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Mitochondrial DNA phylogeny of red and green rock lobsters (genus *Jasus*)

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Abstract. A cladistic analysis of nucleotide substitutions in the *16S* ribosomal RNA and cytochrome oxidase subunit I genes of mitochondrial DNA does not support the monophyly of species within each of the '*lalandii*' and '*frontalis*' subgroups of *Jasus*; the subgroupings may need revision. The recently described species *J. caveorum* is most closely related to *J. tristani* and *J. paulensis*, and not to *J. frontalis* with which it shares an eastern South Pacific distribution and morphological similarity. Two species, *J. tristani* and *J. paulensis*, are so genetically similar that further genetic, morphological and behavioural analyses are needed to assess their status as separate species.

Introduction

Jasus rock lobsters inhabit coasts and seamounts in the southern Indian, Atlantic and Pacific Oceans and are a morphologically homogeneous genus of economic importance. Typical of palinurids, their life cycle is triphasic: a long-lived (12–24 months) macroplanktonic (phyllosoma) larva; a short-lived (about 1 month) transitional postlarval (puerulus) settlement stage; and a benthic juvenile–adult phase which is a dominant part of the macrofauna (Booth and Phillips 1994). Except for *J. verreauxi* and *J. edwardsii*, the species have allopatric distributions (Fig. 1): *J. edwardsii*, New Zealand and southern and south-eastern Australia; *J. lalandii*, southern Africa; *J. frontalis*, Juan Fernandez Archipelago and nearby islands; *J. paulensis*, St Paul and Amsterdam Is and nearby seamounts; *J. tristani*, Tristan da Cunha Archipelago, Gough I. and Vema Seamount; and *J. verreauxi*, mid-eastern Australia and New Zealand (Holthuis and Sivertsen 1967; George and Kensler 1970; Webber and Booth 1988; Holthuis 1991). A new species, *J. caveorum*, has recently been described from a seamount in the eastern South Pacific Ocean (Webber and Booth 1995). *Jasus* larvae are found patchily throughout the upper 200 m of the southern oceans and have potentially sympatric larval distributions (Booth and Grimes 1991; Booth 1994; Pollock *et al.* 1995).

Descriptions of *Jasus* evolution have been made difficult by the apparent lack of appropriate morphological characters for phylogenetic analysis. The green rock lobster (*J. verreauxi*) is morphologically distinct from the remainder of the genus, is the only warm-water form, and is the sole member of the '*verreauxi*' group (Holthuis and Sivertsen 1967). Diagnostic characters for the six remaining species, which are all 'red' rock lobsters belonging to the '*lalandii*' group, include the amount of sculpturing on the dorsal surface of the abdominal segments, the shape and size of carapace spines and supraorbital horns and the presence or absence of turbercles and setae on the leg surfaces (George and Kensler 1970). The three species of the '*frontalis*' subgroup are the insular populations of *J. frontalis*, *J. tristani* and *J. paulensis*, and are distinguished by the size and shape of carapace spines, the shape of the transverse groove, and particularly by the relatively small amount of

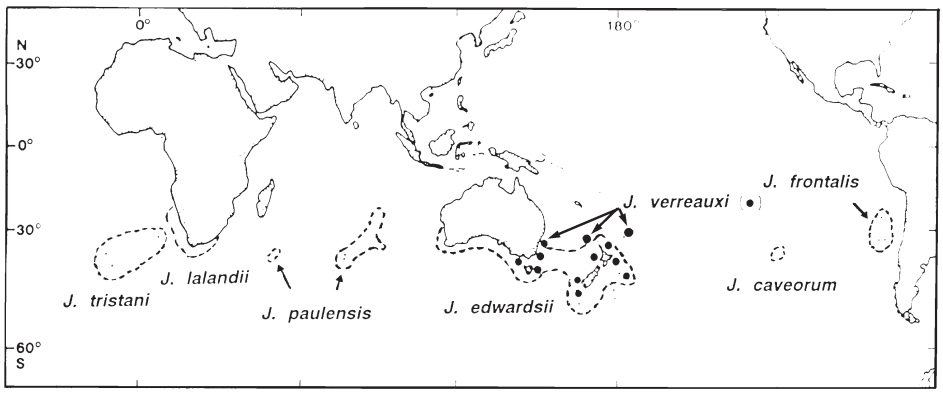

Fig. 1. Distribution of *Jasus* rock lobsters based on Holthuis and Sivertsen (1967), George and Kensler (1970), Webber and Booth (1988) and Holthuis (1991).

