonstrated that exemplars of New

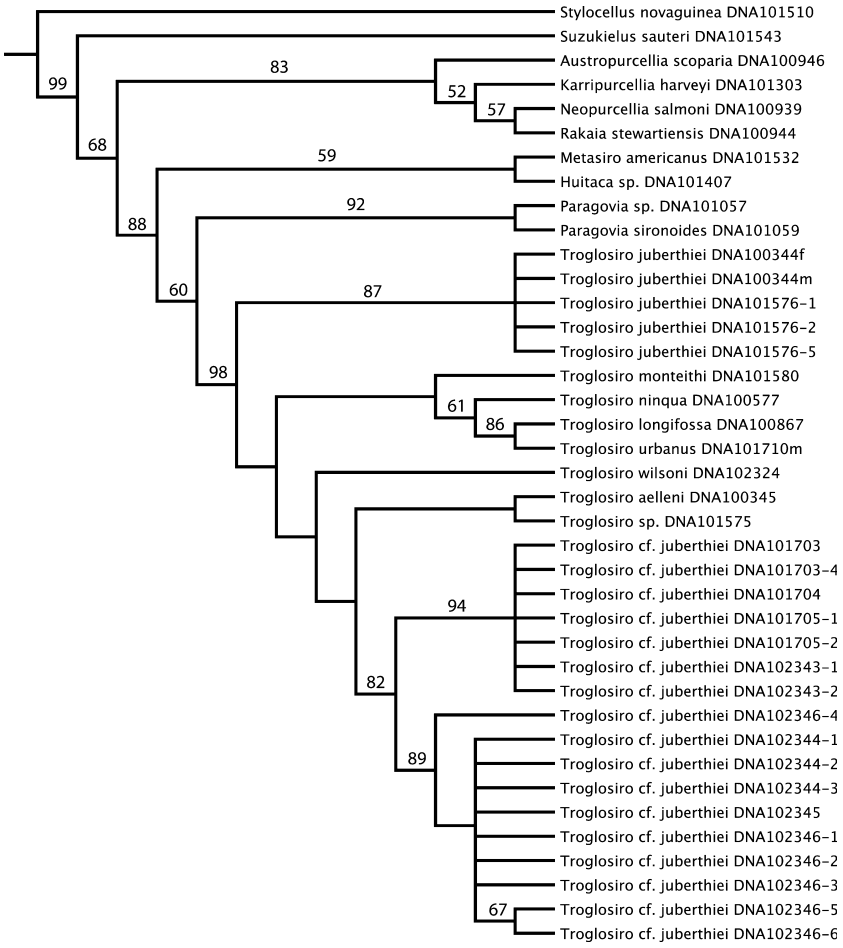

Fig. 3. Phylogenetic relationships of Troglosironidae based on the strict consensus of the single most parsimonious tree (parameter set 121, 1875 weighted steps) of the 16S rRNA data partition. Numbers on branches indicate jackknife support values.

Caledonian biota, far from being relictual and Gondwanan, have resulted from recent (*ca*. Pliocene) diversifications, preceded by dispersal to the Grande Terre.

The Troglosironidae of New Caledonia are consequently paradoxical and pivotal for three reasons. First, Cyphophthalmi are demonstrably poor dispersers; no unambiguous cases of transoceanic dispersal are known among this group (Boyer and Giribet, 2007; but see Clouse and Giribet, 2007 for a possible case), and Cyphophthalmi have never been reported on oceanic islands. Second, Neogoveidae, which are distributed in present-day Tropical Gondwana (West Africa, northern South America and the south-eastern USA), split from Troglosironidae, its sister group, *ca*. 124–221 Ma (Boyer et al., 2007b). The ancient relationship to the distant Neogoveidae, rather than the geographically closer Australian Pettalidae, Japanese Sironidae, or Southeast Asian Stylocellidae was unexpected, starkly contrasting phylogenetic relationships of typical biota of New Caledonia, which largely comprise descendants of Australian or Southeast Asian stock (Walley and Ross, 1991; Edgecombe and Giribet, in press). Third, molecular dating analyses repeatedly obtain a comparatively ancient diversification date for Troglosironidae in the Eocene. For all these reasons, a scenario of recent dispersal to New Caledonia, characteristic of other recently studied arthropod groups (Murienne et al., 2005, 2008; Balke et al., 2007a,b; but see Buckley et al., 2008, for a *ca*. 20 Ma diversification event), is unlikely.

Unlike some New Caledonian taxa that were once considered relictual but subsequently proven otherwise (e.g. Araucariaceae, *Angustonicus*; Setoguchi et al., 1998; Murienne et al., 2005; reviewed in Grandcolas et al., 2008), Troglosironidae demonstrate the hallmarks of relicts: they constitute an endemic, monophyletic group; their sister group is presently located across the expanse of the Pacific; their origin is ancient, estimated in the Late Triassic; however, their diversification occurs much later, in the Eocene. The reconstruction of troglosironid evolutionary history, particularly the long branch (*ca*. 80–170 Myr) between origin and

