

# Phylogenetic relationships of mid-oceanic ridge and continental lineages of *Lasaea* spp. (Mollusca: Bivalvia) in the northeastern Atlantic

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**ABSTRACT:** Direct-developing lineages of the genus *Lasaea* are common constituents of both oceanic island and continental rocky shore crevice faunas in the eastern North Atlantic. We utilized mitochondrial gene sequence variation to flesh out the phylogenetic relationships of individuals sampled from 2 Macaronesian archipelagos (Azores, Madeira) and from downstream continental (Iberian) sites. There was no evidence for colonization of the islands by upstream western North Atlantic congeners. Of 5 *Lasaea* clades detected in Iberia, 1 was also present on Madeira, whereas 4 of the 5 had representatives on the Azores. Madeira did not share haplotypes with the other sampling locations. In contrast, the Azorean and Iberian samples shared multiple haplotypes and our phylogenetic tree topologies were consistent with a minimum of 6 inferred migration events across the >1400 km oceanic expanse separating these 2 regional populations. Three of the putative migration events involved a predominantly island clade whose topology was consistent with colonization by ancestral continental lineages, extensive island cladogenesis, and secondary downstream migrations back to the mainland. The remaining 3 inferred migration events were distributed across the tips of the phylogenetic trees, a topology consistent with evolutionarily recent migrations against the prevailing current fields. Our results indicate that the pattern of easterly surface flow in the study area may generate differentially effective dispersal filters downstream of the Azorean and Madeiran archipelagos. Evidence for countercurrent migration in marine populations should be assessed in light of the totality of surface-flow patterns in the study system, not merely the prevailing one.

**KEY WORDS:** Biogeography · 16S rDNA · Azores · Madeira · Oceanic island

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## INTRODUCTION

Understanding spatiotemporal patterns of biotic diversity represents one of the central challenges facing ecologists and systematists (Cracraft 1994). In the marine environment, this goal is complicated by the prevalence of sibling species among benthic invertebrates (Knowlton 1993, Klautau et al. 1999), by the

latent potential for long-distance dispersal events (Palumbi 1992, Carlton & Geller 1993, Lessios et al. 1998, Ó Foighil et al. 1999), and by the enormous geographic distributions attained by many taxa (Kohn & Perron 1994, Benzie 1999). Phylogenetic analyses of comparative molecular datasets can address these issues by helping to resolve marine sibling species complexes (Muricy et al. 1996, Ó Foighil et al. 1996, Davidson & Haygood 1999) and by placing the genetic structuring of marine taxa into an historical context (Avice 1994, Cunningham & Collins 1994).

Important novel phylogeographic insights have been gained from recent molecular studies of diverse marine

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invertebrates (Palumbi 1996, 1997, Chase et al. 1998, Hellberg 1998, Benzie 1999), although the emerging picture is complex. Some taxa show a genetic structure consistent with high dispersal rates among geographically distant populations, e.g., Atlantic *Eucidaris* spp. echinoids (Lessios et al. 1999). Others exhibit heterogeneous patterns in which previously unsuspected genetic discontinuities are apparent (Avisé 1994, Lavery et al. 1996, Chase et al. 1998, Gopurenko et al. 1999) and inferred that patterns of gene flow may be inconsistent with present day patterns of oceanic circulation (Palumbi et al. 1997, Benzie 1999, Lessios et al. 1999, Ó Foighil & Jozefowicz 1999). In this study, we utilize mitochondrial gene sequence variation to flesh out the phylogenetic relationships of oceanic island and continental direct-developing lineages of the intertidal clams *Lasaea* spp. in the northeastern Atlantic.

The Azorean archipelago comprises 9 major oceanic islands of volcanic origin (estimated geological ages range from 0.3 million years before present (myr bp) for Pico [Chovellon 1982] to 5.27–8.12 myr bp for Santa Maria [Féraud et al. 1984, Abdel-Monem et al. 1975 respectively]), extending 615 km from west to east across the mid-Atlantic Ridge (approximately 37 to 40° N, 25 to 31° W). It is situated 1800 and 1584 km distant from the respective nearest points on the continental margins of North America (Newfoundland) and Europe (Portugal) and 860 km from the nearest sub-aerial habitat, the Madeiran group of oceanic islands

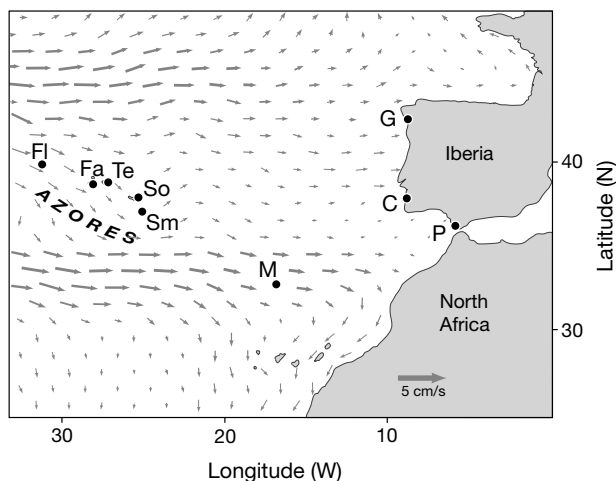


Fig. 1. Northeast Atlantic showing Azorean (Fl: Flores; Fa: Faial; Te: Terceira; So: São Miguel; Sm: Santa Maria), Madeiran (M) and Iberian (G: Galicia; C: Cabo de Sines; P: Las Palomas) sampling sites. Arrows: surface distribution of the annual mean thermal wind field, referenced to 2000 dbar (modified from Alves & de Verdière 1999). Note that 2 zones of pronounced easterly flow bracket the Azores: the Azores Current to the south, and the North Atlantic Current to the north, of the archipelago

