Large-scale gene flow in the barnacle *Jehlius cirratus* and contrasts with other broadly-distributed taxa along the Chilean coast

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Abstract

We evaluate the population genetic structure of the intertidal barnacle *Jehlius cirratus* across a broad portion of its geographic distribution using data from the mitochondrial cytochrome oxidase I (COI) gene region. Despite sampling diversity from over 3000km of the linear range of this species, there is only slight regional structure indicated, with overall Φ_{CT} of 0.036 (p<0.001) yet no support for isolation by distance. While these results suggest greater structure than previous studies of *J. cirratus* had indicated, the pattern of diversity is still far more subtle than in other similarly-distributed species with similar larval and life history traits. We compare these data and results with recent findings in four other intertidal species that have planktotrophic larvae. There are no clear patterns among these taxa that can be associated with intertidal depth or other known life history traits.

Introduction

A persistent question in marine biogeography and population biology involves the interaction of species life history, geographic range, and trait or genealogical diversity within that range. In some cases, genealogical diversity or "structure" (Wares 2016) *within* a species is informative of mechanisms that act to limit other species' distributional ranges (Dawson 2001; Wares 2002; Wares *et al.* 2001). Of course, these studies often find that organisms with limited larval or juvenile dispersal have greater amounts of structure and less extensive ranges, but there are often exceptions (Marko 2004). It is the variation among species, and the exceptions to the rules, that offer continued opportunity to understand marine diversity.

Early approaches to comparative phylogeography (Dawson 2001; Wares 2002; Wares & Cunningham 2001) focused primarily on regions of co-diversification of intraspecific lineages, e.g. the regions across which species were likely to exhibit structure. Subsequently, Marko (2004) noted that even when species had apparently identical life history and dispersal mechanisms, the distribution of a species across habitats (e.g. intertidal height) could influence their persistence in distinct glacial refugia. However, certainly to understand these associations more taxa should be compared, and Kelly and Palumbi (2010) made simple comparisons of diversity for 50 species along the Pacific coast of North America to suggest that species high in the intertidal were more likely to exhibit spatial genetic structure than those at lower depths.

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The particular spatial structure of these species varies; however, there is often concordance of population structure among species (Pelc *et al.* 2009; Small & Wares 2010) on this coast. Other regions that have been similarly explored have fewer instances of strong population structure aside from regions that are also biogeographic transitions (Altman *et al.* 2013; Díaz-Ferguson *et al.* 2009). One example of this was recently published by Haye et al. (2014), looking at species with short-dispersing larval forms around the well-characterized biogeographic transition near 30°S latitude along the coast of Chile. Again, the structure of diversity within species was informative to the mechanisms – including shifts in upwelling intensity and nutrient availability (Navarrete *et al.* 2005) – that may limit the distribution of other taxa.

Evaluating broad-scale diversity structure on the Chilean coast is of key interest as there are so many oceanographic and biogeographic comparisons to be made between this well-studied coastline and the well-studied Pacific coast of North America (Navarrete *et al.* 2008). However, until recently there were few data available for species that spanned most of the length of the Chilean coastline, and that could span across both major biogeographic transitions (the other is closer to 42°S; (Thiel *et al.* 2007) etc.).

Some of the first such work was done in the direct-developing gastropod *Acanthina monodon* (Sanchez *et al.* 2011) and another gastropod *Concholepas concholepas*

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(Cardenas et al. 2009). In *Acanthina*, which has low dispersal potential among locations, strong concordance of intraspecific diversity with the 30°S biogeographic boundary was found, but association with the 42° boundary was less clear. Nevertheless, statistically significant genetic structure and shifts in phenotypic diversity are associated with this region. The gastropod *Concholepas concholepas*, on the other hand, has high potential for pelagic larval dispersal, is similarly distributed along the coast of Chile, but exhibits no significant genetic structure at all (Cardenas *et al.* 2009). These contrasts are wholly in line with predictions based on larval life history.