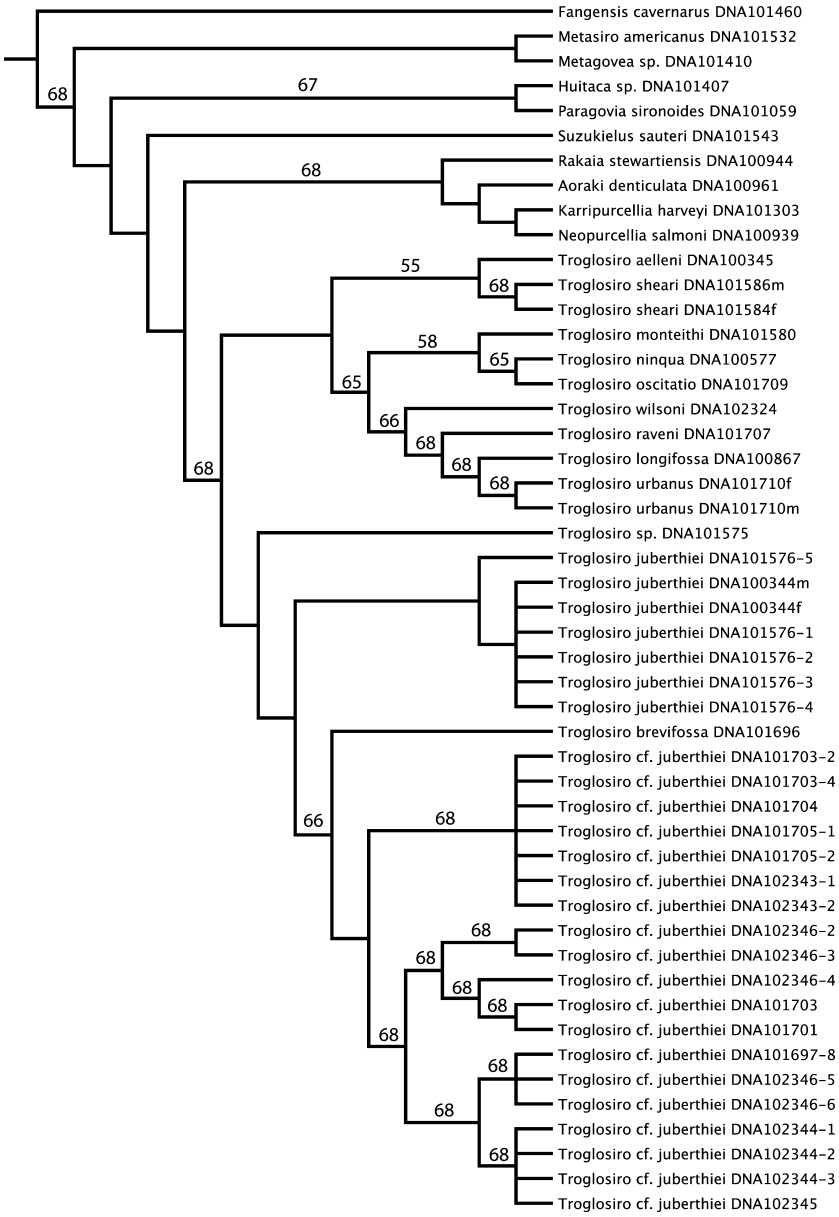

Fig. 4. Phylogenetic relationships of Troglosironidae based on the strict consensus of the single most parsimonious tree (parameter set 121, 2753 weighted steps) of the COI data partition. Numbers on branches indicate jackknife support values.

diversification, is indicative of extensive extinction events in this lineage. We discuss each of these elements in turn.

Compared with most Cyphophthalmi, the diversification of Troglosironidae (*ca.* 49 Myr) is recent; other families of Cyphophthalmi diversified well before the Cenozoic (Boyer et al., 2007b). Diversification in the Eocene has been attributed to a possible catastrophic event (Boyer et al., 2007b). Geographically, the pattern of diversification loosely follows a clinal/step-ladder process, with basal lineages in the north and derived lineages in the south. This pattern is recurrent among New Caledonian biota (Murienne et al., 2005) and has been hypothesized to result from progressive recolonization of the island with ultramafic substrate after Eocene ophiolithic obduction (Paris, 1981; Morat et al., 1986). The timing of these events is roughly contemporaneous with the diversification of Troglosironidae, estimated to the early Eocene. The estimated dates of diversification of the Recent clades within Troglosironidae similarly fit this pattern (Table 2). However, the diversification of the northern lineage is consistently estimated to be younger than the diversification of the other lineages. This may be an artefact of inadequate sampling if the northern species presently available for

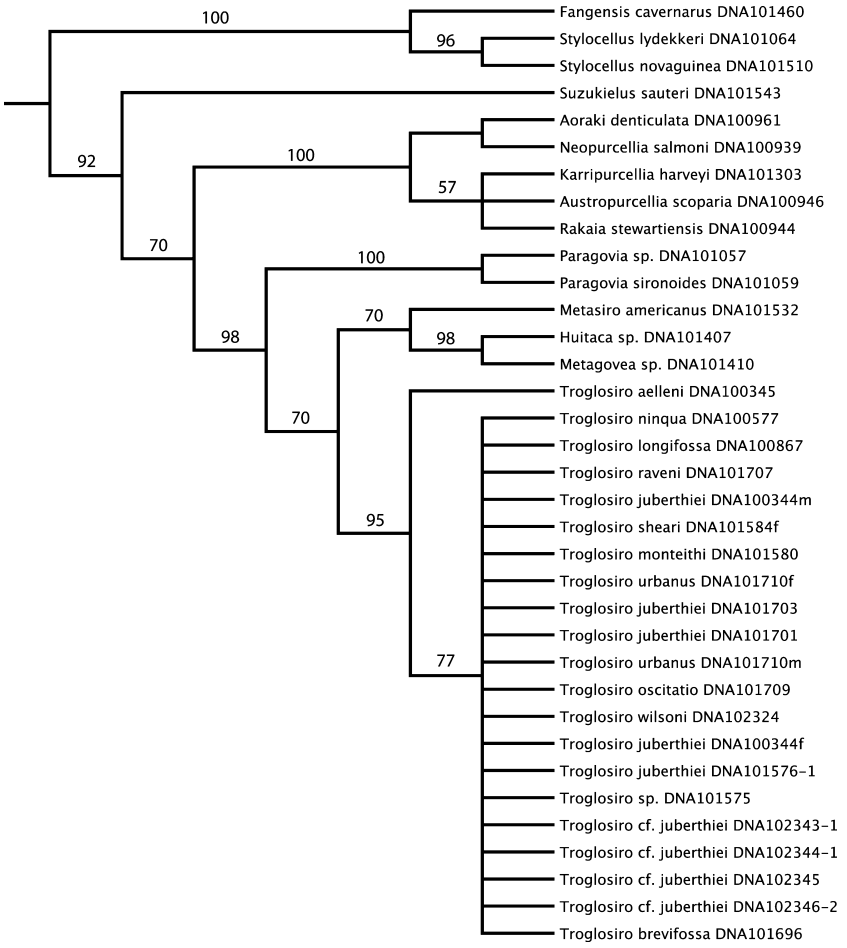

Fig. 5. Phylogenetic relationships of Troglosironidae based on the strict consensus of 75 most parsimonious trees (parameter set 121, 1349 weighted steps) of the nuclear ribosomal (18S rRNA + 28S rRNA) data partitions. Numbers on branches indicate jackknife support values.