(Borges & Brown 1999). Oceanic islands have never been connected to continental landmasses (Nunn 1994) and receive their biotas solely through dispersal from geographically distant source populations and from subsequent *in situ* diversification (Paulay 1994). The Azorean biota contains a significant number of endemic species; however, the marine fauna has explicit phylogenetic ties to the European/North African mainlands and to the other northeastern Atlantic (Macaronesian) islands (Miller 1984, Prud'homme van Reine 1988, Gofas 1990, Gosliner 1990, Moss 1992, Wirtz & Martins 1993, Lee & Vacquier 1995, Francisco-Ortega et al. 1996, de Wolf et al. 1998a,b, 2000, Krapp-Schickel & Vader 1998, Morton et al. 1998, Salas & Gofas 1998, Borges & Brown 1999, Bernardi et al. 2000). This is somewhat surprising because, based on predominant present day surface circulation patterns, the archipelago is upstream from eastern putative source populations. The Azores are bracketed by 2 eastward flowing branches of the North Atlantic Gulf Stream System: the North Atlantic Current to the north (44 to 54° N) of the archipelago and the Azores Current (a subtropical jet flowing eastward at 30 to 35° N; Alves & de Verdière 1999) to the south (see Fig. 1). A net eastward surface circulation passes through the archipelago; however, local current fields are dominated by mesoscale meanders and eddies rather than a fast steady flow (Krauss & Meincke 1982, Richardson 1985, Krauss 1986, Santos et al. 1995, Alves & de Verdière 1999).

Atlantic populations of the genus *Lasaea* lack pelagic larval development (Ó Foighil 1989), and northeastern Atlantic continental populations are composed of sympatric clones (Crisp & Standen 1988, Tyler-Walters & Crisp 1989) that represent highly divergent phylogenetic lineages (Ó Foighil & Smith 1995, Ó Foighil & Jozefowicz 1999). *Lasaea* are 'extremely abundant' in Azorean rocky shore coralline algal tufts (Bullock et al. 1990) and are also common constituents of the intertidal crevice fauna of the European mainland. Ó Foighil & Jozefowicz (1999) recently tested colonization hypotheses for North Atlantic oceanic island *Lasaea* populations by constructing molecular phylogenetic gene trees incorporating 2 continental putative source populations (Florida; Galicia, Spain) and 2 oceanic island populations (Bermuda; São Miguel, Azores). No amphiatlantic genotypes were detected. Two phylogenetically distinct *Lasaea* lineages were present on São Miguel, which nested within a regional northeastern Atlantic clade but exhibited divergent levels of genetic distinctiveness from mainland sister taxa. This result is consistent with a minimum of 2, temporally distinct, inferred colonization events of São Miguel by direct-developing eastern Atlantic continental ancestors across >1400 km of open ocean and against the prevailing

present day surface circulatory patterns in the eastern North Atlantic (Ó Foighil & Jozefowicz 1999).

Ó Foighil & Jozefowicz's (1999) limited sampling of genetically diverse eastern Atlantic *Lasaea* spp. lineages did not permit a detailed reconstruction of the frequency and tempo of gene flow among Azorean and Iberian populations. Our present study aims to flesh out the genetic inter-relationships of Mid-Atlantic Ridge and continental *Lasaea* populations by incorporating homologous molecular data from 7 additional populations, 4 on the Azorean archipelago, 1 from the Madeiran archipelago and 2 from Iberia. Specifically, we aimed to comprehensively test the preliminary finding that Azorean *Lasaea* lineages are exclusively of eastern North Atlantic continental origin (Ó Foighil & Jozefowicz 1999), to infer the minimum number and polarity of transoceanic migration events among the study populations.

## MATERIALS AND METHODS

This study incorporates specimens of direct-developing *Lasaea* spp. sampled from rocky shore mid-zone intertidal crevices at 9 North Atlantic study locations (Fig. 1). Samples from 2 of these sites (Aguíño, Ria de Arousa, Galicia, Spain; Vila Franca do Campo, São Miguel, Azores) were collected by T. Backeljau in 1995 and were genetically characterized in an earlier North Atlantic *Lasaea* phylogeographic study (Ó Foighil & Jozefowicz 1999). Additional collections were taken by the corresponding author in August 1997 from coralline algal mats at 4 Azorean (Flores, Santa Cruz das Flores; Faial, Horta; Terceira, Praia da Vitória; Santa Maria, Vila do Porto) and 2 Iberian (Cabo de Sines, Portugal; Las Palomas, Spain) locations, and in February 1999 from a single Madeiran location (Ponta de San Lourenço).

Specimens were taken from a number of microsites on each shore and pooled, prior to preservation in 95% ethanol, in order to obtain a locally representative sample of genetic diversity. From each of the 9 study populations, 30 individuals were genetically characterized by amplifying and directly sequencing a fragment (462 nucleotides, nt) of the mitochondrial large ribosomal subunit (16S) gene. A subsample of representative 16S genotypes were additionally sequenced for a 624 nt fragment of another mitochondrial (mt) gene, cytochrome oxidase III (CO III), in order to place the North Atlantic lineages into a global phylogenetic framework (Ó Foighil & Smith 1995). Details of the molecular techniques used for generating 16S and CO III sequencing templates are respectively available in Ó Foighil et al. (1996) and Ó Foighil & Smith (1995). Both strands of the target fragments were cycle-

sequenced using a DNA sequencing kit (PE Applied Biosystems, Foster City, CA), and electrophoresed on an ABI 377 automated DNA sequencer. Haplotype nomenclature was based on the geographical distribution of individual mitochondrial genotypes, e.g., the PFlSmSo haplotype co-occurred in the Las Palomas (P) sample in addition to the Azorean Flores (Fl), Santa Maria (Sm) and São Miguel (So) samples.

