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MOLECULAR PHYLOGENETIC ANALYSIS OF 28S rDNA SUPPORTS A GONDWANAN ORIGIN FOR AUSTRALASIAN HYRIIDAE (MOLLUSCA: BIVALVIA: UNIONOIDA)

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GONDWANALAND
NEW ZEALAND
HYRIIDAE
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MOLECULAR PHYLOGENETICS
VICARIANCE
BIOGEOGRAPHY

ABSTRACT. – The Hyriidae (Mollusca: Bivalvia: Unionoida) have a disjunct distribution, occurring on South America, Australia, and New Zealand. Most previous macroevolutionary studies of the Hyriidae pre-dated widespread acceptance of both continental drift and phylogenetic systematics. For this study, we applied molecular phylogenetic techniques to test the hypothesis that the observed disjunction of Australasian hyriids across the Tasman Sea is due to the disintegration of Gondwanaland (>80 million years ago). We sequenced a fragment of 28S rDNA for representative hyriid Velesunionini (Australia), Hyridellini (Australia and New Zealand), and Hyriinae (South America) and for outgroups belonging to the unionoid families Margaritiferidae and Unionidae. The topology of the single 28S tree [i.e., (Margaritiferidae, Unionidae, (Velesunionini, (Hyridellini, Hyriinae)))] recovered by both maximum parsimony and maximum likelihood did not support a monophyletic Australasian clade, and the branch lengths were consistent with Mesozoic vicariance. We also acquired COI sequences for the Australian subset of mussels to corroborate the 28S branch lengths. Our results suggest that (1) the Hyriidae pre-date the break up of Gondwanaland and (2) the New Zealand Hyridellini are relics rather than colonizers. Alternative long-distance dispersal hypotheses are discussed in the context of our results, historical geology, and mussel life history.

GONDWANA
NOUVELLE ZÉLANDE
HYRIIDAE
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RÉSUMÉ.– Les Hyriidae (Mollusca: Bivalvia: Unionoida) ont une répartition disjointe en Amérique du Sud, en Australie et en Nouvelle Zélande. La plupart des études passées sur la macroévolution datent d'avant l'acceptation générale de la dérive des continents et de la systématique phylogénétique. Dans cette étude nous avons appliqué des techniques de phylogénie moléculaire pour tester l'hypothèse selon laquelle la répartition disjointe des Hyriidés d'Australie et de Nouvelle-Zélande séparés par la mer tasmanienne est due à la désintégration du Gondwana il y a environ 80 millions d'années. Nous avons séquencé un fragment du 28 S rADN de représentants des Hyriidés Velesunionini (Australie), Hyridellini (Australie et Nouvelle Zélande) et des Hyriinae (Amérique du Sud), et pour les outgroups, des Unionoidés Margaritiferidae et Unionidae. La topologie du seul arbre 28 S [(Margaritiferidae, Unionidae, (Velesunionini, (Hyridellini, Hyriinae)))] obtenue par la parcimonie du maximum de vraisemblance et de « Maximum Likelihood » ne conforte pas la monophylie du clade australasien, et la longueur des branches est en faveur d'une vicariance datant du Mésozoïque. Nous avons également traité les séquences COI pour le sous-ensemble des Moules d'Australie afin de corroborer les longueurs du branchement 28S. Nos résultats suggèrent (1) que les Hyriidae existaient avant la désintégration du Gondwana et (2) que les Hyridellini de Nouvelle Zélande sont des relictes plutôt que des colonisateurs. Les hypothèses alternatives de dispersion à grande distance sont discutées dans le contexte de nos résultats, de la géologie historique et des traits d'histoire de vie des Moules.

INTRODUCTION

Freshwater mussels (Unionoida) are a globally distributed, ancient group of strictly continental bivalves. Their diversity and unique parasitic larvae have attracted a great deal of ecological study, especially from a conservation perspective [see Kat (1984) and Watters (1994) and references cited

Table I. - Taxonomy and Distribution of the Hyriidae. The nomenclature of the Hyriidae has been updated to standardize the views of Iredale (1934), McMichael & Hiscock (1958), Parodiz & Bonetto (1963), and Graf (2000): the Australian family and subfamilies have been demoted to a subfamily with four tribes. † Indicates presence on Tasmania. Distribution data references: [1]McMichael & Hiscock (1958), [2]Walker *et al.* (2000), [3]McMichael (1956), [4]McMichael (1958), [5]Parodiz &Bonetto (1963).

