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Molecular evolutionary relationships between partulid land snails of the Pacific

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Adaptive radiation of partulid land snails in the tropical Pacific has produced an extraordinary array of distinctive morphological, ecological and behavioural types. Here we use part of the nuclear ribosomal RNA gene cluster to investigate the relationships within and between the three partulid genera, *Partula*, *Samoana* and *Eua*. The genera cluster separately, with *Samoana* and *Partula* forming monophyletic groups. With one exception, the molecular data generally support the previous generic classification based on genital morphology, even in species that show a number of characteristics otherwise atypical of the genus. Convergent evolution explains morphological similarities between members of different genera. The phylogeny suggests that *Samoana* has colonized the Pacific from west to east, originating in the area where *Eua*, believed to be the most ancient partulid genus, is found. An unexplained anomaly is the reported occurrence of a single species of *Samoana* in the Mariana Islands of the western Pacific. The genus *Partula* has a disjunct distribution, encompassing islands both to the east and west of the range occupied by *Eua*. *Partula* seems to have spread both eastward and westward after the splitting of the *Partula* lineage.

Keywords: Partulidae; rRNA phylogeny; convergent evolution

1. INTRODUCTION

Partulid land snails of the Pacific Ocean islands have provided excellent opportunities to study adaptation and speciation (Garrett 1884; Crampton 1916, 1925, 1932). Many species from the Society Islands in French Polynesia have been studied, however, little is known about the evolutionary relationships between other members of the family. There are three genera within the Partulidae: *Partula*, which is widespread on the high islands of the Pacific from Belau in the west to the Austral Islands in the east; *Samoana*, which stretches from Samoa in the central Pacific to the Marquesan Islands in the east; and *Eua*, which occurs only in the Tongan and Samoan archipelagos, including the island of 'Eua, after which it was named (figure 1). *Eua* is considered to be the most ancient genus within the Partulidae on the basis of morphology, allozymes and distribution (Pilsbry 1909–1910; Kondo 1968; Kondo & Burch 1971; Johnson *et al*. 1986, 2000; Cowie 1992). A recent, large-scale phylogenetic analysis based on the nuclear large subunit ribosomal RNA (rRNA) gene (Wade *et al*. 2001) also places *Eua* in a basal position relative to the other partulids.

The three partulid genera have been separated largely by the anatomy of their genitalia (Kondo 1968). There is also a suite of characters, including the thickness of the shell, the pigmentation of the mantle, the type of mucus and the length of the tentacle, that, in the Society Islands, are loosely correlated with the generic classification. In these islands, *Samoana* often have thin, unpigmented shells, spotted mantles, long tentacles and sticky mucus, whereas *Partula* have thick, pigmented shells, shorter tentacles and less-sticky mucus. However, some *Samoana* from the Marquesas Islands, where *Partula* are absent, have thick, pigmented and banded shells, short tentacles and less-sticky mucus, characteristics more commonly associated with *Partula* elsewhere. In contrast, *P. arguta*, *P. exigua* and *P. turgida* from the Society Islands are thin shelled, with long tentacles and sticky mucus, features more commonly associated with *Samoana*. Data from allozymes suggest that the classification according to genitalia is usually correct (Johnson *et al*. 1986, 2000; but for an exception, see Johnson *et al*. 1993*a*) and that other morphological resemblances are the consequences of adaptive convergence.

In this study, we present the first use of DNA sequences to study evolution within the Partulidae. We present a phylogeny of 18 partulid taxa from all three genera, *Partula*, *Samoana* and *Eua*. The genetic relationships between taxa allow us to re-examine the origins of characteristics, such as shell thickness, that have apparently evolved more than once, and to infer the order in which partulids have colonized the Pacific.

2. METHODS

(a) *Sample collection, DNA extraction, polymerase chain reaction amplification and sequencing*

Specimens and their localities are given in table 1. Many species are now extinct in the wild (reviewed by Cowie 1992) and the only specimens available to us had been stored at −20 °C for up to 18 years before use. Several of the more recent samples were stored in ethanol.