Initial alignments were performed using the CLUSTAL option of Sequence Navigator 1.0.1 (Kececioglu & Myers 1994) and then manually adjusted to minimize mismatches. Best trees were assessed with the maximum parsimony (MP) optimality criterion (PAUP\* 4.0b2a, Swofford 1998) using representatives of 3 other galeommatoid genera (*Kellia laperousi* [GenBank No. AF112264], *Galeomma turtoni* [GenBank No. AF215772], and *Chlamydoconcha orcutti* [GenBank No. AF215772]), as outgroups. Analyses were performed as unweighted heuristic searches using random stepwise addition with 50 replications. Inferred sequence gaps were coded as missing characters and consensus trees were constructed using the strict consensus option in PAUP. Branch support levels were estimated using nonparametric bootstrapping (500 heuristic iterations with random stepwise addition with 10 replications) and Bremer support (decay index) values (Bremer 1994). The TreeRot program (Sorenson 1996) was used to establish a constraint statement for each node in the strict consensus tree. Support indices were calculated by subtracting the number of steps in the shortest unconstrained tree from the number of steps found in each of the constrained searches.

## RESULTS

From the 7 study sites, 210 *Lasaea* spp. individuals were sequenced for the target 16S gene fragment. Combining these novel results with those of Ó Foighil & Jozefowicz (1999) for Azorean (São Miguel) and Iberian (Ria de Arousa, Galicia) *Lasaea* samples yielded a total of 270 individuals from the study area (180 Macaronesian, 90 Iberian), comprising 40 mt 16S genotypes (Table 1). The majority of haplotypes (29 of 40) were restricted to 1 of the 9 populations sampled, including all 5 Madeiran haplotypes detected. Of the 11 mt genotypes occurring in more than 1 site, 3 were restricted to the Azorean archipelago, 4 to the Iberian Peninsula and an additional 4 were recovered from both Azorean and mainland populations, including the most common haplotype in our dataset (PFaFlTe: 51 specimens).

MP analysis of the entire North Atlantic *Lasaea* 16S mt dataset (including all of Ó Foighil & Jozefowicz's [1999] mt genotypes) revealed a major phylogenetic

Table 1. *Lasaea* spp. Frequency and distribution patterns of all 40 mitochondrial 16S rDNA haplotypes encountered in the 3 Iberian (G: Galicia; C: Cabo de Sines; P: Las Palomas) and 6 Macaronesian (Fl: Flores; Fa: Faial; Te: Terceira; So: São Miguel; Sm: Santa Maria; M: Madeira) samples. \*Haplotype nomenclature used for 11 of the Azorean and Iberian mitochondrial genotypes in an earlier study (Ó Foighil & Jozefowicz 1999); Gen. No.: GenBank Accession No.

Haplotype	C	G	P	So	Sn	Te	Fa	Fl	M	Total	Gen No.
<b>Iberian</b>											
GC (IBd)*	14	3								17	AF112269
GP1 (IBa)*		3	6							9	AF112270
GP2 (IBe)*		1	1							2	AF112270
CP	4		1							5	AF215748
G1 (IBb)*		17								17	AF112271
G2 (IBc)*		4								4	AF112272
C1	5									5	AF215742
C2	1									1	AF215743
C3	1									1	AF215744
C4	1									1	AF215745
C5	1									1	AF215746
C6	1									1	AF215747
P1			3							3	AF215749
P2			1							1	AF215750
P3			3							3	AF215751
P4			1							1	AF215752
P5			1							1	AF215753
P6			1							1	AF215754
<b>Azorean/Iberian</b>											
PFaFlTe			7			18	16	10		51	AF215755
GCPFaSo (AZc)*	2	2	3	3			1			11	AF112276
PSm			1		3					4	AF215756
PFISmSo (AZa)*			1	11	2			1		15	AF112274
<b>Azorean</b>											
SmFaFlTe					5	10	7	16		38	AF215757
SmSo (AZd)*				12	20					32	AF112277
FaSo (AZb)*				2			1			3	AF112275
Fa1							1			1	AF215758
Fa2							1			1	AF215759
Fa3							2			2	AF215760
Fa4							1			1	AF215761
Fl1								2		2	AF215762
Fl2								1		1	AF215763
So1 (AZe)*				1						1	AF112278
So2 (AZf)*				1						1	AF112279
Te1						1				1	AF215764
Te2						1				1	AF215765
<b>Madeiran</b>											
M1									15	15	AF215766
M2									9	9	AF215767
M3									2	2	AF215768
M4									3	3	AF215769
M5									1	1	AF215770
Total:	30	30	30	30	30	30	30	30	30	270	

dichotomy in which each primary branch supported 3 terminal clades (Fig. 2). None of the 6 terminal clades contained haplotypes from both western (Florida/Bermuda) and eastern (Azores/Madeira/Iberia) North Atlantic sites. The western samples formed a single shallow clade sister to an Australian indirect-developing species (*L. australis*, sampled in Sydney, NSW: GenBank No. AF215774). The remaining 5 terminal

clades were all present in the eastern continental samples, and 4 of the 5 also had representatives on the Azores. A congruent topology was obtained when exemplar mt lineages (C1, GC, FLA, PFIFmSo, GP2, SO1) from each of the 6 terminal 16S clades in Fig. 2 were additionally characterized for a 624 nt portion of another mt gene, CO III, and added to a preexisting CO III dataset containing homologous sequences from