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dorsal abdominal sculpturing (Holthuis and Sivertsen 1967). The two '*lalandii*' subgroup species are *J. lalandii* and *J. edwardsii*, with continental distributions. *J. edwardsii* was originally separated into two species on either side of the Tasman Sea by the presence or absence of broad, pale bands on the antennal flagellum and by slightly less sculpturing on the terga (George and Kensler 1970). *J. caveorum* has yet to be assigned to a subgroup, although Webber and Booth (1995) noted that it has particularly little abdominal sculpturing.

Genetic analyses have tested aspects of *Jasus* speciation and phylogeny. Smith *et al.* (1980), followed by Booth *et al.* (1990), found no significant difference in allozyme frequencies of red rock lobsters from Australia and New Zealand (*J. novaehollandiae* and *J. edwardsii*). Proposed synomony of the two species was accepted after further analyses of morphological and other characters (Booth *et al.* 1990), and after restriction-site variation in the mitochondrial genome (Ovenden *et al.* 1992; Ovenden and Brasher 1994) showed no consistent patterns of variation across the Tasman Sea. These results shed doubt on the validity of the remaining species, because variation in selected morphological characters within Australian and New Zealand red rock lobsters was as great as that used to define species in the two subgroups of group '*lalandii*' (George and Kensler 1970). Using the mitochondrial genome, Brasher *et al.* (1992) produced the first phylogenetic analysis of the genus to show that allopatric populations of red rock lobsters were most probably biologically meaningful species. The magnitude of mitochondrial DNA (mtDNA) restriction-site variation between putative species pairs was well within the range of that normally expected for metazoan species. Brasher *et al.* (1992) were unable to test the validity of the '*lalandii*' and '*frontalis*' subgroups because *J. paulensis* and *J. frontalis* were not included in the analysis.

Descriptions of the possible evolution of species within *Jasus* have been proposed by George and Main (1967), Pollock (1990, 1993) and Newman (1991). Vicariant events such as the tectonic closure of what is now Drake Passage (between South America and Antarctica) and the sea to the south of Tasmania may have assisted the formation of species (Pollock 1990), although Newman (1991) argued that an amphitropical origin is also possible. Pollock (1990) believed that entrainment of planktonic larvae in circular, basin-specific currents, and the transition of habitats from warm to cold water may also have been important speciation mechanisms.

The aims in the present study were to use recently developed genetic techniques to establish the phylogenetic relationship among all *Jasus* species and to test evolutionary hypotheses in the genus. In particular, we assessed the validity of the '*frontalis*' and '*lalandii*' subgroups and the appropriate position for the new species, *J. caveorum*. We used cladistic analysis of nucleotide sequence from two mtDNA genes: those of the 16S ribosomal RNA subunit (*16S*) and the cytochrome oxidase subunit I (*COI*). Nucleotide sequence variation in mitochondrial genes has been used to provide detailed phylogenies that are independent of morphological characters and are free from the environmental influence that may bias topologies (Simon *et al.* 1994). MtDNA sequence data are analogous to restriction-site data, except that the latter sample small portions (four to six nucleotides) of sequence across the whole mitochondrial genome, whereas direct sequencing provides large fragments of information from the same place in each genome.

Methods

Late-juvenile or adult specimens of each *Jasus* species were collected during 1989–96 (Table 1). Leg or abdominal muscle was dissected and stored in an excess of 70% ethanol. Tissue was shipped to Hobart and stored at 4˚C. As intraspecific sequence variation had previously been surveyed in four of the seven species (Brasher *et al.* 1992), one individual per species was chosen, except for *J. edwardsii* (n = 3). *J. verreauxi* was considered divergent enough to act as an outgroup to the balance of the genus in the phylogenetic analysis; suitable specimens of *Projasus*, the most closely related genus to *Jasus*, were unprocurable, and species from another spiny lobster genus (*Panulirus*) proved to be too distantly related.