In order to overcome problems of polymerase chain reaction (PCR) inhibition by mucopolysaccharides, we extracted DNA with CTAB (hexadecyltrimethylammonium bromide). For each sample, a small piece of foot tissue (*ca*. 25 mm^3) was sliced finely, placed in 300 µl 100 mM Tris/HCl, 1.4 M $NaCl_2$, 20 mM ethylenediaminetetraacetic acid, 2% CTAB, 0.2% b-mercaptoethanol with 0.01 mg Proteinase K, and incubated at 60 °C for 2–3 h, shaking vigorously every hour. Proteins were removed by extraction using 300 µl chloroform, centrifuging at 13 000 rpm for 15 min and then removing the aqueous layer for two further extractions using 300 µl 1:1 liquid phenol–chloroform mix followed

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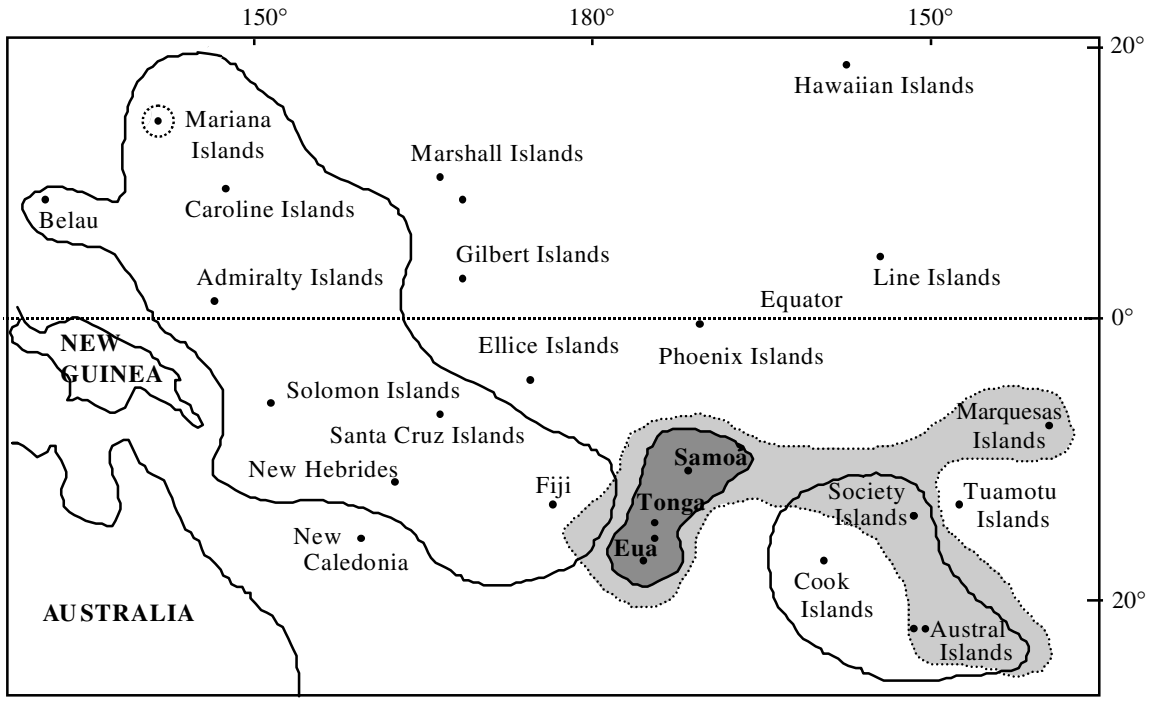

Figure 1. Distributions of *Partula* (solid line), *Samoana* (light-grey area within dotted line) and *Eua* (dark-grey area within solid line) in the Pacific (taken from Cowie 1992). Dots mark approximate locations of major Pacific archipelagos.

Table 1. *Specimens collected and their localities*

(All Society Island specimens were collected by B. C. Clarke, J. J. Murray and M. S. Johnson apart from *P. hebe* and *P. tristis* from Raiatea, which were collected by D. Clarke. *S. strigata*, *S. bellula* and *S. ganymedes* were collected by D. Clarke and W. Spencer. *S. conica* and *E. zebrina* were provided by R. Cowie. *P. langfordi*, *P. radiolata* and *P. gibba* were provided by S. Wells. Of the *Samoana* species, *S. strigata* and *S. ganymedes* have a particularly *Partula*-like appearance, with a thick shell.)