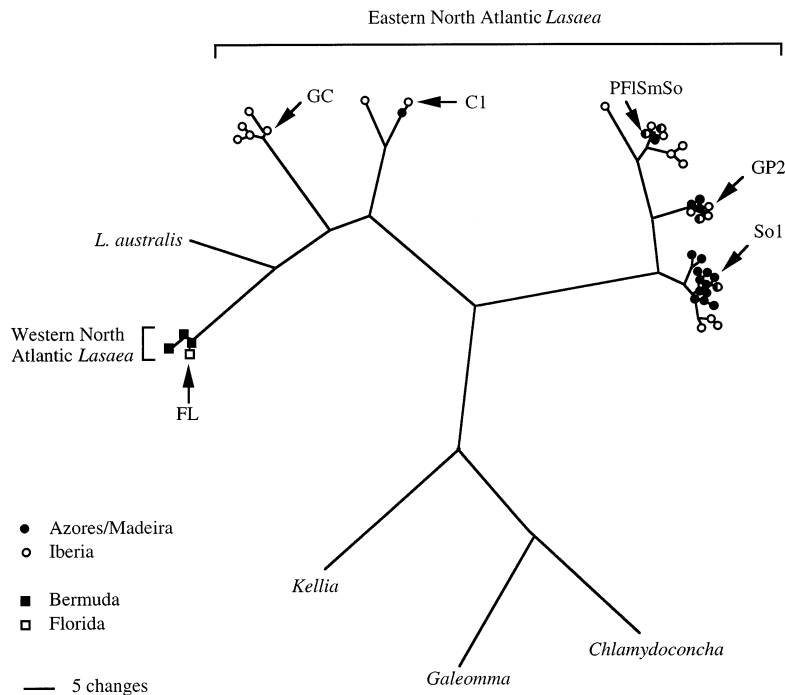


Fig. 2. *Lasaea* spp. Unrooted phylogram of 1 of the 6 most parsimonious trees (388 steps, CI = 0.623, Retention Index [RI] = 0.892) obtained with the entire North Atlantic 16S rDNA mt dataset (including all of Ó Foighil & Jozefowicz's 1999 dataset = total of 44 haplotypes representing 330 North Atlantic individuals) together with a representative haplotype of the indirect-developing Australian species *L. australis*. *Kellia laperousi*, *Galeomma turtoni* and *Chlamydoconcha orcutti* were designated outgroups, and the 5 other most parsimonious topologies differed only in minor rearrangements within the terminal clades. The positioning of an exemplar haplotype for each clade, used in global *Lasaea* phylogenetic analyses (Fig. 3), is indicated

globally sampled congeners (Ó Foighil & Smith 1995, Ó Foighil & Jozefowicz 1999, Taylor & Ó Foighil 2000). Although clearly comprised of 2 phylogenetically distinct lineages, all of the 5 exemplar eastern haplotypes were robustly, and exclusively, sisters to previously characterized (Ó Foighil & Smith 1995) north-eastern Atlantic mt genotypes from Ireland (IR) and the French Mediterranean (FEMED), including the novel 16S rDNA terminal clade represented by C1 (Fig. 3).

Further phylogenetic analyses were restricted to the Azorean/Madeiran and Iberian lineages, and Fig. 4 shows a strict consensus of the 16 most parsimonious trees (309 steps, CI = 0.676, Retention Index [RI] = 0.895) obtained for the eastern North Atlantic dataset. Note that the pronounced phylogenetic dichotomy and 5 eastern terminal clades evident in Fig. 2 were recovered. When the island/continental status of the 40 ingroup lineages was traced on the tree topology, a minimum of 6 inferred migration events across the oceanic expanse separating Macaronesian (Azores/

Madeiran) and continental populations could be made. These migration inferences are premised on the assumption that specific oceanic island populations of globally distributed taxa such as *Lasaea* spp. (Ó Foighil 1989) do not originate de novo (Paulay 1994), but represent descendants of colonists from geographically distant source populations (eastern North Atlantic continental lineages in this case).

Of the 2 major branches in the tree topology (Fig. 4), 1 supports Terminal Clades I and II which, together, encompass 8 of 40 mt genotypes and 33 of 270 individuals of the eastern North Atlantic dataset. The geographic distribution of Clade I and II haplotypes was highly skewed; 7 of 8 constituent haplotypes and 27 of 33 individuals were found in a single mainland site, Cabo de Sines (Fig. 4, Table 1). Indeed, only 3 of the 30 Cabo de Sines specimens clustered in Terminal Clades III, IV and V (Fig. 4, Table 1), which collectively constitute the large bulk (237 of 270 individuals) of the dataset. Clade I contained a single Macaronesian lineage (Fa3) which had 2 representatives in the Faial sample. Clade II was restricted to continental sites and, although dominated by Cabo de Sines specimens, it had representatives in all 3 mainland locations (Fig. 4, Table 1). Fa3 nested within an otherwise

exclusively mainland section of the 16S tree topology, and differed by 2 nucleotide substitutions from mainland lineage C1. These data are consistent with placement of an evolutionarily recent migration event, against the predominant present day current flow, on the terminal branch leading to Fa3 (Fig. 4).