Table 1. Collection details for *Jasus* rock lobsters
Sample size is 1, except for *J. edwardsii* (n = 3) (SA = South Africa)

Species	Locality	Date
J. edwardsii	SE Tasmania	Sept. 1996
J. caveorum	Foundation Seamounts	June 1995
J. paulensis	Amsterdam I.	Dec. 1992
J. frontalis	Juan Fernandez I.	May 1993
J. tristani	Tristan da Cunha	Sept. 1989
J. lalandii	Cape Province, SA	Sept. 1989
J. verreauxi	NE Tasmania	Sept. 1996

Total DNA was extracted from specimens using a modification of the CTAB (hexadecyltrimethylammonium bromide) protocol of Folmer *et al.* (1994) which includes chloroform and isopropanol extractions. Using the polymerase chain reaction (PCR; Saiki *et al.* 1988) and total DNA as a template, we amplified two regions of the mtDNA genome near the 3′ termini of the *16S-rRNA* and cytochrome oxidase I (*COI*) genes. A Perkin Elmer Cetus thermal cycler was used with the following cycle profiles for the *16S* gene: 4 min at 94˚C followed by 35 cycles of 15 s at 94°C, 30 s at 55°C and 60 s at 72°C. For the COI gene it was: 1 cycle of 4 min at 95°C, 45 s at 60°C and 2 min at 72°C followed by 35 cycles of 30 s at 95°C, 1 min at 40°C and 2 min at 72°C. Each reaction was performed in sterile 200-μL tubes in 48 μL of reaction mix containing 20 mM Tris pH 8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% triton-X, 0.75 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 mM of each primer, 0.2 units of Taq DNA polymerase (Promega) plus 2 μL of diluted template DNA (Palumbi 1996).

Two pairs of primers were used to generate double-stranded DNA: 16sar-L, 5′ CGCCTGTTTATCAAAAACA 3′; 16sbr-H 5′ ACGTGATCTGAGTTCAGACCGG 3′ (*16S*, Simon *et al.* 1994);

L-CO1490 5′ GGTCAACAAATCATAAAGATATTG 3′; and H-CO2198 5′ TAAACTTCAGGGTGACCAAAAAATCA 3′ (*COI*, Folmer *et al.* 1994). The same primers were used for sequencing, except that for the *COI* gene only one primer was used. Sequences were obtained with an ABI automated sequencer using the chain-termination method with dye terminators.

The sequences from all taxa and from each gene were aligned manually. The Molecular Evolutionary Genetics Analysis (MEGA) software package (Kumar *et al*. 1993) was used to generate pairwise haplotype sequence divergences and standard errors by using Kimura's (1980) two-parameter model (with transitions given twice the probability of transversions). PAUP 3.1.1 (Swofford 1993) with the 'exhaustive' search option was used to search for the most parsimonious (MP) trees of haplotype relationship. Differences in total MP tree length for alternative topologies were verified with MacClade 3.06 (Maddison and Maddison 1992). The neighbour-joining (NJ) algorithm (Saitou and Nei 1987) from the MEGA package was also used to estimate phylogeny.

Results

mtDNA sequence variation

There was no evidence of insertions or deletions among the *Jasus* sequences from the *16S* and *COI* regions. The sequence of the 485 base pair (bp) region from the *16S* gene for the seven species varied at 17.5% of the nucleotide positions. For the 520 bp region sequenced from the *COI* gene, 26.7% of the nucleotide positions varied. For the protein-coding gene (*COI*), third positions in codons were the most variable (83% of changes on the MP tree), followed by first positions (16.3%), and only one change was observed in second positions. As and Ts were found at 59.5% and 62% of all sites across all taxa within the *COI* and *16S* regions respectively. The percentage of [A+T]s in this *Jasus* sequence is less than that reported for insects (*COI*, 68–75%, Lunt *et al.* 1996; *16S*, 77–82%, Simon *et al*. 1994). A high A+T bias has the potential to reduce the amount of phylogenetic information in the data because of the increased chance of homoplasious changes.