Taxon	New Guinea[1,2,3]	Australia[1,2]	New Zealand[1,2,4]	South America[5]
Hyridellinae				
Hyridellini	X	X†	X	
Cucumerunionini	X	X	X	
Velesunionini	X	X†		
Lortiellini		X		
Hyriinae				
Hyriini [= Prisodontini]				X
Diplodontini				X
Castaliini				X

therein]. Much of that research was focused on the Nearctic mussel assemblage and, until very recently, has lacked a modern evolutionary context (Graf & Ó Foighil 2000). This is surprising given that the age, distribution, and diversity of the Unionoida provide ample pattern with which to test hypotheses of macroevolutionary processes such as character evolution and biogeography.

Of special interest is the zoogeography of the freshwater mussel families confined to the Southern Hemisphere: Etheriidae, Iridinidae, and Hyriidae (Etherioidea). These mussels are presently restricted to the southern continents, although there is paleontological data suggesting their Mesozoic inhabitance of North America (Henderson 1935, Morris & Williamson 1988). The modern distribution of the Etherioidea led Graf (2000) to speculate about the influence of the disintegration of Gondwanaland on the evolution of those mussels. His morphological cladistic study principally tested the relationships of the Hyriidae, a family found in both South American and Australasian fresh waters. However, it did little to clarify the relationships within the Hyriidae. The object of this paper is to address the evolution and biogeography of Australasian hyriid clades, especially the problem of disjunction of New Zealand freshwater mussels across the Tasman Sea.

Based upon their morphology, there seems little doubt that the Hyriidae is monophyletic (Graf 2000). If this is true, however, their present distribution presents a dramatic disjunction: the Neotropical Hyriinae and the Australasian Hyridellinae (Table I). Unfortunately, nearly all discussion of these families pre-dated both (1) the widespread acceptance of phylogenetic systematics as a scientific means to discover organismal relationships and (2) the recognition of continental drift as a potential mechanism for vicariance. Thus, the narrative of hyriid macroevolution has yet to be formally purged of problematic hypotheses involving waif dispersal or migration of hypothetical "ancestral stocks" across post-Mesozoic "land bridges" (e.g., Ortmann 1921, Modell 1942, McMichael & Hiscock 1958, McMichael & Iredale 1959, Parodiz & Bonetto 1963).

While the Hyriinae is limited to South America, the Hyridellinae presents its own disjunction. The Australian Hyriidae occur on Australia, Tasmania, New Guinea, and the Solomons on the western and northern sides of the Tasman Sea, and New Zealand on the eastern side. Two of the eight genera that inhabit the region occur on New Zealand: *Hyridella menziesi, H. aucklandica,* and *Cucumerunio websteri* (McMichael 1958). The consensus has been that the observed disjunction among the Hyridellinae is due to late Tertiary long-distance dispersal via phoresy upon migratory birds (McMichael 1954, 1958, McMichael & Hiscock 1958) or host fish (Walker *et al.* 2000) from Australia.

Until the relatively recent realization of continental drift (Wegener 1966), trans-oceanic dispersal or migration across "land bridges" would have been the only options available to explain the disjunctions of the Hyriidae (e.g., Darlington 1957). Modern biogeographic theory suggests an alternative. The Hyriidae may have been distributed widely on Mesozoic Gondwanaland, the

southern supercontinent composed of what are now South America, Africa, Madagascar, India, Antarctica, Australia, and New Zealand. When that landmass rifted apart, the respective hyriid faunas of South America, Australia, and New Zealand were isolated and have persisted into modern times. A review and chronology of the disintegration of Gondwanaland can be found in Storey (1995: Fig. 1) and Brown & Lomolino (1998: Fig. 6.17). In summary, rifting and sea floor spreading around 160 million years ago (Mya) (Jurassic) split South America and Africa from the rest of Gondwanaland. However, southern South America remained in close proximity to Antarctica into the Tertiary. On the other side of Antarctica around 100 Mya (Cretaceous), Australia began to separate from Antarctica. New Zealand remained locked to both Australia and Antarctica until roughly 90 Mya. Since 80 Mya, New Zealand has been isolated from Australia; and since 60 Mya, the two have been separated by a minimum distance of over 1000 km (Cooper *et al.* 1993). Vicariance hypotheses to explain the distributions of southern continent freshwater mussels have received almost no attention and have gone largely untested (McMichael 1967, Graf 2000, Walker *et al.* 2000).