The other major branch in the consensus tree topology (Fig. 4) supports Terminal Clades III, IV and V, each of which contained a mix of mainland and island haplotypes in addition to at least 1 mt genotype occurring in both locations. Terminal Clade III was a numerically substantial component of the dataset (16 of 40 mt genotypes; 104 of 270 individuals). Unlike the other 4 terminal eastern North Atlantic clades, it was predominantly composed of exclusively island haplotypes (12 of 16), including all 5 Madeiran lineages detected. The Madeiran samples were paraphyletic, and 2 of the Madeiran haplotypes (M2, M5) occupied a basal position within the clade. The most common Clade III haplotype, SmFaFIte (n = 38), was restricted to the Azores and was present in 4 of the 5 Azorean sites sampled.

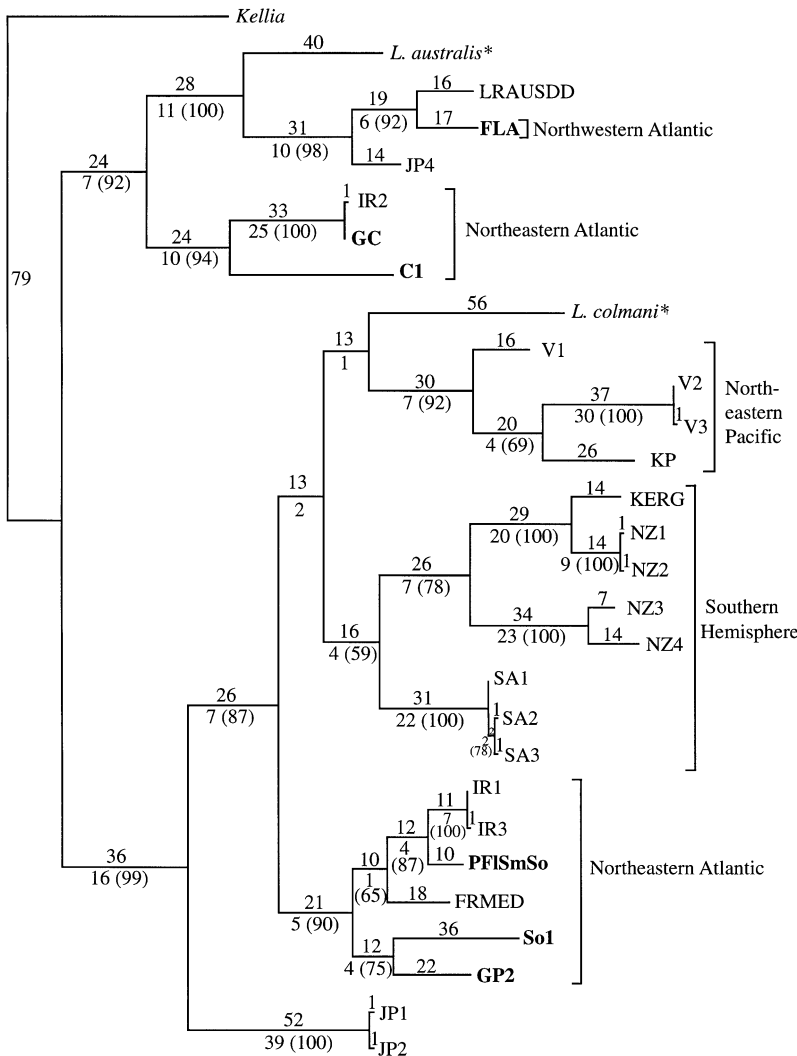


Fig. 3. *Lasaea* spp. The most parsimonious tree (1045 steps, CI = 0.444, RI = 0.675) obtained by a heuristic search for optimal trees (PAUP) using mt CO III partial sequences (624 nt) from North Atlantic exemplar lineages of all 6 terminal clades in Fig. 2 (C1, GC, FLA, PFIFmSo, GP2, SO1), together with a homologous dataset from previously studied global *Lasaea* populations (Ó Foighil & Smith 1995, Taylor & Ó Foighil 2000). *Kellia laperousi* was employed as an outgroup; \*: the 2 known sexual diploid Australian species, *L. australis* and *L. colmani*. Sampling origins of previously studied clonal, polyploid, direct-developing lineages are as follows: LRAUSDD, Australia; JP, Japan; IR, Ireland; FRMED, French Mediterranean; KERG, Kerguelen Island, southern Indian Ocean; NZ, New Zealand; SA, South Africa; V1–3 and KP, British Columbia, Canada. The respective numbers of steps are indicated above each branch and the decay index and bootstrap values supporting each node are respectively presented below the branches

Mainland haplotypes were nested in 2 derived positions within Clade III. A single haplotype (PSm) was present in both Azorean (Santa Maria) and continental (Las Palomas) samples. In another section of the Clade III topology, 3 mainland haplotypes (C2, P1 and P6) co-clustered and differed by a minimum of 4 sub-