Table 2
Estimated divergence times (Ma)

	Constraints					
	1–4	1, 2, 4	1, 3, 4	2–4	1, 4	3, 4
Pet	212.98	212.98	212.98	215.00	212.98	228.53
TroNeo	221.00	221.00	221.00	221.04	221.00	221.37
Neo	192.29	192.29	192.29	179.00	192.29	179.00
Troglo	49.00	49.00	49.00	49.00	49.00	49.00
North	28.12	28.12	28.12	28.12	28.12	28.11
Central	25.87	25.87	25.87	25.86	25.87	25.86
SG	22.53	22.53	22.53	22.53	22.53	22.53
Apex	6.70	6.70	6.70	6.70	6.70	6.70
NotN	36.27	36.27	36.27	36.26	36.27	36.26

Pet, Pettalidae; TroNeo, Troglosironidae + Neogoveidae; Neo, Neogoveidae; Tro, Troglosironidae; North, northern clade (*T. aelleni* + *T. sheari*); Central, central clade (*T. monteithi* + *T. oscitatio* + *T. ninqua*); SG, clade comprising (*T. longifossa* + *T. urbanus* + *T. raveni* + *T. wilsoni*); Apex, clade comprising (*T. longifossa* + *T. urbanus*); NotN, clade sister to northern clade. The base of the clade containing *Paragovia* and *Metasiro* was fixed at 165 Myr. Constraints are as follows: (i) 124 < TroNeo < 221; (ii) 120 < Pet < 215; (iii) 109 < Neo < 179; (iv) Tro < 49.

study represent a derived clade within the northern lineage.

The consistently retrieved relationship between Troglosironidae and the Tropical Gondwanan family Neogoveidae from multiple data sets in multiple studies (Giribet and Boyer, 2002; Boyer and Giribet, 2007; Boyer et al., 2007b) is corroborated in the present study. This result is currently the greatest challenge to the study of cyphophthalmid biogeography, upsetting the close correlation between the phylogenetic and geographical proximity characteristic of Cyphophthalmi. A relationship between New Caledonia and Tropical Gondwana has never been proposed on either biological or geological grounds. A model of diversification by vicariance is strongly supported for most Cyphophthalmi lineages (Boyer and Giribet, 2007; Giribet and Kury, 2007), but the present-day distributions of Neogoveidae and Troglosironidae are difficult to attribute to vicariance alone without postulating massive extinction. Boyer et al. (2007b) proposed a pan-Gondwanan distribution of Neogoveidae + Troglosironidae

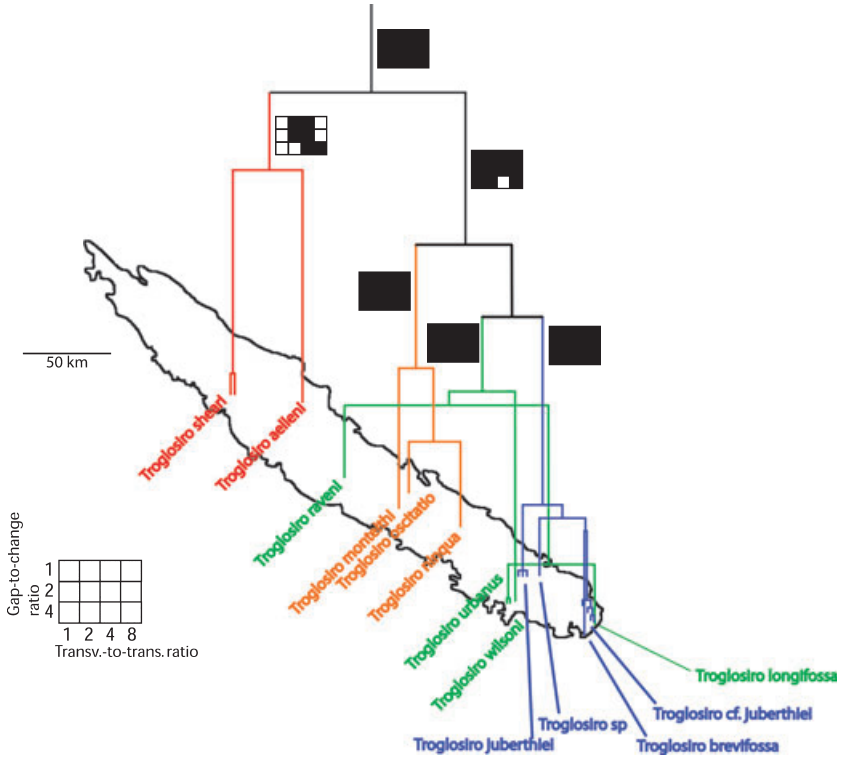

Fig. 6. Cladogram of Troglosironidae on the New Caledonian mainland, based on the strict consensus of two most parsimonious trees (parameter set 121, 6031 weighted steps). Colours correspond to monophyletic lineages. Terminal locations correspond to collecting localities. Navajo rugs on nodes indicate monophyly (black square) or non-monophyly (white square) for selected clades under explored parameter sets.

that fragmented 124–221 Ma (estimated 221–245.95 Ma in the present study), possibly preceding the break-up of Gondwana. The authors conceded that the hypothesis was disputable, given the absence of corroboratory evidence from other poorly dispersing taxa of Gondwanan origin. Apropos, a number of unrelated biota has been found to emulate this peculiar distribution, in particular linking Australia–New Zealand– Tasmania to Chile (reviewed in McCarthy et al., 2007). McCarthy (2003, 2005) identified numerous distributions linking taxa across the Pacific Ocean and some lines of geological evidence, and ultimately favored a reconstruction of Gondwana (McCarthy et al., 2007) with a closed Pacific in the Upper Triassic–Lower Jurassic. This radical reconstruction is part of the "expanding Earth" hypothesis, which proposes that the planet increased in volume from the pre-Mesozoic through the Cenozoic (McCarthy, 2005).