Recently, large data sets have become available for other commonly encountered taxa in the Chilean intertidal. Microsatellite data were analyzed in the mussel *Perumytilus purpuratus* (Guiñez *et al.* 2016), which both spawns gametes and has a long-lived planktotrophic larva, and this ecosystem engineer exhibited significant structure with two main lineages (separated at close to 40°S) and isolation by distance within each lineage. Similarly, Ewers-Saucedo et al. (2016) explored genetic variation in the high intertidal barnacle *Notochthamalus scabrosus*, with nauplius larvae that have high pelagic larval dispersal potential, and found two primary lineages that mirror the dominant biogeographical pattern of Chile: in the northern Peruvian region only one lineage is found, while both are found in the Intermediate Area that represents the overlap of the Peruvian and Magellanic regions, and only the southern lineage is found south of 42°S. Another barnacle, the edible *picoroco* (*Austromegabalanus psittacus*) exhibits only slight structure along

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most of the Chilean coast (Pappalardo *et al.* 2016), but nevertheless the structure is statistically significant and associated with the northern biogeographic transition.

To these data we add one more layer: Zakas et al. (2009) had explored mitochondrial sequence population structure in the high intertidal barnacle *Jehlius cirratus*, a species that is biologically and ecologically very similar to *Notochthamalus* but found slightly higher in the intertidal (Lamb *et al.* 2014; Shinen & Navarrete 2010, 2014). Zakas et al. (2009) found that unlike *Notochthamalus*, there was very little apparent genetic structure in *J. cirratus*. However, that analysis comprised only a small section of the Chilean coast from ~28-34°S. Here, we expand the sampling of *J. cirratus* to include diversity from ~3500km of coastline, including most of the known distribution (Häussermann & Försterra 2009). As chthamalid barnacles have a propensity to harbor cryptic genetic diversity (Dando & Southward 1981; Meyers *et al.* 2013; Tsang *et al.* 2008; Wares *et al.* 2009; Zardus & Hadfield 2005), we specifically look for any phylogeographic structure that may add to our understanding of coastal biodiversity in Chile. We then more directly compare the whole-coast data described above for the ecological implications of the population structure identified within and among taxa.

Methods

Specimens of *J. cirratus* were collected from the intertidal in 2004-2013. Sequences of cytochrome oxidase I (n=153) from Zakas et al (2009) were used in this study (Genbank GU126073 – GU126226); additional sequences (n=187) were generated from subsequent samples collected in 2011-2013 using PCR methods as in Zakas et al. (2009) and were submitted to NCBI via Bankit. Samples were mostly collected in central Chile (Figure 1, Table 1), but this additional effort also added substantially to information from northern Chile and northern Patagonia.

After quality control and alignment of sequence data using CodonCode Aligner v6.0.2 (CodonCode Corporation), data were formatted for analysis using Arlequin (Excoffier *et al.* 2005) to identify population structure. Pairwise Φ_{ST} was calculated for all sites and compared to a matrix of pairwise geographic distance for signal of isolation by distance (Wright 1943). In addition, analysis of molecular variance (AMOVA) was performed to identify maximal structure along the coast as in (Dupanloup *et al.* 2002) and Zakas et al (2009), using an iterative approach for K contiguous spatial groups, increasing K until there were no significant patterns of Φ_{SC} within each group. From the results of AMOVA, a haplotype network was generated using PopArt (http://popart. otago.ac.nz). Haplotypes were coded by sample location and by regions separated by the iterative AMOVA results that maximize Φ_{CT} to visually identify components of diversity associated with each regional group. Population diversity was also assessed at each sampled location; nucleotide diversity (π) and haplotype diversity (H) are estimated at each location

using Arlequin.

Results

New sequences are archived in Genbank under accession numbers KX014910 -
KX015034. Site-specific diversity is presented in Table 1; pairwise values of Φ_{ST} are
presented in Table 2. Only a single sequence was recovered from the northernmost
collection site of Arica, so this sequence was included in the Antofagasta sample
(results identical when excluded) for statistical purposes. Values of Φ_{ST} are very low
and in general not statistically significant (Table 2); the only exceptional locations
are Guanaqueros (30°S) and Pichilemu (34°S), each of which tend to exhibit higher
differentiation from a broader set of other locations. Testing these results for a
pattern of genetic isolation by distance was not significant (p>0.05).