For this study, we attempted to falsify vicariance as the biogeographic mechanism of hyriid disjunction across the Tasman Sea. From the alternative biogeographic process hypotheses (i.e., dispersal vs. vicariance), we derived predictions of molecular phylogenetic pattern. If the vicariance hypothesis is true, the origin of the genus *Hyridella* must predate the barrier. We predicted that if the New Zealand hyriids achieved their present distribution by vicariance 80 Mya (i.e., the rifting of New Zealand from Australia and Antarctica), then the branch lengths separating New Zealand *Hyridella* spp. from their Australian congeners should be long relative to the internal branch supporting the clade. We presumed that the length of these terminal branches should be of the same order (or longer) than the branch lengths presented by other late Mesozoic freshwater mussel splits. This assumes a reasonably constant rate of molecular evolution, allowing branch length to serve as a loose proxy for time (i.e., long branch . long time, and vice versa). Strictly speaking, clades containing both New Zealand and Australian species should be "leafy" (Salisbury 1999). Short terminal branch lengths for New Zealand hyriids or a"stemmy" topology would reject a vicariance hypothesis and support more recent dispersal.

Several studies have demonstrated the value of nucleic acid characters in recovering the family-level phylogeny of freshwater mussels (Rosenberg *et al.* 1994, 1997, Lydeard *et al.* 1996, Hoeh *et al.* 1998, Graf & Ó Foighil 2000). To test the vicariance hypothesis, we sequenced domain 2 of 28S rDNA from South American, Australian, and New Zealand hyriids, as well as representative northern continent unionoids to serve as outgroups and for branch length comparisons. That gene fragment has been successfully employed to recover late Mesozoic phylogenetic branching patterns among the Bivalvia (Park & Ó Foighil 2000). In our preliminary work, we discovered some hyriids exhibit unexpectedly high levels of intraspecific 28S rDNA variation. As an independent test of branch lengths among the Hyridellinae, we also sequenced a stretch of cytochrome oxidase subunit I (COI) for our Australian and New Zealand species. Our results allow us to reevaluate the story of the evolution of the Hyriidae from a modern biogeographic perspective.

METHODS AND MATERIALS

Acquisition of Nucleotide Sequences: Domain 2 of 28S rDNA sequences were obtained from 11 species of freshwater mussels, including five outgroups (Table II). PCR and cycle-sequencing primers for 28S were D23F and D4RB (Park & Ó Foighil 2000 for PCR and sequencing protocol). We also harvested partial COI sequences from the Australasian Hyridellini and Velesunionini (Table II) using the Folmer *et al.* (1994) primers LCO1490 and HCO2198 as described in Graf & Ó Foighil (2000). All sequences are available through GENBANK (National Center for Biotechnology Information, National Institutes of Health; http://www.ncbi.nlm.nih.gov). Multisequence alignments were compiled and manipulated using Sequence Monkey 2.8.0 (available from http://www.members.tripod.com/sequence_monkey) and Clustal_X (Thompson *et al.* 1994, 1997; available from http://ncbi.nlm.nih.gov) and refined manually where necessary. The matrices (in NEXUS file format) are available from the corresponding author.

Phylogenetic Analysis: Phylogenetic analyses were carried out using PAUP* 4.0b3 (Swofford 1998). Maximum Parsimony (MP) and Maximum Likelihood (ML) optimality criteria were both applied to recover the phylogeny of the Hyriidae. MP searches ran as branch-and-bound with gaps in the alignment treated as missing data. ML searches (heuristic searches, 5 random sequence additions) were performed under the HKY model (Hasegawa *et al.* 1985) with rate heterogeneity. The transition: transversion ratio, proportion of invariable sites, and gamma shape parameter were estimated by maximum likelihood. Analogous searches were performed for both 28S and COI. However, the COI matrix was composed only of the hyridellid taxa from Australia and New Zealand and was intended only as an independent test of the branch lengths obtained from the 28S phylogeny.