The evidence for McCarthy's reconstruction (McCarthy, 2003, 2005) has been extensively refuted on geological and biological grounds (Briggs, 2004, 2006; Ali, 2006). While the distribution of Troglosironidae could readily be explained by McCarthy's reconstruction of Gondwana (particularly the juxtaposition of New Caledonia and tropical South America), we reject this model as a plausible explanation for troglosironid distribution due to inadequate geological evidence.

Nevertheless, the ancient origin and limited vagility of Troglosironidae and their sister clade Neogoveidae hint at some elements of Gondwanan geography that may have been overlooked, and which require further investigation. While invoking extinction events to reconcile the distribution of Troglosironidae with a putative Gondwanan origin might seem *post hoc*, evaluating a scenario of dispersal in this taxon does little in the way of providing an alternative, discussed below.

The possibility of dispersal

A separate conundrum altogether lies in the geological evidence pointing to total submersion of the New Caledonian mainland in the Paleocene long after its isolation from potential islands in the vicinity that could have served as refugia (Paris, 1981; Brothers and Lillie, 1988; Cluzel et al., 2001; Lee et al., 2001), which would have ensured the extinction of Troglosironidae present on the island theretofore. How, then, did the troglosironids persist? One possibility is that extensive volcanism created ephemeral islands that harboured metapopulations of Troglosironidae until the reemergence and subsequent recolonization of the mainland in the Eocene (Heads, 2005)—a hypothesis that paradoxically requires some oceanic dispersal ability in the ancestors of extant Troglosironidae. Like the

pan-Gondwanan distribution invoked to explain the relationship between Neogoveidae and Troglosironidae (Boyer et al., 2007b), this hypothesis is limited in evidence. However, given the characteristics of Troglosironidae, and the Cyphophthalmi at large, it is all the more implausible to invoke a single, long-range dispersal in the Eocene to account for the diversification of Troglosironidae subsequent to the Paleocene drowning. Significantly, the geography of the region also accords with the existence of ephemeral islands, given the demonstrable presence of spreading ridges, subduction zones, and associated volcanic activity in and around the region that is presently occupied by the Grand Terre throughout the Cenozoic (Schellart et al., 2006). Short-range dispersals among a group of ephemeral islands is a far more parsimonious hypothesis than to postulate a single ''jump'' dispersal event between the Neotropics and New Caledonia across the expanse of the Pacific.

The overarching problem with dismissing the distribution of Troglosironidae as the consequence of a one-time trans-Pacific dispersal event between tropical South America and New Caledonia is that this hypothesis cannot be falsified. This is not to say that dispersal is an unimportant process in the study of biogeography. Nor do the authors of the present study subscribe to the untenable view that dispersal is mere ''noise'' amidst biota formed overwhelmingly by vicariance. On the contrary, dispersal has clearly played a formative role in Pacific biogeography (Cowie and Holland, 2006) as well as New Caledonian biogeography (Grandcolas et al., 2008). However, dispersal is just as demonstrable a process as vicariance in the distributions of Pacific island biota. Dispersal has been demonstrated by the collection of species, especially endemics, from oceanic islands isolated from continents or continental islands (Goodacre and Wade, 2001; Garb and Gillespie, 2006), or by molecular dating of clades that post-date geologically documented vicariant events (Barker et al., 2007), or both (Harbaugh and Baldwin, 2007). Consequently, the authors of the present study do not rule out the possibility of dispersal. However, given the documented diversity and distribution of Troglosironidae and Neogoveidae, combined with the results of the dating analysis, the hypothesis of an ancient, Gondwanan origin of Troglosironidae that diversified in accordance with Eocene events is better supported than the hypothesis of a single ''jump'' dispersal. Furthermore, the former is fully falsifiable; ongoing and future collection efforts on nearby volcanic and continental islands (e.g. the Loyalty Islands, Lord Howe Island, the Isle of Pines), and improved dating analysis subsequent to contributions to the recorded biodiversity of Cyphophthalmi, can falsify the hypothesis of an ancient biotic link between New Caledonia and Tropical Gondwana. It is insufficiently rigorous to dismiss the evolutionary history of Troglosironidae by invoking inexplicable migration across the Pacific Ocean without further evidence.

Conclusion

Contrary to the results of numerous studies demonstrating recent origin and diversification in New Caledonia's endemic biota, and subsequently rejecting its supposedly relictual nature (reviewed in Grandcolas et al., 2008), phylogenetic analysis of troglosironid evolutionary history demonstrates the inclusion of evolutionary relicts among New Caledonia's opiliofauna. The persistence of this ancient lineage through the Paleocene/Eocene bottleneck speaks to the ability of Cyphophthalmi to persist through evolutionary time. The unique evolutionary history of Troglosironidae seems quite puzzling in the absence of validation by other taxa with similar geographical distributions. Similarly, the connection between New Caledonia and Tropical Gondwana, as well as the mechanism of persistence of relictual taxa through Paleocene drowning, remain outstanding questions for biogeography. To understand the processes that engendered this curious lineage, it is imperative to identify taxa with similar evolutionary histories that may shed light on the biogeography of New Caledonia.

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References

Ali, J.R., 2006. Biogeographical and geological evidence for a smaller, completely-enclosed Pacific basin in the Late Cretaceous: a comment. J. Biogeogr. 33, 1670–1674.

Balke, M., Wewalka, G., Alarie, Y., Ribera, I., 2007a. Molecular phylogeny of Pacific Island Colembetinae: radiation of New Caledonian and Fijian species (Coleoptera, Dytiscidae). Zool. Scr. 36, 173–200.