Although only slight structure is exhibited along the Chilean coast in *J. cirratus*, there
is significant regional structure detectable with the increased power of sampling at
that regional scale. Our implementation of spatial AMOVA (Zakas et al. 2009)
recovered two contrasts for K=2 regions in which $\Phi_{CT} > 0.035$ and p<0.01, though
similar results are found if the separation among regions is near to either of these
locations (Table 3). These local maxima in Φ_{CT} separate Guanaqueros (30°S) and
sites to the north from all locations to the south; and Pichilemu (44°S) and all sites
to the south from all locations to the north. No significant Φ_{SC} is exhibited in these
comparisons. If K=3 groups are chosen using these same delineations, Φ_{CT} is
comparable (0.03661, p <0.001).

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From these results, a haplotype network (minimum spanning tree) is presented in Figure 2; "northern" diversity (from Guanaqueros northward), "southern" diversity (including Pichilemu and southward sites), and "central" diversity (locations in between), for visualization.

Discussion

As noted in Zakas et al. (2009) there is only slight population structure in *J. cirratus*. Previous efforts had also noted that using alternate statistics such as Hudson's (Hudson 2000) Snn also recovered no signal of structure or pattern of isolation by distance (Wares 2014). Here, we do identify statistically significant structure that is roughly associated with the 30°S biogeographic transition between the Peruvian and "Intermediate" zones, and there may also be structure further south – but not associated with the boundary at 42°S.

Excluding the direct developer *A. monodon* from further consideration, the studies reviewed earlier and current study include 5 intertidal species with high larval dispersal potential that are distributed and analyzed along the length of the Chilean coast. Unfortunately, there is no clear pattern associated with intertidal depth; the species with no or slight population genetic structure (*J. cirratus*, this study; *A. psittacus*, Pappalardo *et al.* 2016; *C. concholepas*, Cardénas *et al.* 2009) are in the highest reaches of the intertidal (*J. cirratus*) and the low intertidal (*A. psittacus* and *C. concholepas*). The two species that exhibit significant structure, each with two primary lineages and evidence for isolation by distance within each lineage, are in

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the high-to-middle intertidal (*N. scabrosus*, Ewers-Saucedo *et al.* 2016; *P. purpuratus*, Guiñez *et al.* 2016).

Clearly a sample of only 5 taxa is insufficient for statistical consideration. However, what we can indicate is that all 3 barnacles (*A. psittacus*, *J. cirratus*, and *N. scabrosus*) have at least some signal associated with the 30-32° oceanographic transition in upwelling (Lagos *et al.* 2005; Navarrete *et al.* 2005); however the two molluscs, the mussel *P. purpuratus* and abalone *C. concholepas* do not. The association of genetic structure with the southern biogeographic boundary near 42°S (Thiel *et al.* 2007) is far more varied; other taxa with shorter distributional ranges that span this biogeographic transition, such as the mussel *Mytilus chilensis*, show little spatial structure at mitochondrial or other putatively neutral markers (L. Besch and Bockrath, unpublished; Areneda *et al.* (2016) but can be distinguished among different coastal environments by outlier markers (Araneda *et al.* 2016) and expression profiling (Núñez-Acuña *et al.* 2012). Ewers-Saucedo *et al.* (2016) note that environmental transitions and current-mediated larval dispersal in this region, where trans-oceanic currents are separated as they reach the continental margin (Acha *et al.* 2004), are likely to transport regionally-differentiated diversity along a broad swath of this coastline. Thus, identifying concordant intraspecific diversity patterns among taxa may require a different analytical approach that is model-driven as in Ewers-Saucedo *et al.* (2016).

There is an expanding interest in exploration of genetic diversity within and among

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regional populations of intertidal species along the coast of Chile (see Haye et al. 2014 for a recent synthesis). Such data are being used to explore the underlying causes of biogeographic transition (Cardenas *et al.* 2009; Ewers-Saucedo *et al.* 2016; Zakas *et al.* 2009), to inform management and aquacultural concerns (Haye & Munoz-Herrera 2013; Núñez-Acuña *et al.* 2012; Pappalardo *et al.* 2016), and better understand how the dynamics of a coastal ocean influence local diversity (Aiken & Navarrete 2014; Broitman *et al.* 2001; Navarrete *et al.* 2005). For example, even with variation among the data and taxa evaluated here, there is a concordance between the genetic transitions exhibited in these taxa and regions of strong upwelling along coastal Chile (Navarrete *et al.* 2005).