species	collecting locality
Eua	central Pacific
E. zebrina (Gould)	Samoa (Alava/Maugaloa ridge, Tutuila)
Samoana	central Pacific
S. conica (Gould)	Samoa (Alava/Maugaloa ridge, Tutuila)
	Society Islands
S. attenuata (Pease)	Moorea (Fareaito valley)
	Marquesas Islands
S. strigata (Pease)	Ua Huka
S. bellula (Hartman)	Ua Pou
S. ganymedes (Pfeiffer)	Hiva Oa
Partula	western Pacific
P. gibba (Férussac)	Saipan, Mariana Islands (Navy Hill)
P. radiolata (Pfeiffer)	Guam, Mariana Islands (Tumon Bay)
P. langfordi (Kondo)	Aguijan, Mariana Islands
P. turneri (Pfeiffer)	Tanna Island, New Hebrides
	Society Islands
P. tristis (Crampton & Cooke)	Raiatea (Tevaitoa valley)
P. hebe (Pfeiffer)	Raiatea (Hotopuu valley)
P. rosea (Broderip)	Huahine (Mahuti valley)
P. varia (Broderip)	Huahine (Tevairahi valley)
P. mooreana (Hartman)	Moorea (Atimaha valley)
P. suturalis (Pfeiffer)	Moorea (Haapiti valley)
P. taeniata (Mörch)	Moorea (Mouaputa valley)
P. mirabilis (Crampton)	Moorea (Fareaito valley)
P. tohiveana (Crampton)	Moorea (Fareaito valley)
P. otaheitana (Bruguière)	Tahiti (Vaihiria valley)

by a final extraction in the same manner as the first. DNA was precipitated using two volumes of 100% ice-cold ethanol and left on ice for 10 min. The DNA was pelleted by spinning at 13 000 rpm for 15 min; the pellet was then washed in 70% ethanol, dried, resuspended in 50 µl distilled water and stored at −20 °C; 1 µl was used as a template for PCR reactions.

Amplification of *ca.* 1460 base pairs (bp) of the nuclear rRNA genes (90 bp of the 5.8S rRNA gene, the complete internal transcribed spacer 2 (ITS2) region of 530 bp and 840 bp of the large subunit (LSU) gene) was performed using two primer pairs, LSU1 & LSU3 and LSU2 & LSU4 (details in Wade & Mordan 2000). Part of the ITS region was found to be particularly variable and so an additional primer pair (LSUmicro1 & LSUmicro2) was designed to amplify this region in some specimens. Primer sequences are as follows: LSU-1, 5′-CTAGCTGCGAGAATTAATGTGA-3′ (sense 1, position 2990–3011 of the rat rDNA sequence (GenBank accession number X00133); LSU-2, 5′-GGGTTGTTTGGGAATGCAGC-3′ (sense 2, position 4143–4162 of the rat rDNA sequence); LSU-3, 5′-ACTTTCCCTCACGGTACTTG-3′ (antisense 1, position 4221–4240 of the rat rDNA sequence); LSU-4, 5′-GTTAGACTCCTTGGTCCGTG-3′ (antisense 2, position 5085–5104 of the rat rDNA sequence); LSUmicro1, CTCAGCGTTCTTCCACTGC (sense 3, position 280–299 of the partulid ITS2 region); and LSUmicro2, TAACCTCGTCCGATCTGAG (antisense 3, position 80–99 of the partulid LSU gene).

All PCR reactions were carried out in a total volume of 50 µl containing 1 unit of Taq DNA polymerase (Boehringer Mannheim, Roche Diagnostics, Lewes, UK), 2.5 mM $MgCl_2$, 0.5 mM dNTP and 400 nM of each primer in a buffer of 10 mM Tris/HCl, 500 mM KCl at pH 8.3 and 20 °C. An initial denaturation at 94 °C for 1 min was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 or 50 °C for 20 s and extension at 72 °C for 30 s. The lower annealing temperature was used if PCR amplification did not produce enough product at the higher temperature. PCR products were prepared for sequencing using Qiagen® PCR purification columns (Qiagen, Crawley, UK) and *ca.* 50 ng in 10 µl distilled water was used for automated sequencing reactions. Direct automated sequencing used the Perkin-Elmer d'Rhodamine sequencing mix (Perkin-Elmer, Warrington, UK).