Balke, M., Pons, J., Ribera, I., Sagata, K., Vogler, A.P., 2007b. Infrequent and unidirectional colonization of hyperdiverse

Papuadytes diving beetles in New Caledonia and New Guinea. Mol. Phylogenet. Evol. 42, 505–516.

Barker, N.P., Weston, P.H., Rutschmann, F., Sauquet, H., 2007. Molecular dating of the 'Gondwanan' plant family Proteaceae is only partially congruent with the timing of the break-up of Gondwana. J. Biogeogr. 34, 2012–2027.

de Bivort, B.L., Giribet, G., 2004. A new genus of cyphophthalmid from the Iberian Peninsula with a phylogenetic analysis of the Sironidae (Arachnida: Opiliones: Cyphophthalmi) and a SEM database of external morphology. Invertebr. Syst. 18, 7–52.

Bouchet, P., Lozouet, P., Maestrati, P., Heros, V., 2002. Assessing the magnitude of species richness in tropical marine environments: exceptionally high numbers of molluscs at a New Caledonia site. Biol. J. Linn. Soc. 75, 421–436.

Boyer, S.L., Giribet, G., 2007. A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. Cladistics 23, 337–361.

Boyer, S.L., Karaman, I., Giribet, G., 2005. The genus *Cyphophthalmus* (Arachnida, Opiliones, Cyphophthalmi) in Europe: a phylogenetic approach to Balkan Peninsula biogeography. Mol. Phylogenet. Evol. 36, 554–567.

Boyer, S.L., Baker, J.M., Giribet, G., 2007a. Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread "mite harvestman" defies DNA taxonomy. Mol. Ecol. 16, 4999–5016.

Boyer, S.L., Clouse, R., Benavides, L., Sharma, P., Schwendinger, P., Karunarathna, I., Giribet, G., 2007b. Biogeography of the world: a case study of globally distributed arachnids. J. Biogeogr. 34, 2070–2085.

Briggs, J.C., 2004. The ultimate expanding earth hypothesis. J. Biogeogr. 31, 855–857.

Briggs, J.C., 2006. Another expanding Earth paper. J. Biogeogr. 33, 1674–1676.

Brothers, R.N., Lillie, A.R., 1988. Regional geology of New Caledonia. The ocean basins and margins. In: Nairn, A.E.M., Stehli, F.G., Uyeda, S. (Eds.), The Pacific Ocean. Plenum Press, New York, Vol. 7B, pp. 325–374.

Buckley, T.R., Attanayake, D., Bradler, S., 2008. Extreme convergence in stick insect evolution: phylogenetic placement of the Lord Howe Island tree lobster. Proc. R. Soc. B. 276, 1055–1062.

Clouse, R.M., Giribet, G., 2007. Across Lydekker's line – first report of mite harvestmen (Opiliones, Cyphophthalmi, Stylocellidae) from New Guinea. Invertebr. Syst. 21, 207–227.

Cluzel, D., Aitchison, J.C., Picard, C., 2001. Tectonic accretion and underplating of mafic terranes in the Late Eocene intraoceanic fore-arc of New Caledonia (Southwest Pacific): geodynamic implications. Tectonophysics 340, 23–59.

Cowie, R.H., Holland, B.S., 2006. Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. J. Biogeogr. 33, 193–198.

Edgecombe, G.D., Giribet, G. 2009. Phylogenetics of scutigeromorph centipedes (Myriapoda: Chilopoda) with implications for species delimitation and historical biogeography of the Australian and New Caledonian faunas. Cladistics. doi: 10.1111/j.1096-0031.2009.00253.x.

Edgecombe, G.D., Giribet, G., Wheeler, W.C., 2002. Phylogeny of Henicopidae (Chilopoda: Lithobiomorpha): a combined analysis of morphology and five molecular loci. Syst. Entomol. 27, 31–64.

Farris, J.S., 1997. The future of phylogeny reconstruction. Zool. Scr. 26, 303–311.

Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D., Kluge, A.G., 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12, 99–124.

Fleischer, R.C., McIntosh, C.E., Tarr, C.L., 1998. Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Mol. Ecol. 7, 533–545.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R.C., 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Garb, J.E., Gillespie, R.G., 2006. Island hopping across the central Pacific: mitochondrial DNA detects sequential colonization of the Austral Islands by crab spiders (Araneae: Thomisidae). J. Biogeogr. 33, 201–220.

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

Giribet, G., 2003. Stability in phylogenetic formulations and its relationship to nodal support. Syst. Biol. 52, 554–564.

Giribet, G., 2005. Generating implied alignments under direct optimization using POY. Cladistics 21, 396–402.

Giribet, G., Boyer, S.L., 2002. A cladistics analysis of the cyphophthalmid genera (Opiliones, Cyphophthalmi). J. Arachnol. 30, 110–128.

Giribet, G., Kury, A.B., 2007. Phylogeny and biogeography. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), Harvestmen: The Biology of Opiliones. Harvard University Press, Cambridge, MA, USA, pp. 62–87.

Giribet, G., Carranza, S., Baguñà, J., Riutort, M., Ribera, C., 1996. First molecular evidence for the existence of a Tradigrada + Arthropoda clade. Mol. Biol. Evol. 13, 76–84.

Goloboff, G.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics 15, 415–428.

Goloboff, G.A., 2002. Techniques for analyzing large data sets. In: Desalle, R., Giribet, G., Wheeler, W. (Eds.), Techniques in Molecular Systematics and Evolution. Brikhäuser Verlag, Basel, pp. 70–79.

Goodacre, S.L., Wade, C.M., 2001. Patterns of genetic variation in Pacific island land snails: the distribution of cytochrome *b* lineages among Society Island *Partula*. Biol. J. Linn. Soc. 73, 131–138.

Grandcolas, P., Murienne, J., Robillard, T., Desutter-Grandcolas, L., Jourdan, H., Guilbert, E., Deharveng, L., 2008. New Caledonia: a very old Darwinian island? Philos. Trans. R. Soc. B 363, 3309–3317.

Harbaugh, D.T., Baldwin, B.G., 2007. Phylogeny and biogeography of the sandalwoods (*Santalum*, Santalaceae): repeated dispersals throughout the Pacific. Am. J. Bot. 94, 1028–1040.