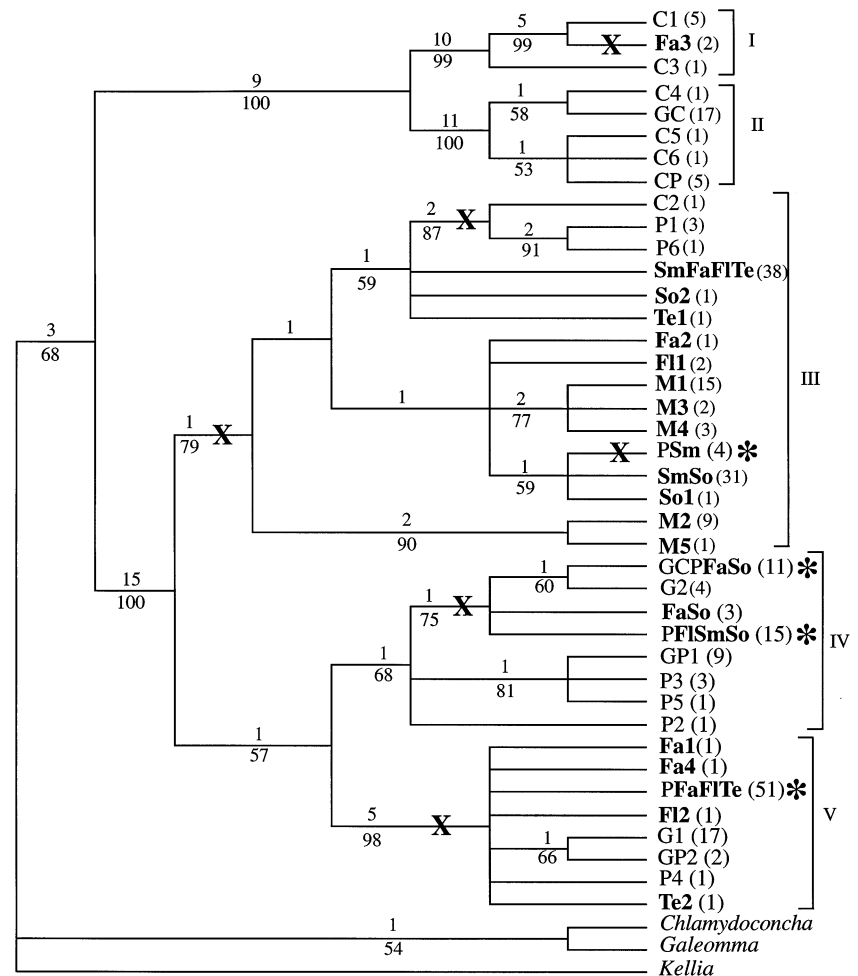
stitutions (C2/SmFaFlTe) from Clade III island haplotypes. The topology of Clade III is consistent with placement of a single inferred colonization event of the islands by ancestral eastern continental lineages along the Clade III stem branch, followed by inter-archipelago exchange and cladogenesis of island lineages and by 2 separate secondary downstream migrations back to the eastern continental margin habitats (Fig. 4).

Terminal Clade IV contained approximately 20% (8 of 40 mt genotypes and 47 of 270 individuals) of the eastern North Atlantic dataset. Of the 8 constituent haplotypes, 5 occurred exclusively in continental samples, including the most divergent lineage (P2,  $\geq 16$  steps from other clade members). A single haplotype (FaSo,  $n = 3$ ) was exclusively Azorean, and differed by 1 step from the least divergent haplotype present on the mainland (PFISmSo). However, the majority of individual clams (26 of 47) in Clade IV represent 2 mt genotypes (GCPFaSo; PFISmSo) that co-occur in both Azorean and continental locations. Clade IV lineages formed a basal polytomy incorporating 3 branches, 1 of which supported all of the mt genotypes present in the Azores (Fig. 4). We interpret this topological and genetic diversity data to indicate a minimum of 1, evolutionarily recent, countercurrent migration event along the branch supporting the mt genotypes present on the Azores (Fig. 4).

The most common haplotype in the dataset, PFaFlTe, accounted for the large majority of individual clams (51 of 75) in Terminal Clade V, and occurred in both Azorean and mainland samples. The remaining Clade V haplotypes consisted of exclusively island (4) and exclusively mainland (3) lineages. Clade V was robustly monophyletic (decay index = 5) however, the 8 constituent haplotypes differed by  $\leq 4$  substitutions and there was very little intraclade phylogenetic structure (Fig. 4). Clade V lineages have

apparently engaged in at least 1 evolutionarily recent migration between Mid-Atlantic Ridge and continental populations. Although the basal polytomy in the rooted analysis (Fig. 4) obscured the polarity of this event, the default polarity is a mainland to oceanic island migration (Paulay 1994). Coalescence analyses can provide

Fig. 4. *Lasaea* spp. Strict consensus of the 16 most parsimonious trees (309 steps, CI = 0.676, RI = 0.895) obtained by a heuristic search for optimal trees of the mt 16S rDNA dataset (Fig. 2), utilizing *Kellia laperousi*, *Galeomma turtoni* and *Chlamydoconcha orcutti* as outgroups. Prefixes indicate the location(s) from which a mitochondrial genotype was obtained (see Fig. 1) and brackets after haplotype labels indicate the number of individuals detected per mt lineage. **Bold** mt genotype label: oceanic island haplotypes; \*: mitochondrial lineages present in both oceanic island and mainland samples (their island locations are indicated by the **bold** component of their composite haplotype label). The 5 clades in this tree topology, referred to in the text, are indicated by the terminal brackets and identified by roman numerals. A minimum of 6 inferred migration events between Azorean and Iberian sites by direct-developing *Lasaea* lineages are indicated (X) on the tree topology. Decay index and bootstrap values (if >50) supporting each node are respectively presented above and below the branches



additional insights into the phylogenetic structure of closely related lineages (Donnelly & Tavaré 1986), and an unrooted parsimony analysis of Clade V was also performed. The resulting tree (Fig. 5) had a starlike topology in which there was a distinct lack of networking; 5 of the 8 haplotypes had only a single connection, and a central topological position was occupied by the dominant haplotype PFaFIte. According to coalescent theory, the probability that a given haplotype is the oldest in a population is a function of its frequency and the centrality of its positioning in topological networks (Crandall & Templeton 1993, 1996, Beckenbach 1994). Haplotype PFaFIte met these criteria best in Terminal Clade V, and received the highest heuristic outgroup weighting (Fig. 5) (Castelloe & Templeton 1994). However, because PFaFIte occurs in both continental and island sites, it cannot be utilized to assign directionality to the inferred migration event(s). We therefore retain the default Iberia to Azores polarity (Paulay 1994) for Clade V transoceanic range extension among the study sites.