(b) *Phylogenetic analysis of sequence data*

Sequences were aligned manually within the data-analysis package GDE (Smith *et al.* 1994). Phylogenetic analyses were performed, using v. 4d65 of PAUP* (Swofford 1999), based on 1186 unambiguously aligned nucleotide sites. Phylogenies were reconstructed by maximum likelihood (Felsenstein 1981), neighbour joining (Saitou & Nei 1987) and maximum parsimony (Fitch 1971). For maximum likelihood and the distance-based methods, multiple hits were corrected using the general time-reversible model incorporating rate variation between sites. The rate matrix, base frequencies, proportion of invariant sites and shape parameter (α) for the gamma distribution, using 16 rate categories, were estimated by likelihood using an iteration procedure based on an initial neighbour-joining tree. Parameter values estimated from the initial tree were incorporated and a new neighbour-joining tree was generated. Parameters were then re-estimated and the process repeated until no further improvement in likelihood was observed. Tree searching for maximum likelihood and maximum parsimony involved a heuristic procedure with tree bisection–reconnection branch swapping. The phylogeny was rooted on *E. zebrina*. *Eua* is considered to be the basal genus within the Partulidae (Pilsbry 1909–1910; Kondo 1968; Kondo & Burch 1971; Johnson *et al.* 1986; Cowie 1992; Wade *et al.* 2001). Bootstrap resampling (1000 replicates, Felsenstein 1985) was used to assign support for particular branches within the tree.

Principal-coordinate analysis of genetic distances in the rDNA data set (assuming the Kimura two-parameter model) used the program PCOORD (Higgins 1992). Relative rates of evolution (Sarich & Wilson 1973) were estimated for the rDNA data set by calculating the difference (d) between the respective distances of pairs of sequences to the out-group, *E. zebrina*. If d is shown to be significantly different from zero, the value expected if all rates are equal, then the assumption of a constant rate is not supported.

(c) *Nucleotide sequence accession numbers*

Nucleotide sequences reported in this study have been assigned the GenBank accession numbers AF310626–AF310645.

3. RESULTS

We have amplified an approximately 1460 nucleotide fragment of the rRNA gene cluster for a total of 18 partulid taxa from the three genera, *Partula*, *Samoana* and *Eua*, distributed widely across the Pacific. Sequences were obtained from 12 species of *Partula*, from Vanuatu, the Mariana Islands, Guam, Saipan and Aguijan in the western Pacific and from Raiatea, Huahine, Moorea and Tahiti in the Society Islands of the eastern Pacific. Sequences were also obtained from five *Samoana* species, from Samoa in the central Pacific, Moorea and the Marquesas Islands in the eastern Pacific, and from *E. zebrina* from the island of Samoa. Shorter sequences (from primers LSU1 and LSU3 only) were obtained for two additional *Partula*: *P. tristis* from Raiatea and *P. mirabilis* from Moorea. The age of the samples and the conditions of storage probably contributed to the low success rate (*ca.* 5%) in extracting good-quality DNA from the specimens.

(a) *rDNA sequence variation*

The guanine and cytosine (GC) content of the sequenced region was in the range 62.2–63.4%, with a mean of 63.1% (table 2). Out of the nucleotide sites, 1186 were unambiguously aligned across all taxa, and subsequent phylogenetic analyses were based on this subset of sites. Out of these sites, 90 positions were variable, 29 of which were parsimony informative. The majority of the variable positions (69) were in the ITS2 region and the remaining 21 were in the LSU region. The ITS2 region also contained most of the insertions and deletions that were present, as well as three microsatellite loci: $(GCG)_n$, $(GTG)_n$ and $(GT)_n$ in which n ranged from 2 to 8.

The LSU region of the alignment contained six insertions–deletions: GTT (position 1267), C (position 1161), C (position 1284), GTGGT (position 1334), GA (position 1359) and AA (position 1486). Two patterns were observed, the first common to all *Samoana* and the second common to all *Partula*. Within the ITS2 region there were five indels characteristic of *Samoana* (positions 122–141, 276, 278–280, 328 and 607 in the alignment).

Within each genus there were changes in ITS2 associated with particular groups of species. In *Samoana* there were indels common to both Society Island and

Table 2. *Means and ranges of genetic distances, transition:transversion Ts:Tv ratios, and guanine and cytosine (GC) contents for the rDNA sequences*

(Distances were calculated using the six-parameter, general time-reversible model allowing for rate variation between sites using a gamma distribution (pinvar = 0.82, $\alpha = 16.3$).)

	genetic distance mean (range)	Ts:Tv mean (range)	GC content (%) mean (range)
within *Samoana*	0.009 (0.005–0.018)	1.94 (1.80–5.00)	63.2 (63.1–63.4)
within *Partula*	0.012 (0.005–0.021)	1.85 (0.78–9.00)	63.1 (62.2–63.2)
between *Samoana* and *Partula*	0.017 (0.010–0.031)	2.47 (1.57–10.00)	—
overall (*Samoana*, *Partula* and *E. zebrina*)	0.015 (0.005–0.031)	2.19 (0.71–10.00)	63.1 (62.2–63.4)