To gauge the "robustness" of the topology recovered from the 28S MP analysis, Jackknife resampling analysis (50% character deletion each replication; 1000 replications, heuristic searches, 10 random additions each) was run using PAUP*. Also, Bremer-Decay Index (BDI) values were calculated using TreeRot (available from http://mightyduck.bu.edu/TreeRot), which creates a

Table II. - Taxa from which sequences were acquired. See text for explanation of protocol and references.

Taxon	Locality	GENBANK Accession # 28S	COI
Velesunionini			
Velesunio ambigua (n = 2)	New South Wales, Australia	AF305378	AF305371
		AF305379	AF305372
Hyridellini			
Hyridella australis (n = 2)	New South Wales, Australia	AF305373	AF305367
		AF305374	
H. depressa (n = 3)	New South Wales, Australia	AF305375	AF156496
			AF305368
H. menziesi (n = 2)	North Island, New Zealand	AF305376	AF305369
	South Island, New Zealand	AF305377	AF305370
Diplodontini			
Diplodon chilensis	Chile, South America	AF305380	
Castaliini			
Castalia sp.	Paraguay, South America	AF305381	
outgroups			
Cumberlandia monodonta	Minnesota, USA	AF305382	
Unio pictorum	Austria	AF305383	
Pygamodon grandis	Michigan, USA	AF305384	
Amblema plicata	Michigan, USA	AF305385	
Lampsilis cardium	Michigan, USA	AF305386	

constraint file for PAUP*. For each node, BDI indicates the difference in length of the next shortest tree without that node. The larger the BDI, the better the support (Bremer 1995).

Alternative topologies were constructed using MacClade 3.07 (Maddison & Maddison 1997). These were scored with PAUP* under both parsimony and the likelihood model derived from the ML search. Kishino & Hasegawa (1989) tests were used to gauge the significance of alternative topologies. The likelihood of the optimal 28S phylogeny was also analyzed both with and without a molecular clock enforced, and a Likelihood Ratio Test (LRT) was applied to test the significance between the two models. The molecular clock analysis was rooted between the Hyriidae and (*Cumberlandia*, Unionidae) based on Graf 2000, Graf & Ó Foighil 2000.

Stemminess of the *Hyridella* clade was calculated similar to the methods of Salisbury (1999), where stemminess equals the average internal to terminal branch length ratio. Terminal branch length is the distance from each terminal taxon to the node in question. Stemminess was estimated from the branch lengths assigned by PAUP* (ACCTRAN character-state optimization) on the 28S phylogeny for both MP and ML. Stemminess values greater than 1.0 indicate a "stemmy" topology with short terminals relative to the internal branch; less than 1.0 indicates a "leafy" topology with long terminal branches.

RESULTS

Fourteen partial 28S rDNA sequences acquired from 11 species were aligned into a matrix of 446 characters. On average, outgroup sequences were slightly longer than those of the ingroup. Whereas the five outgroup sequences averaged 430.2±2.2 nucleotides (nt) in length, the median ingroup sequence length was 412 nt, with only *Diplodon* (413 nt), *Castalia* sp. (387), and one of the *Hyridella menziesi* (307) deviating. In the case of *Castalia*, the missing nt were from the ends of the gene fragment; we truncated these due to a high number of ambiguous bases. The *H. menziesi* from the North Island of New Zealand, on the other hand, had a deletion extending from positions 234 to 349 in the