## DISCUSSION

Our novel findings substantially refine and extend the preliminary phylogenetic conclusions reached by Ó Foighil & Jozefowicz (1999) for Azorean/Iberian lineages of the genus *Lasaea*. They robustly corroborate 1 of the primary findings of the earlier study: Azorean *Lasaea* samples co-clustered exclusively with eastern North Atlantic congeners and there was no evidence for transoceanic colonization by upstream western North Atlantic source-populations. According to Ó Foighil & Jozefowicz, the absence of sister western North Atlantic/Azorean *Lasaea* lineages implies that either the distances involved (>4000 km) are too great for the successful transport of intact rafting platforms from upstream source populations, or else that unfavorable Azorean environmental conditions have prevented establishment of rare upstream colonists. The Madeiran sample represents a potential control population for distinguishing among these 2 proposed limiting factors because, unlike the Azores, Madeira shares

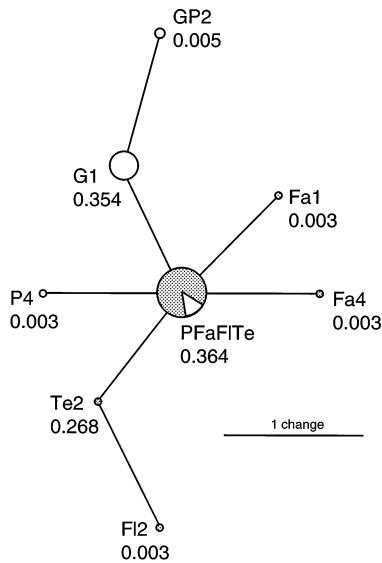


Fig. 5. *Lasaea* spp. Unrooted cladogram of the 8 16S haplotypes in Clade V (Fig. 4). Diameter of the spherical symbol associated with each haplotype is proportional to its relative frequency (Table 1), and the numerical values give the outgroup weighting estimate (Castelloe & Templeton 1994) for each haplotype. Shaded and unshaded circles, or portions thereof, indicate presences in Azorean and Iberian samples respectively. Note that the most common haplotype, PFaFiTe, which co-occurs on both the Azores and the Iberian mainland, has attained a central positioning and displays the highest outgroup weighting and the greatest degree of connectedness a result consistent with it being the oldest haplotype in the cladogram

many prominent shallow water faunal constituents with the Caribbean (Saldanha et al. 1986, Wirtz et al. 1988, Shipp 1990, Wirtz & Martins 1993, Wirtz 1998). Nevertheless, our results reveal that Madeiran *Lasaea* lineages have unambiguous phylogenetic ties to Azorean and eastern North Atlantic continental congeners, indicating that the breadth of the North Atlantic separating Madeira from upstream source populations represents an effective dispersal filter for Caribbean lineages of *Lasaea*.

The more comprehensive sampling of regional *Lasaea* populations in the present study yielded a much fuller understanding of the complex phylogenetic ties connecting Macaronesian and Iberian lineages and allowed a comparative evaluation of gene flow estimates among the study populations. Tripling the sampling intensity of Iberian populations uncovered 1 novel regional clade (the relatively rare Clade I, Fig. 4) and also mainland lineages of Clade III, the predominantly Macaronesian clade which Ó Foighil & Jozefowicz (1999) had speculated might be endemic to the Azores. Expanding the Azorean sampling effort 5-fold increased the number of known Azorean haplotypes from 6 to 22, revealed the presence of 4 of the 5

regional eastern North Atlantic *Lasaea* clades on the archipelago, and raised the inferred number of trans-oceanic Iberian/Azorean migration events from 2 to 6.

Of the minimum of 6 inferred migration events across the >1500 km of open ocean separating the Azores from Iberia, 2 were placed towards the tips of the predominantly Macaronesian Clade III (Fig. 4) and are consistent with downstream colonization of continental habitats. These are of interest because they imply that the proximal cause of the well-documented affinities of Azorean and Iberian marine biotas (Wirtz & Martins 1993) may not always be mainland to island colonization. A third, earlier, migration event is postulated on the stem branch of Clade III, and represents an inferred colonization of the Macaronesian islands by ancestral eastern continental lineages against the predominant flow of present day regional surface currents (Krauss & Meincke 1982, Pollard & Pu 1985, Richardson 1985, Santos et al. 1995, Alves & de Verdière 1999). Based on available molluscan molecular clock estimates, Ó Foighil & Jozefowicz (1999) speculated that this earlier migration may date from the late Pliocene, soon after the first appearance of the Azores. Absence of basal continental Clade III haplotypes in the expanded dataset is congruent with this hypothesis of 'early' Macaronesian colonization by ancestral Clade III lineages. The Madeiran haplotypes M2, M5 occupy the basal position in Clade III (Fig. 4), indicating that Clade III ancestral lineages may have colonized Madeira and the Azores in a stepping-stone fashion. Interestingly, Madeiran haplotypes were exclusively nested within the Clade III topology, and evidence for phylogenetic exchange between the 2 archipelagos is restricted to this clade.