Marquesan *Samoana* (at positions 206 and 641–647) and another common to Marquesan and Samoan *Samoana* (at position 451–462); one was found in *S. conica* alone (position 374–385). There were ten indels within *Partula*, three of which occurred only in *P. turneri* (at positions 158–171, 293–299 and 500–507), one in both *P. taeniata* and *P. rosea* (at position 262), one in *P. radiolata* (at position 466–469), one shared by *P. turneri*, *P. hebe*, *P. taeniata*, *P. suturalis* and *P. tristis* (at position 536), one in *P. otaheitana* (at position 650–651), one in *P. mooreana* (at position 652), one in *P. mirabilis* and *P. tristis* (at position 669–672) and one in *P. mooreana* and *P. tohiveana* (at position 675–677). Four indels were shared between *Samoana* and *Partula* (at positions 119–121, 393–423, 555–557 and 666–668).

The most variable part of the ITS region was amplified in eight *P. taeniata*, three *P. mooreana*, two *P. tohiveana*, two *P. rosea*, three *P. varia*, three *P. langfordi*, two *S. attenuata* and two *S. bellula* specimens. Five different sequence variants were identified in the eight *P. taeniata* sequenced. Single base changes within the five *P. taeniata* types were found at positions 437 (T–A), 446 (A–G), 509 (G–C) and 528 (T–G). Indels were found at position 536 (T) and position 668 (A). Two sequence variants, differing by an A–T change at position 437 were detected in *P. tohiveana*. The two variants in *P. mooreana* also differed by an A–T change at position 437. No intraspecific variation was found in the two *P. rosea*, three *P. varia*, three *P. langfordi*, two *S. attenuata* and two *S. bellula* sequenced.

(b) *rDNA genetic distances*

Maximum-likelihood estimates of the gamma shape parameter (α) and the proportion of invariant sites (pinvar) for the 1186 nucleotide rDNA data set were $\alpha = 16.3$ and pinvar = 0.82. These values indicate that a large proportion of sites (around 80%) are conserved, and that the rates of change of the remaining sites can be approximated by a normal distribution with a small standard error. Genetic distances were calculated using the six-parameter, general time-reversible model with the above values of α and pinvar. Genetic distances and transition:transversion (Ts:Tv) ratios for intra- and intergeneric comparisons are presented in table 2. Genetic distances were, on average, slightly higher between genera than within a genus. Mean Ts:Tv ratios were slightly higher between genera than within genera.

(c) *rDNA phylogenetic analyses*

A maximum-likelihood phylogeny incorporating all sequenced taxa from the three partulid genera is presented in figure 2. The tree shows that *Samoana*, *Partula* and *Eua* separate well. This is supported by all three methods (maximum likelihood, neighbour joining and parsimony). The separation is also supported by principal coordinate analysis (figure 3). When only *Samoana* and *Partula* are considered, 1265 nucleotide sites can be aligned and the separation of *Partula* from *Samoana* is supported in 95% of bootstrap replicates (data not shown).

Samoana forms a distinct monophyletic group within the tree, supported in 80% of bootstrap replicates (based on a neighbour-joining analysis). Although only a few *Samoana* specimens were studied, they represent taxa from the easternmost and westernmost limits of their range (although a putative *Samoana* species has also been identified in Guam, far to the west, as described in Kondo (1970)). *S. attenuata* (Society Islands) and *S. conica* (Samoa) fall away from the Marquesan species, with *S. conica* occupying the most basal position (supported in 68% of bootstrap replicates in the neighbour-joining analysis). Interestingly, *S. strigata* and *S. ganymedes*, which both have a characteristically *Partula*-like thick shell, cluster together with other Marquesan *Samoana* and not with *Partula* (as also indicated by allozymes, Johnson *et al.* (2000)).