Mean estimated sequence divergences between *J. verreauxi* and congeners were 18.2% (*16S* region) and 24.0% (*COI* region, Table 2). Excluding *J. verreauxi*, mean sequence divergence between pairs of *Jasus* species varied from a high of 3.8% (*16S*) and 10% (*COI*) both between *J. caveorum* and *J. frontalis* to 0.7% (*J. caveorum*/*J. paulensis*, *16S*) and 1.1% (*J. paulensis*/*J. tristani*, *COI*). Intraspecific sequence divergence within *J. edwardsii* was 0.3% (*16S*) and 1.12% (*COI*). The pairwise sequence divergences, consistently smaller in the *16S* than in the *COI* regions, indicate that in *Jasus*, as in all other species examined to date, the *16S* gene is more highly conserved than the *COI* gene (Simon *et al*. 1994). For both the *16S* and the *COI* regions sequenced, nucleotide transitions (A to G; C to T) outnumbered transversions (A to C or T; G to C or T). For the *16S* region, transversions were observed only in comparisons of *J. verreauxi* to the remainder; all remaining nucleotide variations were transitions.

Phylogeny

An exhaustive search using equal weighting of all nucleotide substitutions returned two MP trees for the *16S* region (lengths of 95 from 85 variant characters). Bootstrap replicates (500) of the *16S* trees showed that the data did not support the monophyly of any group except one containing *J. caveorum*, *J. paulensis* and *J. tristani*. Examination of the variant characters for the *16S* sequence showed that 70% (60/85) of them were fixed between *J. verreauxi* and the

Table 2. Interspecific sequence divergence estimates for pairs of *Jasus* species from the 16S ribosomal RNA subunit (*16S*) and the cytochrome oxidase I (*COI*) regions

Above diagonal: Kimura's (1980) two-parameter model. Below diagonal: s.e.

	Species	(A)	(B)	(C)	(D)	(E)	(F)	(G)
16S								
(A)	*J. edwardsii*[A]		0.0231	0.0207	0.0231	0.0278	0.0091	0.1763
(B)	*J. caveorum*	0.0074		0.0068	0.0375	0.0137	0.0231	0.1915
(C)	*J. paulensis*	0.0070	0.0040		0.0351	0.0068	0.0207	0.1854
(D)	*J. frontalis*	0.0074	0.0096	0.0092		0.0375	0.0184	0.1793
(E)	*J. tristani*	0.0082	0.0056	0.0040	0.0096		0.0231	0.1946
(F)	*J. lalandii*	0.0046	0.0074	0.0070	0.0066	0.0074		0.1793
(G)	*J. verreauxi*	0.0221	0.0235	0.0230	0.0225	0.0238	0.0225	
COI								
(A)	*J. edwardsii*[A]		0.0929	0.0868	0.0522	0.0868	0.0403	0.2561
(B)	*J. caveorum*	0.0173		0.0645	0.0999	0.0583	0.0800	0.2279
(C)	*J. paulensis*	0.0167	0.0141		0.0937	0.0112	0.0740	0.2294
(D)	*J. frontalis*	0.0126	0.0181	0.0176		0.0937	0.0462	0.2331
(E)	*J. tristani*	0.0167	0.0133	0.0056	0.0176		0.0740	0.2294
(F)	*J. lalandii*	0.0109	0.0158	0.0152	0.0117	0.0152		0.2327
(G)	*J. verreauxi*	0.0317	0.0290	0.0294	0.0297	0.0294	0.0296	

[A]Comparisons involving *J. edwardsii* are averages of the three haplotypes sequenced

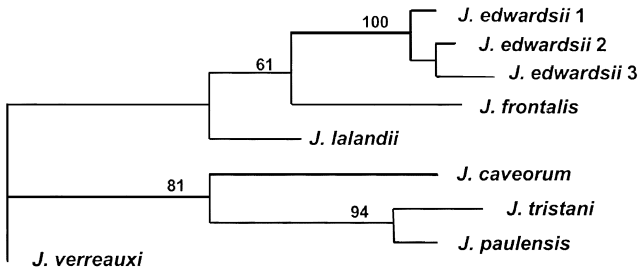

Fig. 2. One of the two most parsimonious trees of mtDNA haplotype relationships in *Jasus* rock lobsters based on equal weighting of all nucleotide substitutions in the *COI* and *16S* sequence. Branch lengths are proportional to the number of unambiguous changes. Bootstrap percentages indicated only for nodes > 50%.

remainder. Only 14% (12/85) of the variant characters were synapomorphic among the non-*verreauxi* species. Because of the lack of phylogenetic signal in the 16S sequence, the 16S and the COI data were combined for each individual.