The remaining 3 inferred continental/Macaronesian migration events are distributed across the tips of the consensus tree topology in Clades I, IV, and V (Fig. 4), involve Iberian/Azorean haplotypes which exhibit modest to zero genetic divergence values, and are consistent with evolutionarily recent genetic exchange. A countercurrent polarity has been assigned to all 3 inferred migrations. However, this interpretation is tenuous for Clade V, in which the presumed ancestral haplotype has a joint continental/Azorean distribution (Fig. 5), and no basal lineages intersect the robust stem branch leading to the Terminal Clade V polytomy (Fig. 4). Our Clade V dispersal polarity assignment would be falsified if further genetic characterization uncovered basal lineages in the Azores, but would be corroborated by the discovery of such in continental populations.

The inferred countercurrent dispersal events in Clades I, IV, and V are inherently enigmatic because they involve the Azores, but apparently not Madeira (based on the available data), and because gene tree



topologies alone often cannot distinguish among potential dispersal modes (Palumbi 1997), e.g., human introduction and/or spontaneous rafting events. Ó Foighil & Jozefowicz (1999) entertained a potential role for anthropogenic introduction to account for the presence of Clade IV haplotype GCPFaSo in their São Miguel (eastern Azorean island group) and Galician samples. Subsequent discovery of this haplotype also in Faial (middle Azorean island group) makes its historical introduction to the Azores by humans less likely, because of the low probability of 2 independent introductions, or of extensive inter-island spread of these direct-developers, within the past half-millennium (Duncan 1972). Human-mediated introduction is least plausible for other mt genotypes, such as PFaFITE (the most common haplotype in the dataset) and PFISmSo, which were recovered from 1 mainland site (Los Palomas) and from a variety of Azorean islands spanning the extremities of the archipelago (Fig. 1, Table 1). Furthermore, it is not clear why anthropogenic introduction might be pervasive in the Azores but undetectable in Madeira. Our novel results indicate that the pattern of prevailing easterly surface flow in the study area may generate differentially effective dispersal filters downstream of the Azorean and Madeiran archipelagos.

How might rafted Iberian *Lasaea* propagules reach the Azores against the prevailing surface flow? Recent detailed studies of circulation patterns in the Azores Front Current System have revealed it to be a region of complex and dynamic mesoscale circulation (Santos et al. 1995, Cromwell et al. 1996, Fernandez & Pingree 1996, Alves & de Verdière 1999, Pingree et al. 1999). The Azores Current (AzC) proper is a permanent shallow, almost zonal, subtropical jet which transports an estimated 13 Sv ( $\text{Sv} = 10^6 \text{ m}^3 \text{ s}^{-1}$ ) (Alves & de Verdière 1999). Associated with this jet, and just north of it, is the smaller (2 Sv) westward-flowing and eddy-driven subsurface Azores Countercurrent (AzCC, not evident in Fig. 1) with maximum intensity between 200 and 500 m depth (Alves & de Verdière 1999). Significantly, elements of the westward flow may also extend, at least temporarily, to surface waters (Santos et al. 1995, Alves & de Verdière 1999). For instance, Cromwell et al. (1996) used altimetry and hydrographic data to detect persistent westward surface flow over a 3 mo period in late winter/early spring at  $35^\circ \text{N}$ ,  $28^\circ \text{W}$ , which they attributed to retroflexion of water from the northern edge of the AzC. The AzC extends eastward to the Gulf of Cadiz (Alves & de Verdière 1999), and northern-edge retroflexion events might act as an intermittent westward conduit for rafted *Lasaea* propagules entrained in this section of the Azores Front Current System. Madeira lies within the main eastward flow of the AzC (Fig. 1), and is therefore less

likely to be routinely approached by eddy-generated surface westerly flow at the northern edge of this subtropical jet. At our present state of knowledge, northern AzC edge retroflexion events represent a potential explanation for the prevalence of *Lasaea* haplotypes PFaFITE ( $n = 51$ ), PFISmSo ( $n = 15$ ) and GCPFaSo ( $n = 11$ ) in the Las Palomas and Azorean archipelago samples.

Molecular studies of a variety of marine taxa have inferred patterns of gene flow that are inconsistent with present day oceanic circulation patterns (Palumbi et al. 1997, Benzie 1999, Lessios et al. 1999). Our Macaronesian *Lasaea* samples provide a striking example of such incongruence in that we find no evidence for colonization by upstream western North Atlantic lineages. Benzie (1999) referred to similar patterns of range extension by coral reef species as 'ghosts of dispersal past', implying that they are likely to have resulted from dispersal under different current patterns to those prevailing at present. Such an interpretation may be pertinent in the case of the predominantly Macaronesian Clade III, found in both Madeira and the Azores, in which the Iberian haplotypes occupy derived positions (Fig. 4). However, it does not seem to apply to Clades IV and V, which are dominated by haplotypes that co-occur in Azorean and Iberian samples (GCPFaSo, PFISmSo, PFaFITE, Fig. 4), a result consistent with evolutionarily recent migration against the prevailing surface current polarity. We propose that eddy-driven westward retroflexion of surface water from the northern edge of the AzC may underlie this unexpected pattern. The comparative phylogenetic isolation of our Madeiran sample indicates that impingement by the northern edge of the AzC may be a more important factor in enhancing contemporary westward *Lasaea* gene flow than the relative geographic proximity of the individual Macaronesian island groups to continental source-populations.

It is not clear at present how specific our findings are to the taxon, and to the particular section of the North Atlantic Gyre System, we investigated. If edge retroflexion of the AzC is an important regional mechanism of generating westward gene flow, then we predict that additional phylogeographic characterization of Macaronesian/Iberian benthic marine taxa will yield patterns congruent with our *Lasaea* results. In the meantime, we suggest that evidence for evolutionarily recent, countercurrent gene flow in marine populations be assessed in light of the totality of surface-flow patterns in the study system, not merely the prevailing one.

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