The *Partula* species form a monophyletic group (figure 2). The sequence of *P. turneri* differs more from the other *Partula* taxa than does *Eua*. As a consequence, it is likely to be inconsistently placed in bootstrap trees. If *P. turneri* is excluded from the analysis, the *Partula* clade is supported in 83% of bootstrap replicates. Within the *Partula* group there is little internal phylogenetic structure but some groups are supported by all tree-construction methods and by high bootstrap values. These include the group of *P. gibba*, *P. langfordi* and *P. radiolata* from the Mariana islands (supported in 82% of bootstrap replicates), the group of *P. rosea* and *P. varia* from Huahine (76% bootstrap support) and the group of *P. tohiveana*, *P. mooreana* and *P. otaheitana*, sinistral snails from Tahiti and Moorea (69% bootstrap support). Based on a partial alignment of 682 sites, *P. tristis* from Raiatea groups with *P. hebe*, also from Raiatea, and *P. mirabilis* from Moorea groups with the sinistral snails from Tahiti and Moorea. The overall structure of the tree produced by analysis of the shorter DNA section was similar to that produced using full-length sequences.

The branch leading to *P. turneri* in the rDNA tree is strikingly long and may lead to errors in the placement of this lineage due to 'long-branch attraction' (Felsenstein 1978; Philippe & Laurent 1998). Re-amplification and sequencing of the rDNA region was performed on this

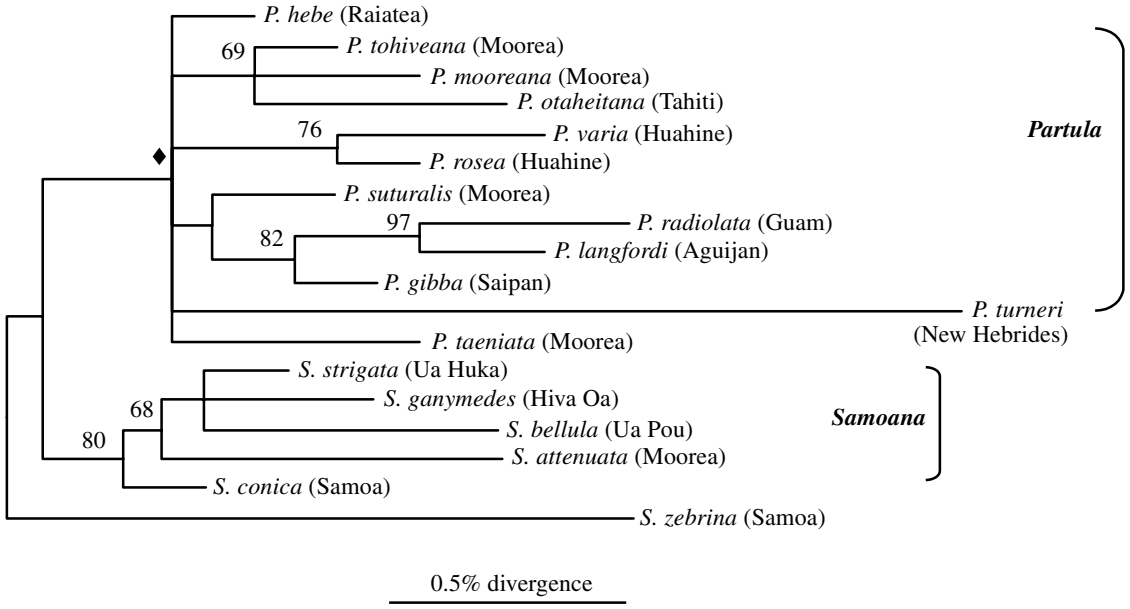

Figure 2. Evolutionary tree showing the phylogenetic relationships within the Partulidae. The phylogeny was reconstructed using maximum likelihood, based on an analysis of 1186 sites of the nuclear rDNA (general time-reversible model; pinvar = 0.82; $\alpha = 16.3$). Values on the tree indicate the bootstrap support for individual branches (based on the neighbour-joining method) in 1000 bootstrap replicates (only values above 50% are shown). The phylogeny is rooted on *E. zebrina*. *Eua* is considered to be the basal genus within the family Partulidae. Diamond symbol, support for the monophyletic *Partula* group increases to 80% when *P. turneri* is excluded.