One of the two MP trees (length of 284 from 220 variant characters), which was found by an exhaustive search of the combined 16S and COI data for the nine *Jasus* haplotypes, is shown in Fig. 2. The two MP trees varied only in the relative placement of the three *J. edwardsii* haplotypes within their monophyletic clade and their topology was identical to that of a NJ tree. The topology of the trees showed *J. caveorum* to be part of a monophyletic group that contained the sister taxa *J. paulensis* and *J. tristani*. This group was supported by 81% and 97% of bootstrap replicates on the MP and NJ (not shown) trees, respectively. The *J. caveorum/J. paulensis/J. tristani* clade was a sister group to *J. edwardsii*, *J. lalandii* and *J. frontalis*. The relative branching order of *J. edwardsii*, *J. lalandii* and *J. frontalis* was not resolved by this analysis of sequence variation in the mitochondrial genome. However, the degree of bootstrap support for the clade, 73% on the NJ tree at least, suggests that they may have shared a common ancestor which was not the same as that shared by members of the other major clade (*J. caveorum/J. paulensis/J. tristani*). MP tree length increased from 284 to 301 if *J. caveorum* and *J. frontalis* (geographic neighbours) were constrained to be the only members of a monophyletic group. Similarly, tree length increased to 290 if a monophyletic group was constructed for *J. frontalis*, *J. caveorum*, *J. paulensis* and *J. tristani* (possible '*frontalis*' subgroup). In all trees constructed, *J. verreauxi* formed a sister lineage to the remainder.

Discussion

Phylogenies produced from mitochondrial sequence variation representing all known species of the genus *Jasus* indicate that the '*frontalis*' subgroup (*J. frontalis*, *J. tristani* and *J. paulensis*) is not monophyletic. The degree of sculpturing on the dorsal abdominal surface, which has been used as a character for placing *J. frontalis*, *J. tristani* and *J. paulensis* in the same subgroup, may not be a shared similarity that is due to common ancestry. It may instead be an example of a homoplasious character that has undergone parallelism or convergence, possibly due to selective pressures. In addition to morphological characteristics, the '*frontalis*' subgroup members are also defined by occurrence on islands, as opposed to continental margins; this feature also may have arisen more than once during the evolution of *Jasus*.

These results indicate that the new *Jasus* species, *J. caveorum*, is most closely related to *J. tristani* and *J. paulensis* and not to its nearest geographic neighbour and morphologically most similar species, *J. frontalis*. The larvae of the common ancestor of *J. caveorum*, *J. tristani* and *J. paulensis* may have been able to recognize non-continental metamorphosis cues and to colonize circumpolar mid-ocean habitats during periods of maximum inter-ocean advection by west wind drift during glacial periods. Speciation may have occurred when gene flow among mid-ocean populations was reduced possibly by alterations in the west wind drift during inter-glacials.

Depending on the interpretation of the branching pattern in the other major *Jasus* clade (*J. frontalis*, *J. lalandii* and *J. edwardsii*; Fig. 2), the present classification of the '*lalandii*' subgroup may be paraphyletic because it may contain *J. frontalis*. Paraphyletic groups are biologically meaningless because they do not contain all descendants of a recent, common ancestor. Resolution may lie in including *J. frontalis* in the '*lalandii*' subgroup. A revision of '*lalandii*' and '*frontalis*' subgroupings is needed, and should include extensive cladistic analysis of morphological characters, and be accompanied by observations on the behaviour and ecology of the species. The branching pattern within the clade may be further resolved by increasing the numbers of individuals sequenced or by increasing the amount of sequence obtained from each individual from both mitochondrial and nuclear genomes.

The genetic divergence between *J. verreauxi* and its conspecifics is large (18–24%) and, in keeping with this, sub-generic status has recently been proposed for this species (*Jasus (Sagmariasus) verreauxi*; Holthuis 1991). If an inherent rate of cladogenesis is assumed, there are at least two explanations for the magnitude of this divergence: extinction may have eliminated a large number of *Jasus* species of intermediate relatedness, or evolution in *J. verreauxi* may somehow have been accelerated.