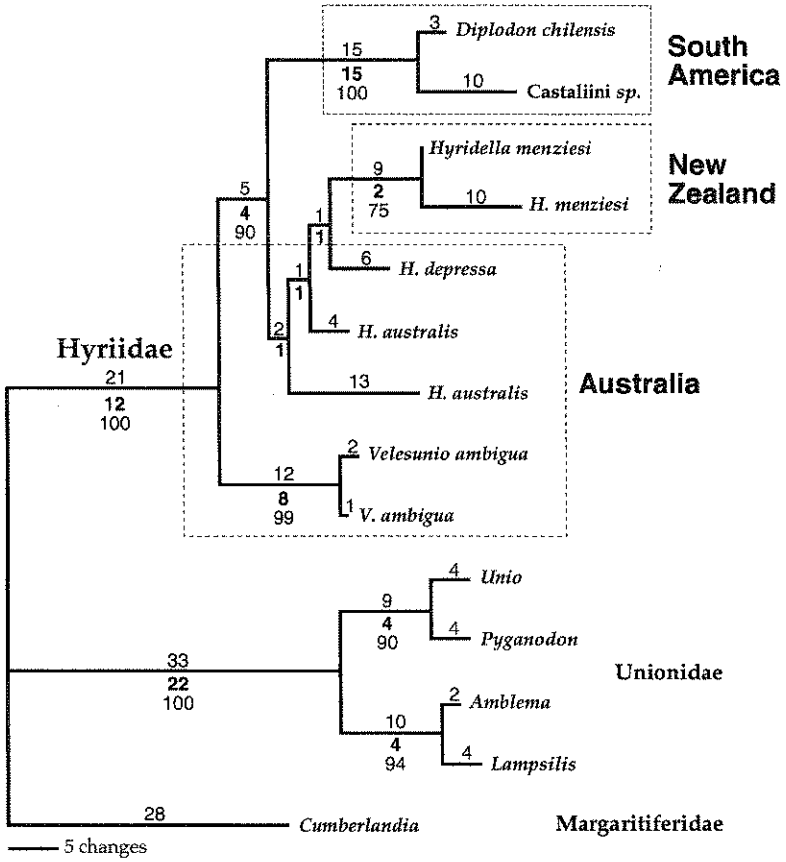

Fig. 1. - 28S rDNA Domain 2 Phylogeny of the Hyriidae. Numbers above the branches are MP branch lengths; those below are BDI (boldface) and Jackknife resampling values also from the MP analysis. The ML analysis recovered the same branching order.

aligned matrix. In addition to sequence length variation, we also uncovered unexpected intraspecific sequence divergence among the Australasian hyriids: *H. australis* (4.4%), *H. menziesi* (3.3%), and *Velesunio ambigua* (0.7%). All three *H. depressa* individuals we studied exhibited the same 28S rDNA sequence.

MP and ML analysis of the 28S data set recovered a single phylogeny (Fig. 1). The *Hyriidae* were recovered as monophyletic. However, contrary to Graf's (2000: Fig. 1) phylogeny, the Australasian "hyridellinae" were paraphyletic relative to the Hyriinae. In addition, *H. australis* was recovered as paraphyletic. Analysis of an alternate topology with a monophyletic *H. australis* was found to be insignificantly different from the optimal tree under both MP and ML, while a monophyletic Hyridellinae was significantly different from the optimal topology only under MP (Table III). The ML model is provided in Table III. The 28S terminal branch lengths among *Hyridella* spp. are long relative to the internal branch supporting that clade (Fig. 1). The Stemminess value for *Hyridella* is decidedly "leafy" regardless of whether it is calculated from MP (0.22) or ML (0.17) branch lengths (Table V). These 28S data are not consistent with a perfect molecular clock (Table III).

Seven COI sequences were obtained from nine individuals representing four species of Australian and New Zealand hyriids (Table II). We aligned these into a matrix of 638 nt that contained no insertion-deletions. Mitochondrial DNA, as we had suspected *a priori*, proved of little value in recovering the phylogeny of the Australian region Hyridellinae, as evidenced by the insignificantly different topologies favored by the two optimality criteria. Results of phylogenetic analyses of COI are presented in Table IV and Fig. 2. However, COI served to put the observed 28S rDNA variation into perspective. For example, whereas the 28S sequences of the two *H. australis* differed by > 4%, the COI haplotypes of these two individual mussels were identical. In addition, the mean uncorrected, interspecific, pairwise distance among the COI haplotypes was 14.0 ± 1.0%. This corresponds to the average divergence between the Pleurobemini and Lampsilini (Graf & Ó Foighil, unpublished), a suspected Cretaceous split (Haas 1969, Lydeard *et al.* 1996).