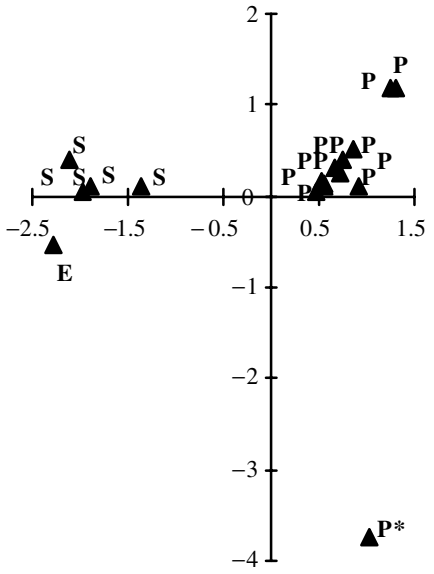

Figure 3. Principal coordinate analysis of rDNA data using PCOORD (Higgins 1992). Genetic distances were calculated using the Kimura two-parameter model. E, *Eua*; S, *Samoana*; P, *Partula*; P*, *P. turneri*. Only the first and second axes, explaining 23% and 16%, respectively, of the variation are shown. The subsequent three axes explain 12%, 10%, and 6% of the variation. Remaining axes each explain less than 5% of the total variation.

specimen to ensure that the sequence was not erroneous. The results from the two attempts were identical. Genetic distances between *P. turneri* and the other *Partula* taxa (mean 0.022, range 0.018–0.026) were, on average, lower than between *P. turneri* and *Samoana* (mean 0.026, range 0.023–0.031). Ts:Tv ratios for all comparisons within *Partula* and *Samoana*, with few exceptions, were between 1.0 and 5.0. However, all values for comparisons with *P. turneri* were in the range 0.7–1.4. Saturation in transitional changes could explain the lower ratios and would suggest that the length of the *P. turneri* branch is likely to have been underestimated rather than overestimated.

There are two alternative explanations for the existence of the long branch in *P. turneri*. The first is that there has been particularly fast evolution of the gene in this lineage. The second is that the long branch represents more evolutionary time. If so, *P. turneri* may be basal within the partulids, and could conceivably form an alternative outgroup. However, it is morphologically unremarkable and appears to be a typical *Partula*. There is no evidence from relative-rate tests of a different rate in the *P. turneri* lineage ($d < 0.014$, s.d. = 0.0075, $p > 0.05$), although the test has low statistical power (Robinson *et al*. 1998).

4. DISCUSSION

The radiation of land snails in the Pacific has interested many evolutionary biologists (Gulick 1905; Garrett 1884; Crampton 1916, 1925, 1932; Clarke & Murray 1969; Peake 1982; Johnson *et al*. 1993*b*). On a broad scale, information about the history of land snails in the region can be inferred from the distribution of species, if the relationships between them are known. On a smaller scale, the relationships between species from islands of archipelagos can be informative about how they were colonized. Land snails such as the Pacific partulids are particularly interesting because they appear to have radiated repeatedly. Moreover, the level of endemism is high and individual islands are often highly speciose (Johnson *et al*. 1993*b*). Despite the high level of variability, a suite of characteristics that includes shell thickness, shape, colour, tentacle length,

condition of the mucus and even genital structure appears to have evolved more than once.

(a) *Evolutionary relationships within the Partulidae*

The present study clearly separates the genera *Partula* (including the highly diverged *P. turneri*) and *Samoana* from each other and from *Eua*. In addition to the phylogeny presented in figure 2, the presence of six genus-specific indels in the LSU gene, which is expected to be under structure-conserving selection, is also evidence that *Partula* and *Samoana* represent separate lineages. The results agree well with the classification based on genital anatomy and with more recent work on allozymes (Johnson *et al*. 2000). rDNA sequences confirm the monophyletic nature of *Samoana*, though the *Partula* clade is less well resolved. All methods support the monophyly of *Partula*, but bootstrap support is weak due to the inconsistent placement of the long branch leading to *P. turneri*. Within *Samoana*, the most western species, *S. conica*, appears to be the most basal. The eastern species, *S. ganymedes*, *S. bellula* and *S. strigata* from the Marquesas, and perhaps also *S. attenuata*, fall into a separate group. Given that a recent, large-scale phylogenetic analysis, which includes genera outside the Partulidae, places *Eua* in a basal position with respect to the other partulid genera (Wade *et al*. 2001), the tree can be used to infer a direction of colonization by *Samoana*. Taxa from the central and eastern part of the *Samoana* range (the Society and Marquesas Islands) appear to be the most derived, and those from the west (Samoa) appear to be the most basal, suggesting that colonization has been from the west to the central and eastern Pacific. Data from allozymes also favour this hypothesis (Johnson *et al*. 2000).