The phylogenetic analysis and the small amount of sequence divergence between *J. tristani* and *J. paulensis* indicate that these two species diverged recently from a common ancestor and are the most closely related species pair within the genus. The amount of divergence between

them is so small as to be similar to that between the three haplotypes of *J. edwardsii*. George and Kensler (1970) and Pollock (1990) emphasized the similarity between *J. tristani* and *J. paulensis* in their levels of abdominal sculpturing and in other aspects. They occupy oceanic insular and seamount habitats, are located relatively close to each other in the southern Atlantic and Indian Oceans, and may be closely linked hydrographically (Collette and Parin 1991). It is possible that *J. tristani* and J. *paulensis* are not separate species, and further analyses may suggest their synonymy in the same way as *J. edwardsii* and *J. novaehollandiae* have been synonomized.

The magnitude of mtDNA sequence divergence measured from *Jasus 16S* and *COI* regions in this study is similar to that measured by Brasher *et al*. (1992) from restriction-site variation. Those authors measured intraspecific divergence in *J. edwardsii* as 0.44–0.89% compared with the present measurements of 0.3% (*16S*) and 1.12% (*COI*). Similarly, *J. verreauxi* diverged from the rest of the genus by 14.9–16.46% as measured from restriction-site variation (Brasher *et al*. 1992) and 18.2% and 24.0% measured from the *16S* and *COI* regions respectively. The divergences calculated by Brasher *et al*. (1992) were based on an average of 39 restriction sites per haplotype, each of about six bases. This is equivalent to indirectly surveying nucleotide variation at about 230 positions, whereas the present study directly surveyed 1005 positions in two separate genes. Demonstrated correspondence such as this between the results of two popular methods of assessing mtDNA sequence variation (restriction-site analysis and PCR followed by direct sequencing) connects past and present studies, thereby increasing the power of mtDNA for phylogenetic and population analyses.

Brower (1994) has recently proposed an underlying constant mutation rate of 1.1–1.2% per million years per lineage for silent sites for arthropod mtDNA. Most of the data used in the calibration, and his own work with the neotropical butterfly *Heliconius erato*, were derived from cytochrome oxidase regions. Sequence divergence for *Jasus* cytochrome oxidase I, both among the three *J. edwardsii* haplotypes and between *J. tristani* and *J. paulensis*, is about 1.1%. On the basis of Brower's calibration and a large number of assumptions as discussed by him, the corresponding time since divergence within the *J. edwardsii* clade and within the *J. tristani*/*J. paulensis* clade may be 0.5 million years. Pollock (1990) proposed that, following local extinction during glacial maxima, recolonization of *J. tristani* and *J. paulensis* habitat from ancestral northern *J. tristani* populations occurred about 700 000 years ago; the mtDNA data may support this.

Pollock (1990) observed that allopatric speciation, where land masses in the southern ocean acted as barriers to dispersal, has not been a major evolutionary mechanism in *Jasus*. Marine species, in general, have characteristics (high vagility, large population sizes, huge distributional range and fecundities equal to millions of eggs per female) that are thought to break down the extrinsic barriers to dispersal that are essential precursors to allopatric speciation. However, it is possible that entrainment of larvae in oceanic gyres may provide significant barriers to dispersal and be implicated in speciation events (Palumbi 1994). Some planktonic invertebrate larvae are also thought to be able to control their own dispersal to some degree. A good example of this is the annual distribution of spiny lobster (*Panulirus cygnus*) larvae in south-western Australian offshore waters. These larvae are advected offshore by their apparent selection, by means of vertical movement, of appropriate currents. The reverse occurs towards the end of their larval life when metamorphosis and recruitment to inshore adult habitat occurs (Phillips and McWilliam 1986). Active selection of particular water masses as well as passive entrainment may account for some degree of population subdivision, which ultimately may lead to speciation in *Jasus*. No evidence was found for population subdivision in a survey of mtDNA variation in *J. edwardsii* across 4600 km of the southern Australian and New Zealand coastlines (Ovenden *et al*. 1992), but subdivision over short distances has been reported in other invertebrate species with planktonic larvae (e.g. the oyster *Crassostrea virginica*; Reeb and Avise 1990).

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