What remains unsatisfying is our ability to predict – based on what we know of life history, ecology, and other parameters of a given taxon – which species are likely to exhibit structure across a certain region. Haydon et al. (1994) first noted the problem of both stochastic and deterministic contributions to biogeography and overall population structure. Certainly some 'significant' phylogeographic structure may simply represent the interaction of genealogical processes and modest limitations on gene flow (Irwin 2002). However, the most direct contrast of the taxa included here involves the barnacles *N. scabrosus* and *J. cirratus*, which are ecologically nearly indistinguishable (Lamb *et al.* 2014; Shinen & Navarrete 2010, 2014) with little known distinction in larval life history. In fact, though *N. scabrosus* exhibits significant phylogeographic structure (Ewers-Saucedo *et al.* 2016), the larvae of *N. scabrosus* appear to require longer times in the plankton and longer times for cyprid metamorphosis than *J. cirratus* (Venegas *et al.* 2000). Whether the

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cause for this contrast in population structure is ecological, physiological, or simply

fortune remains unclear.

Acknowledgments

Literature Cited

Acha EM, Mianzan HW, Guerrero RA, Favero M, Bava J (2004) Marine fronts at the
continental shelves of austral South America: Physical and ecological
processes. *J. Marine Systems* **44**, 83-105.

Aiken CM, Navarrete SA (2014) Coexistence of competitors in marine
metacommunities: environmental variability, edge effects, and the dispersal
niche. *Ecology* **95**, 2289-2302.

Altman S, Robinson JD, Pringle JM, Byers JE, Wares JP (2013) Edges and overlaps in
Northwest Atlantic phylogeography. *Diversity* **5**, 263-275.

Araneda C, Larraín MA, Hecht B, Narum S (2016) Adaptive genetic variation
distinguishes Chilean blue mussels (*Mytilus chilensis*) from different marine
environments. *Ecology and Evolution*.

Broitman BR, Navarrete SA, Smith F, Gaines SD (2001) Geographic variation in
southern Pacific intertidal communities. *Marine Ecology Progress Series* **224**,
21-34.

Cardenas L, Castilla JC, Viard F (2009) A phylogeographical analysis across three
biogeographical provinces of the south-eastern Pacific: the case of the marine
gastropod Concholepas concholepas. *Journal of Biogeography* **36**, 969-981.

Dando PR, Southward AJ (1981) Existence of Atlantic and Mediterranean Forms of
Chthamalus-Montagui (Crustacea, Cirripedia) in the Western Mediterranean.
Marine Biology Letters **2**, 239-248.

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Dawson MN (2001) Phylogeography in coastal marine animals: a solution from California? *J. Biogeography* **28**, 723-736.

Díaz-Ferguson E, Robinson JD, Silliman BR, Wares JP (2009) Comparative Phylogeography of East Coast American Salt Marsh Communities. *Estuaries & Coasts* **DOI 10.1007/s12237-009-9220-6**.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Mol. Ecol.* **11**, 2571-2581.

Ewers-Saucedo C, Pringle JM, Sepúlveda HH, Byers JE, Navarrete SA, Wares JP (2016) The oceanic concordance of phylogeography and biogeography: a case study in *Notochthamalus. Ecology and Evolution* **in press**.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47-50.

Guiñez R, Pita A, Pérez M, Briones C, Navarrete SA, Toro JE, Presa P (2016) Present-day connectivity of historical stocks of the ecosystem engineer Perumytilus purpuratus along 4500 km of the Chilean Coast. *Fisheries Research* **7/16**, 322-332.

Häussermann V, Försterra G (2009) *Marine Benthic Fauna of Chilean Patagonia* Nature in Focus, Santiago, Chile.

Haydon DT, Crother BI, Pianka ER (1994) New directions in biogeography? *Trends in Ecology and Evolution* **9**, 403-406.

Haye PA, Munoz-Herrera NC (2013) Isolation with differentiation followed by
expansion with admixture in the tunicate Pyura chilensis. *BMC Evol Biol* **13**,
252.