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

Juberthie, C., 1979. Un cyphophthalme nouveau d'une grotte de Novelle-Calédonie: *Troglosiro aelleni* n. gen., n. sp. (Opilion Sironinae). Rev. Suisse Zool. 86, 221–231.

Lee, D.E., Lee, W.G., Mortimer, N., 2001. Where and why have all the flowers gone? Depletion and turnover in the New Zealand Cenozoic angiosperm flora in relation to paleogeography and climate. Aust. J. Bot. 49, 341–356.

Linton, E.W. 2005. MacGDE: Genetic Data Environment for MacOS X. Software available at http://www.msu.edu/~lintone/macgde.

Lowry, P.P. 1998. Diversity, endemism, and extinction in the flora of New Caledonia: a review. In: Peng, C.I., Lowry, P.P. (Eds.), Proceedings of the International Symposium on Rare, Threatened, and Endangered Floras of Asia and the Pacific. Academica Sinica, Monogr. Ser. no. 16. Institute of Botany, Taipei, Taiwan, pp. 181–206.

Mathews, S., Donoghue, M.J., 1999. The root of angiosperm phylogeny inferred from duplicate phytochrome genes. Science 286, 947–950.

McCarthy, D., 2003. The trans-Pacific zipper effect: disjunct sister taxa and matching geological outlines that link the Pacific margins. J. Biogeogr. 30, 1545–1561.

McCarthy, D., 2005. Biogeographical and geological evidence for a smaller, completely-enclosed Pacific Basin in the Late Cretaceous. J. Biogeogr. 32, 2161–2177.

McCarthy, D., Ebach, M.C., Morrone, J.J., Parenti, L.R., 2007. An alternative Gondwana: biota links South America, New Zealand and Australia. Biogeografía 2, 2–12.

Morat, P., Veillon, J.-M., MacKee, H.S., 1986. Floristic relationships of New Caledonian rainforest phanerogams. Teleopea 2, 631–679.

Murienne, J., Giribet, G., 2009. The Iberian Peninsula: ancient history in a hot spot of mite harvestmen (Arachnida: Opiliones: Cyphophthalmi: Sironidae) diversity. Zool. J. Linn. Soc. doi: 10.1111/j.1096-3642.2008.00512.x.

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

Murienne, J., Pellens, R., Budinoff, R.B., Wheeler, W.C., Grandcolas, P., 2008. Phylogenetic analysis of the endemic New Caledonian cockroach *Lauraesilpha*. Testing competing hypotheses of diversification. Cladistics 24, 1–11.

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

Najt, J., Grandcolas, P. (Eds.), 2002. Zoologica Neocaledonica, 5. Systématique et endémisme en Nouvelle Calédonie. Mém. Mus. Nat. Hist. Nat. 187: 1–282.

Neall, V.E., Trewick, S.A., 2008. The age and origin of the Pacific islands: a geological overview. Philos. Trans. R. Soc. B 363, 3293–3308.

Paris, J.P. 1981. Géologie de la Nouvelle-Calédonie. Un essai de synthèse (Mémoire pour servir notice explicative à la carte géologique de la Nouvelle-Calédonie à l'échelle du 1/200000, avec 2 cartes). Mém. BRGM 113, 1–278.

Prendini, L., Weygoldt, P., Wheeler, W.C., 2005. Systematics of the *Damon variegatus* group of African whip spiders (Chelicerata: Amblypygi): evidence from behaviour, morphology and DNA. Org. Divers. Evol. 5, 203–236.

Qiu, Y.L., Lee, J., Bernasconi-Quadroni, F., Soltis, D.E., Soltis, P.S., Zanis, M., Zimmer, E.A., Chen, Z., Savolainen, V., Chase, M.W., 1999. The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. Nature 402, 404–407.

Raven, P.H., 1979. Plates tectonics and southern hemisphere biogeography. In: Larsen, K., Holm-Nielsen, L.B. (Eds.), Tropical Botany. Academic Press, London, pp. 3–24.

Sanderson, M. 2006. r8s version 1.71. Software and documentation available at http://loco.biosci.arizona.edu/r8s.

Schellart, W.P., Lister, G.S., Toy, V.G., 2006. A Late Cretaceous and Cenozoic reconstruction of the Southwest Pacific region: tectonics controlled by subduction and slab rollback processes. Earth Sci. Rev. 76, 191–233.

Schwendinger, P.J., Giribet, G., 2005. The systematics of the south-east Asian genus *Fangensis* Rambla (Opiliones: Cyphophthalmi: Stylocellidae). Invertebr. Syst. 19, 297–323.

Setoguchi, H., Osawa, T.A., Pintaud, J.C., Jaffré, T., Veillon, J.M., 1998. Phylogenetic relationships within Araucariaceae based on *rbcL* gene sequences. Am. J. Bot. 85, 1507–1516.

Sharma, P., Giribet, G., 2005. A new *Troglosiro* species (Opiliones, Cyphophthalmi, Troglosironidae) from New Caledonia. Zootaxa 1053, 47–60.

Sharma, P. P., Giribet, G., 2009. The family Troglosironidae (Opiliones: Cyphophthalmi) of New Caledonia. Zoologia Neocaledonica 7. Biodiversity Studies in New Caledonia, Paris, pp. 83–123.

Shear, W.A., 1980. A review of the Cyphophthalmi of the United States and Mexico, with a proposed reclassification of the Suborder (Arachnida, Opiliones). Am. Mus. Nov. 2705, 1–34.

Shear, W.A., 1993. The genus *Troglosiro* and the new family Troglosironidae (Opiliones, Cyphophthalmi). J. Arachnol. 21, 81–90.

Soltis, D.E., Soltis, P.S., Chase, M.W., Mort, M.E., Albach, D.C., Zanis, M., Savolainen, V., Hahn, W.H., Hoot, S.B., Fay, M.F., Axtell, M., Swensen, S.M., Prince, L.M., Kress, W.J., Nixon, K.C., Farris, J.S., 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. Bot. J. Linn. Soc. 133, 381–461.