Some groupings of *Partula* are strongly supported in the bootstrap analyses. They include a cluster of sinistrally coiled species (*P. tohiveana*, *P. mooreana* and *P. otaheitana*), the two species from Huahine (*P. varia* and *P. rosea*), and the western species (*P. gibba*, *P. langfordi* and *P. radiolata*). Although the original colonization of the Pacific by the partulids is believed to have been from west to east, the lack of phylogenetic structure in *Partula* provides little evidence for the direction of movement.

(b) *Evolution of similar traits in different lineages*

The repeated evolution of different adaptive traits has been observed in many groups of organisms such as *Anolis* lizards (Losos *et al*. 1998), lacustrine fishes (Taylor & McPhail 1999), Galapagos island finches (Grant 1986), geckos (Radtkey 1996) and Canary island beetles (Juan *et al*. 1996). During the evolution of land snails, the reduction of shells in slugs and characters accompanying the evolution of predation have arisen repeatedly (Watson 1915; Tillier 1989; Wade *et al*. 2001) One of the best-studied examples of parallel evolution in land snails is the G-type clausial apparatus of the Clausiliidae. This confers resistance to desiccation and to high temperatures, and has originated several times (Gittenberger & Schiltzhuizen 1996; Douris *et al*. 1998). Our molecular phylogeny (figure 2) shows that in the Partulidae, the suite of characters incorporating a thick, banded shell, non-sticky mucus and short tentacles must have arisen more than once. These observations support the evidence from allozymes of convergent evolution between *Samoana* and *Partula* (Johnson *et al*. 2000). A similar pattern of evolution may have occurred in the Pacific land snails belonging to the genus *Placostylus* (Pilsbry 1900). Thin-shelled forms occur on several islands, associated with an arboreal habit. Although they have recently been reclassified in a separate genus, it seems likely that they are an example of convergence. The repeated evolution of adaptive traits on islands implies that selection is strong enough to overcome the substantial genetic changes that may be brought about by repeated founder events and subsequent genetic drift.

(c) *Evolutionary history of the Partulidae and colonization of the Pacific*

The route by which the Partulidae colonized the Pacific is obscure. They are not represented outside the Pacific islands and their closest continental relatives are unknown. It is most likely that they have their origins either in South-East Asia or in continental Australia (Gondwanaland) and colonized the Pacific by trans-oceanic dispersal. The islands of New Caledonia and the Tongan island of 'Eua provide a possible route of transit into the Pacific. Recent geological studies have suggested that the 'Eua ridge is continental in origin and has arrived relatively recently in the Tongan archipelago in the central south Pacific (figure 1). The 'Eua ridge is thought to have broken off New Caledonia *ca*. 40 million years ago and migrated eastwards, reaching its present position less than six million years ago (Kroenke 1996). The genus *Eua*, apparently the most ancient genus, is found only on the islands of 'Eua and Samoa in the central Pacific. Consequently, New Caledonia and 'Eua may represent the route by which partulids came into the Pacific (as suggested by B. C. Clarke, personal communication). The restriction of *Samoana* to islands east of 'Eua (excepting one species reported on Guam) suggests that the *Samoana* lineage split from the other partulid taxa after the 'Eua ridge reached its current position. This is consistent with the proposed migration of *Samoana* from the west to the central and eastern Pacific. If the *Partula* split also occurred after the 'Eua ridge reached its present position in the central Pacific, then *Partula* must have spread both east and west, in contrast to the unidirectional movement of *Samoana*. One possible explanation for the difference is that *Partula* species in the western Pacific are recent arrivals, although this assumes that the highly diverged *P. turneri* is a fast-evolving species rather than a basal taxon. Western *Partula* are generally observed to be less polymorphic for shell colour and banding than those in the eastern Pacific (Pilsbry 1909–1910; Kondo 1970; Johnson *et al*. 1993*b*) and tend to occupy only the coastal fringes of islands, on lowland areas close to the sea (Crampton 1925; Peake 1968). This may suggest that they have arrived more recently, although it is also possible that they have been prevented from entering the centres of islands by competitors. *Partula* in the west are not always endemic to single islands (Kondo 1970). This is also suggestive of a recent arrival in the region.

Future molecular studies examining New Caledonian and 'Euan taxa will address the origins of the Partulidae with respect to other land-snail families, and may establish the true route of partulids into the Pacific. The use of more genes will improve the resolution of phylogenetic relationships within the Partulidae and help to reveal the patterns and processes of evolution on the Pacific islands.

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