Table III. - Examination of Alternative 28S rDNA Phylogenetic Topologies and Models. Alternative topologies, constraining the monophyly of taxa not recovered in the optimal tree (Fig. 1), were tested using the Kishino-Hasegawa Test, and the alternative likelihood models (molecular clock vs. no molecular clock) were tested using an LRT. CI is the Consistency Index; *p* indicates the probability of getting a more extreme statistic under the null hypothesis (*i.e.*, no difference between the two trees or models). * Indicates statistical significance at the 95% level.

	Parsimony			Maximum Likelihood	
Topology	Length	CI	*p*	-ln L	*p*
optimal	209	0.890	-	1718.86	-
H. australis monophyletic	210	0.886	0.318	1721.06	0.465
Australian *Hyridella* monophyletic	210	0.886	0.564	1719.57	0.884
Hyridellinae monophyletic	214	0.869	0.025*	1726.77	0.141

Model	ln L	*p*
Molecular clock not enforced	-1718.86	-
Molecular clock enforced	-1736.07	<0.05*

ML model: HKY with rate heterogeneity, ti/tv = 1.261, proportion of invariable sites = 0.108, gamma parameter = 0.909.

Table IV. - Examination of Alternate COI Phylogenetic Topologies. Abbreviations and statistics are as in ML model: HKY with rate heterogeneity, ti/tv = 3,91, proportion of invariable sites = 0.69, gamma parameter = ∞(set to maximum allowable value: 300).

Alternative topologies

MP tree: (*Velesunio*, (*Hyridella menziesi*, (*H. australis*, *H. depressa*)))

ML tree: (*Velesunio*, (*Hyridella australis*, (*H. depressa*, *H. menziesi*)))

	Parsimony			Maximum Likelihood	
Topology	Length	CI	*p*	ln L	*p*
MP	206	0.903	-	-1772.38	0.64
ML	210	0.886	0.37	-1771.40	-

DISCUSSION

The patterns recovered by our phylogenetic analyses cast a new light on the evolution of the Hyriidae (Fig. 1). This is relevant not only to the limited biogeographic problem among *Hyridella* spp. in Australasia, but also to the evolution of the Hyriidae on the southern continents. Our results are consistent with ancient vicariance caused by the rifting of New Zealand from Australia and Antarctica as the mechanism behind the disjunction of freshwater mussels across the Tasman Sea. We find this result incompatible with late Tertiary dispersal as suggested by McMichael 1958, McMichael & Hiscock 1958. Other available evi-

Table V. - MP and ML Stemminess Values for *Hyridella* 28S rDNA. IBL is the internal branch length; TBL is the terminal branch length. See text for an explanation of the stemminess calculation.

	Parsimony			Maximum Likelihood		
species	IBL	TBL	Stemminess	IBL	TBL	Stemminess
(1) *H. menziesi*	2	21	0.10	$4.2x10^{-3}$	$6.5x10^{-2}$	0.06
(2) *H. menziesi*	2	11	0.18	$4.2x10^{-3}$	$2.9x10^{-2}$	0.15
H. depressa	2	8	0.25	$4.2x10^{-3}$	$2.1x10^{-2}$	0.20
(1) *H. australis*	2	13	0.15	$4.2x10^{-3}$	$3.5x10^{-2}$	0.12
(2) *H. australis*	2	5	0.40	$4.2x10^{-3}$	$1.3X10^{-2}$	0.32
Mean			0.22±0.12			0.17±0.10

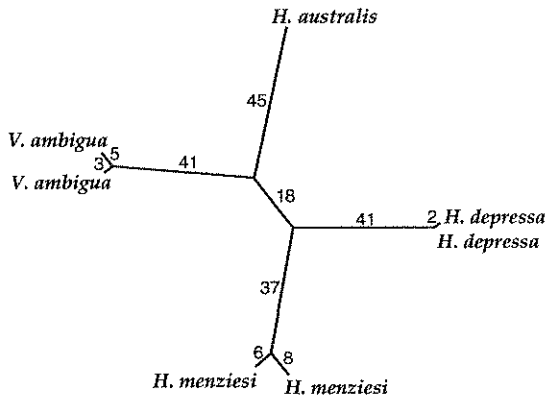

Fig. 2. - COI MP phylogeny of the representative Hyridellinae. Numbers associated with the branches are MP branch lengths. The ML phylogeny has a different topology (Table IV), but the terminal branch lengths are of a similar magnitude relative to the internal branch.

dence, inconsistent with the dispersal model, is reviewed below.