Varón, A., Vinh, L.S., Bomash, I., Wheeler, W.C., 2007. POY 4.0 Beta 2635. Documentation by A. Varón, L. S. Vinh, I. Bomash, W. Wheeler, K. Pickett, I. Tëmkin, J. Faivovich, T. Grant, and W. L. Smith. Software available at http://research.amnh.org/scicomp/projects/poy.php.

Wagner, W.L., Funk, V.A., 1995. Hawaiian Biogeography: Evolution on a Hot Spot Archipelago. Smithsonian Institution Press, Washington, DC.

Walley, A.M., Ross, M.I., 1991. Preliminary reconstructions for the Cretaceous to Caenozoic of the New Zealand–New Caledonian region. Bureau of Mineral Resources, Canberra.

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

Wheeler, W.C., 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics? Cladistics 12, 1–9.

Wheeler, W.C., 2003. Implied alignment: a synapomorphy-based multiple-sequence alignment method and its use in cladogram search. Cladistics 19, 261–268.

Whiting, M.F., Carpenter, J.M., Wheeler, Q.D., Wheeler, W.C., 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46, 1–68.

Xiong, B., Kocher, T.D., 1991. Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Simuliidae). Genome 34, 306–311.

Appendix 1

List of specimens with MCZ accession numbers, locality data, and Genbank accession numbers for the markers employed in this study.

Outgroups	MCZ accession no.	Locality	Coordinates	Collector	16S rRNA	18S rRNA	28S rRNA	COI
Aoraki denticulata (Forster, 1948)	DNA100961	New Zealand	41°48′29′S, 172°50′01′E	ref. GenBank	DQ518069	DQ518001	DQ518040	DQ518126
Austropurcellia scoparia Juberthie, 1988	DNA100946	Australia	16°35′41′S, 145°16′46′E	ref. GenBank	DQ518065	DQ517982	DQ518021	DQ518108
Fangensis cavernarus Schwendinger and Giribet, 2005	DNA101460	Thailand	14°23′54′N, 99°04′53′E	ref. GenBank		DQ133714	DQ133726	DQ133740
Huitaca sp.	DNA101407	Colombia	5°46′46′N, 73°27′13′W	ref. GenBank	DQ518050	DQ518090	DQ825596	DQ518129
Karripurcellia harveyi Giribet, 2003	DNA101303	Australia	34°29′42′S, 115°58′31′E	ref. GenBank	DQ518062	DQ517980	DQ825578	DQ518106
Metagovea sp.	DNA101410	Colombia	1°17′06′N, 78°04′25′W	ref. GenBank		DQ518091	DQ825597	EU887036
Metasiro americanus (Davis, 1933)	DNA101532	Florida, USA	30°33′53′N, 84°57′05′W	ref. GenBank	DQ825616	DQ825542	DQ825595	DQ825645
Neopurcellia salmoni Forster, 1948	DNA100939	New Zealand	44°06′28′S, 169°21′19′E	ref. GenBank	DQ518066	DQ517998	DQ518037	DQ825638
Paragovia sironoides Hansen, 1921	DNA101059	Equatorial Guinea	3°43′32′N, 8°50′17′E	ref. GenBank	DQ518051	DQ518092	DQ825606	DQ518131
Paragovia sp.	DNA101057	Equatorial Guinea	2°07′52′N, 9°52′18′E	ref. GenBank	DQ825621	DQ825547	DQ825602	
Rakaia stewartiensis Forster, 1948	DNA100944	New Zealand	46°53′36′S, 168°06′14′E	ref. GenBank	DQ518080	DQ517994	DQ518028	DQ518117
Stylocellus lydekkeri Clouse and Giribet, 2007	DNA101064	Indonesia	2°43′S, 134°30′E	ref. GenBank		DQ133717	DQ133729	
Stylocellus novaguinea Clouse and Giribet, 2007	DNA101510	Indonesia	0°50′S, 134°02′E	ref. GenBank	DQ825609	DQ825536	DQ825570	
Suzukiellus sauteri (Roewer, 1916)	DNA101543	Japan	35°38′03′N, 139°14′28′E	ref. GenBank	DQ518086	DQ513138	DQ513116	DQ518108
Troglosironidae		New Caledonia						
Troglosiro aelleni Shear, 1993	DNA100345	Aoupinie	21°11′S, 166°28′E	G.B. Monteith	AY639555	AY639497	DQ825580	AY639584
Troglosiro brevifossa Sharma & Giribet, 2009	DNA101696	Cap Ndoua	22º23′S, 166º56′E	G.B. Monteith			EU887117	EU887039
Troglosiro juberthiei Shear, 1993	DNA100344m	Mt Dzumac Road	22°03′S, 166°28′E	G.B. Monteith	EU887077	DQ825540	EU887121	EU887047
Troglosiro juberthiei Shear, 1993	DNA100344f	Mt Dzumac Road	22°03′S, 166°28′E	G.B. Monteith	EU887076	EU887108	EU887122	EU887048
Troglosiro juberthiei Shear, 1993	DNA101576-1	Mt Dzumac Road	22°03′S, 166°28′E	G.B. Monteith	EU887078	EU887109	EU887126	EU887049
Troglosiro juberthiei Shear, 1993	DNA101576-2	Mt Dzumac Road	22°03′S, 166°28′E	G.B. Monteith	EU887079			EU887050
Troglosiro juberthiei Shear, 1993	DNA101576-3	Mt Dzumac Road	22°03′S, 166°28′E	G.B. Monteith				EU887051
Troglosiro juberthiei Shear, 1993	DNA101576-4	Mt Dzumac Road	22°03′S, 166°28′E	G.B. Monteith				EU887052
Troglosiro juberthiei Shear, 1993	DNA101576-5	Mt Dzumac Road	22°03′S, 166°28′E	G.B. Monteith	EU887080			EU887060