Most systematists of the Hyriidae have considered the Hyridellinae of Australasia to be a "well-defined unit" (McMichael & Hiscock 1958: 496). An ancestral hyriid invaded the region from southeastern Asia (Iredale 1934, McMichael & Hiscock 1958) or South America via Antarctica (Parodiz & Bonetto 1963), and the present endemic diversity resulted from speciation on the isolated continent. This model predicts that the Hyridellinae are monophyletic and that the ancestor of the clade was limited to Australasia. This was supported by Graf's (2000) morphological analysis of the Etherioidea.

However, the results of our molecular phylogenetic analyses lead us to reject this model. Fig. 1 shows that the Australasian "hyridellinae" are paraphyletic relative to the South American Hyriinae and that this result is robust. From a vicariance perspective, this topology suggests that hyriids pre-date the disintegration of Gondwanaland and that they were widespread on that supercontinent. The present endemism of the two Australasian tribes represented in our analysis, Velesunionini and Hyridellini, is due to persistence rather than cladogenesis on an isolated continent. Ortmann's (1921), also Modell (1942) "land bridge" scenario is also consistent with our phylogenetic results: the Hyriidae arose on Australia and spread via Antarctica to South America.

McMichael (1954, 1958), McMichael & Hiscock (1958) and McMichael & Hiredale (1959) argued for freshwater mussel phoresy upon migratory birds as the mechanism of dispersal to New Zealand from Australia. Based on the conchological similarity of the New Zealand mussel species to those of modern Australia, he (1958: 430) argued for "fairly recent" dispersal, but did not elaborate on the timing beyond Tertiary. More recently, Walker *et al.* (2000) have also allowed for trans-oceanic dispersal of hyriids upon their host fish. Our results, however, are not consistent with these models, at least as far as *H. menziesi* is concerned.

The 28S phylogeny in Fig. 1 shows a "leafy" *Hyridella* clade. Although this result is complicated by unexpected rapid intraspecific evolution in *H. menziesi* and *H. australis* and by the unconvincing branching order within *Hyridella* (Table III), our COI results corroborate the long 28S branches (Fig. 2). Intraspecific variation in nuclear rDNA is not uncommon but is peculiar under the current paradigm of paralogous sequence homogenization by "concerted evolution" (Hillis & Dixon 1991, Park & Ó Foighil 2000). Our results suggest that perhaps 28S rDNA sequence evolution is mediated by another mechanism in the Hyriidae.

Any correlation between sequence divergence and time would have extremely wide confidence

limits (Hillis *et al.* 1996). But with that in mind, it is interesting to note that the observed 28S and COI branch lengths between *H. menziesi* and its Australian congeners generally match or exceed those between mussel taxa suspected of late Mesozoic divergence [*(Unio, Pyganodon)* and *(Amblema, Lampsilis)* in Fig. 1; Graf & Ó Foighil, unpublished]. This is consistent with vicariance due to the break up of Gondwanaland as an explanation for the disjunction of *Hyridella* across the Tasman Sea. In the same vein, these data also suggest that the split between *H. depressa* and *H. australis* dates to a similarly ancient time.