Appendix 1
Continued

Outgroups	MCZ accession no.	Locality	Coordinates	Collector	16S rRNA	18S rRNA	28S rRNA	COI
Troglosiro cf. *juberthiei*	DNA102343-1	Pic du Pin	22°15′S, 166°49′E	P. Sharma & J. Murienne	EU887087	EU887111	EU887127	EU887062
Troglosiro cf. *juberthiei*	DNA102343-2	Pic du Pin	22°15′S, 166°49′E	P. Sharma & J. Murienne	EU887088			EU887063
Troglosiro cf. *juberthiei*	DNA101703	Pic du Pin	22°15′S, 166°49′E	G.B. Monteith	EU887082	EU887103		EU887045
Troglosiro cf. *juberthiei*	DNA101703-2	Pic du Pin	22°15′S, 166°49′E	G.B. Monteith				EU887055
Troglosiro cf. *juberthiei*	DNA101703-4	Pic du Pin	22°15′S, 166°49′E	G.B. Monteith	EU887083			EU887056
Troglosiro cf. *juberthiei*	DNA101704	Pic du Pin	22°15′S, 166°49′E	G.B. Monteith	EU887084			EU887057
Troglosiro cf. *juberthiei*	DNA101705-1	Pic du Pin	22°15′S, 166°49′E	G.B. Monteith	EU887085			EU887058
Troglosiro cf. *juberthiei*	DNA101705-2	Pic du Pin	22°15′S, 166°49′E	G.B. Monteith	EU887086			EU887059
Troglosiro cf. *juberthiei*	DNA102344-1	Pic du Grand Kaori	22°17′S, 166°54′E	P. Sharma & J. Murienne	EU887089	EU887112	EU887128	EU887064
Troglosiro cf. *juberthiei*	DNA102344-2	Pic du Grand Kaori	22°03′S, 166°28′E	P. Sharma & J. Murienne	EU887090			EU887065
Troglosiro cf. *juberthiei*	DNA102344-3	Pic du Grand Kaori	22°03′S, 166°28′E	P. Sharma & J. Murienne	EU887091			EU887066
Troglosiro cf. *juberthiei*	DNA102345	Pic du Grand Kaori	22°03′S, 166°28′E	P. Sharma & J. Murienne	EU887092	EU887113	EU887129	EU887067
Troglosiro cf. *juberthiei*	DNA102346-1	Foret Nord	22°19′S, 166°55′E	P. Sharma & J. Murienne	EU887093			
Troglosiro cf. *juberthiei*	DNA102346-2	Foret Nord	22°19′S, 166°55′E	P. Sharma & J. Murienne	EU887094	EU887114	EU887130	EU887068
Troglosiro cf. *juberthiei*	DNA102346-3	Foret Nord	22°19′S, 166°55′E	P. Sharma & J. Murienne	EU887095			EU887069
Troglosiro cf. *juberthiei*	DNA102346-4	Foret Nord	22°19′S, 166°55′E	P. Sharma & J. Murienne	EU887096			EU887070
Troglosiro cf. *juberthiei*	DNA102346-5	Foret Nord	22°19′S, 166°55′E	P. Sharma & J. Murienne	EU887097			EU887071
Troglosiro cf. *juberthiei*	DNA102346-6	Foret Nord	22°19′S, 166°55′E	P. Sharma & J. Murienne	EU887098			EU887072
Troglosiro cf. *juberthiei*	DNA101697-8	Foret Nord	22°19′S, 166°55′E	G.B. Monteith				EU887053
Troglosiro cf. *juberthiei*	DNA101701	Foret Nord	22°19′S, 166°55′E	G.B. Monteith		EU887104		EU887054
Troglosiro longifossa Sharma Giribet, 2005	DNA100867	Port Boisé Bay	22°21′S, 166°28′E	G.B. Monteith	DQ518084	DQ518089	DQ825582	DQ825639
Troglosiro monteithi Sharma & Giribet, 2009	DNA101580	Col d'Amieu	21º36′S, 165º43′E	G.B. Monteith	EU887074	EU887101	EU887116	EU887043
Troglosiro ninqua Shear, 1993	DNA100577	Mt Ningua	21°45′S, 166°09′E	G.B. Monteith	DQ518085	DQ518088	DQ825581	DQ518128
Troglosiro oscitatio Sharma & Giribet, 2009	DNA101709	Mt Rembai	21º35′S, 165º51′E	G.B. Monteith		EU887106	EU887124	EU887041
Troglosiro raveni Shear, 1993	DNA101707	Col des Rousettes	21°25′S, 165°28′E	G.B. Monteith		EU887099	EU887120	EU887042
Troglosiro sheari Sharma & Giribet, 2009	DNA101586	Ateou	20°57′S, 164°54′E	G.B. Monteith				EU887037
Troglosiro sheari Sharma & Giribet, 2009	DNA101584	Ateou	20°57′S, 164°54′E	G.B. Monteith		EU887100	EU887115	EU887038
Troglosiro sp.	DNA101575	Riviere Bleue	22°06′S, 166°40′E	G.B. Monteith	EU887081	EU887110	EU887123	EU887046
Troglosiro urbanus Sharma & Giribet, 2009	DNA101710m	Yahoué	22°12′S, 166°30′E	G.B. Monteith	EU887073	EU887105	EU887118	EU887044
Troglosiro urbanus Sharma & Giribet, 2009	DNA101710f	Yahoué	22°12′S, 166°30′E	G.B. Monteith		EU887102	EU887119	EU887040
Troglosiro wilsoni Sharma & Giribet, 2009	DNA102324	Mts Koghis	22°10′S, 166°30′E	P. Sharma & J. Murienne	EU887075	EU887107	EU887125	EU887061

Appendix 2

Sample script typically used in parsimony analyses as implemented in POY ver. 4.

```
wipe ()
set (log: 'err')
read ('Troglosironidae28S.fas')
transform (tcm: '111')
build (1000)
swap (trees:10, alternate)
fuse (iterations:10, replace:best, keep:100, swap
())
select (unique, optimal)
report ('alltrees', trees)
report ('data', treestats)
report ('consensus', graphconsensus)
report ('pictrees', graphtrees)
wipe ()
exit ()
```