Based upon our results, we reject the hypotheses of late-Tertiary/Quaternary dispersal of freshwater mussels by birds or fish across the Tasman Sea – at least for *Hyridella menziesi*. The philosophical hurdle that must be addressed when applying dispersal hypotheses to problems of disjunction is that dispersal, as a biogeographical mechanism, is generally not testable (Croizat *et al.* 1974, Ball 1976). Succinctly put, for each realized falsifiable prediction supporting a vicariance hypothesis, a consistent *ad hoc* dispersalist scenario can also be concocted. This is not to say that individual dispersal hypotheses can not be rejected. Fortunately, vicariance hypotheses are generally falsifiable. They differ fundamentally from dispersal hypotheses in that they describe a temporally and spatially discrete vicariance event: the formation of a barrier. Dispersal, on the other hand, refers to an essentially infinite series of improbable events spanning the entire history of the barrier. Our molecular phylogenetic results alone are not inconsistent with ancient transoceanic dispersal, but post-Gondwana migration is in conflict with other lines of evidence, namely the fossil record, unionoid life history, and the distributions of other southern continent taxa.

While the reality of continental drift had not yet taken hold by the late 1950s, the theory of past connection between the southern continents, especially based on their shared floras (*e.g.* Hooker 1867), enjoyed wide recognition (Brown & Lomolino 1998). McMichael (1958; McMichael & Hiscock 1958) was correct to predict that, for a vicariance (*i.e.*, land bridge) hypothesis, the common ancestor of the New Zealand and Australian hyridellids must have (1) pre-dated the formation of the Tasman Sea (Cretaceous) and (2) been found on both sides of that barrier (Platnick & Nelson 1978). He was, however, incorrect in his assessment of the available fossil evidence. The terrestrial paleontological record for New Zealand is far from complete (Cooper *et al.* 1993, Daugherty *et al.* 1993), and McMichael's (1958) rejection of continuous mussel occupation of New Zealand since the Mesozoic based on the lack of an uninterrupted transitional series seems unfounded. Ideally, the nearest common ancestor of Australian and New Zealand Hyridella would be identified from the fossil record. In practice, that is unlikely. The bottom line is that there are Mesozoic fossil hyriids on New Zealand, including at least one tantalizingly hyridelline specimen (McMichael 1957). While inconclusive, this is still consistent with the vicariance hypothesis.

Among the most damning evidence against long-distance avian dispersal of freshwater mussels is that it has never been observed, it is purely hypothetical. Contrary to historical anecdotes (*e.g.*, Cotton 1934), all available evidence suggests that freshwater mussels are dispersed only via their host fish (Johnson 1970, Graf 1997, 1998). It has been suggested by Walker *et al.* (2000) that *H. menziesi* might have reached New Zealand via *Anguilla*, which the mussel is known to infect (Hine 1978). Infection alone, however, is not a convincing indication of parasitism (Graf 1998), and anguillid catadromy and semelparity make this hypothesis a non-starter (Lake 1971, Bastrop *et al.* 2000). As discussed above, these *ad hoc* dispersal hypotheses are difficult to falsify and, in light of the evidence supporting vicariance, unnecessary. One hundred years ago, mussel phoresy upon migratory birds or fish may have seemed more likely than the possibility of a dynamic continental crust. Our improved understanding of unionoid life histories and historical geology no longer supports this assumption.

It is beyond the scope of this study to provide a detailed analysis of our results in the context of vicariant distributions among other New Zealand taxa (*e.g.*, Platnick & Nelson 1978, Rosen 1978, Craw 1985). Suffice it to say that New Zealand is home to more than a few Gondwanan "relics" – taxa incapable of trans-oceanic dispersal like frogs *(Leiopelma)*, tuataras *(Sphenodon)*, onycophorans *(Peripatus)*, beeches *(Nothofagus)*, *etc.* [reviewed in Cooper *et al.* (1993), Daugherty *et al.* (1993), and Humphries & Parenti (1986)]. *Hyridella* should be added to that list.

Based on our molecular phylogenetic analyses, we failed to reject the hypothesis that vicariance due to the break up of Gondwanaland was the biogeographic mechanism of hyriid disjunction across the Tasman Sea. Taken together, the results of our molecular phylogenetic analyses and our review of the data for and against long-distance dispersal provide a compelling case for vicariance, and, at the same time, demonstrate that there really is no data in favor of the long-distance dispersal model. However, our study was based on only a single clade: *Hyridella menziesi* on New Zealand and a limited sample of its congeners on Australia. Further testing is necessary, especially with regard to the generality of our conclusions to the other two New Zealand freshwater mussels, *H. aucklandica* and *Cucumerunio websteri.*

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