Haye PA, Segovia NI, Munoz-Herrera NC, Galvez FE, Martinez A, Meynard A, Pardo-
Gandarillas MC, Poulin E, Faugeron S (2014) Phylogeographic structure in
benthic marine invertebrates of the southeast Pacific coast of Chile with
differing dispersal potential. *PLos One* **9**, e88613.

Hudson RR (2000) A new statistic for detecting genetic differentiation. *Genetics* **155**,
2011-2014.

Irwin DE (2002) Phylogeographic breaks without geographic barriers to gene flow.
Evolution **56**, 2383-2394.

Kelly RP, Palumbi SR (2010) Genetic structure among 50 species of the northeastern
Pacific rocky intertidal community. *PLos One* **5**, e8594.

Lagos NA, Navarrete SA, Véliz F, Masuero A, Castilla JC (2005) Meso-scale spatial
variation in settlement and recruitment of intertidal barnacles along the
coast of central Chile. *Mar. Ecol. Prog. Ser.* **290**, 165-178.

Lamb EA, Leslie HM, Shinen JL (2014) Both like it hot? Influence of temperature on
two co-occurring intertidal barnacles in central Chile. *Journal of Experimental
Marine Biology and Ecology* **453**, 54-61.

Marko PB (2004) 'What's larvae got to do with it?' Disparate patterns of post-glacial
population structure in two benthic marine gastropods with identical
dispersal potential. *Molecular Ecology* **13**, 597-611.

Meyers M, Pankey MS, Wares JP (2013) Genealogical approaches to the temporal

origins of the Central American Gap: Speciation and divergence in Pacific

Chthamalus. Revista Biologia Tropical **61**, 75-88.

Navarrete SA, Broitman BR, Menge BA (2008) Interhemispheric comparison of

recruitment to rocky intertidal communities: pattern persistence and scales

of variation. *Ecology* **89**, 1308-1322.

Navarrete SA, Wieters EA, Broitman BR, Castilla JC (2005) Scales of benthic-pelagic

coupling and the intensity of species interactions: From recruitment

limitation to top-down control. *PNAS* **102**, 18046-18051.

Núñez-Acuña G, Tapia FJ, Haye PA, Gallardo-Escárate C (2012) Gene expression

analysis in *Mytilus chilensis* populations reveals local patterns associated

with ocean environmental conditions. *J. exp. mar. Biol. Ecol.* **420-421**, 56-64.

Pappalardo P, Pitombo FB, Haye PA, Wares JP (2016) A Rose by Any Other Name:

Systematics and Diversity in the Chilean Giant Barnacle Austromegabalanus

Psittacus (Molina, 1782) (Cirripedia). *Journal of Crustacean Biology* **36**, 180-

188.

Pelc RA, Warner RR, Gaines SD (2009) Geographical patterns of genetic structure in

marine species with contrasting life histories. *J. Biogeogr.* **36**, 1881-1890.

Sanchez R, Sepulveda RD, Brante A, Cardenas L (2011) Spatial pattern of genetic and

morphological diversity in the direct developer *Acanthina monodon*

(Gastropoda: Mollusca). *Marine Ecology Progress Series* **434**, 121-131.

Shinen JL, Navarrete SA (2010) Coexistence and intertidal zonation of chthamalid barnacles along central Chile: Interference competition or a lottery for space? *Journal of Experimental Marine Biology and Ecology* **392**, 176-187.

Shinen JL, Navarrete SA (2014) Lottery coexistence on rocky shores: Weak niche differentiation or equal competitors engaged in neutral dynamics? *American Naturalist* **183**, 342-362.

Small ST, Wares JP (2010) Phylogeography and marine retention. *Journal of Biogeography* **37**, 781-784.

Thiel M, Macaya E, Acuna E, Arntz W, Bastias H, Brokordt K, Camus P, Castilla J, Castro L, Cortes M, Dumont C, Escribano R, Fernandez M, Gajardo J, Gaymer C, Gomez I, Gonzalez A, Gonzalez H, Haye P, Illanes J, Iriarte J, Lancellotti D, Luna-Jorquerai G, Luxoroi C, Manriquez P, Marin V, Munoz P, Navarrete S, Perez E, Poulin E, Sellanes J, Sepulveda H, Stotz W, Tala F, Thomas A, Vargas C, Vasquez J, Vega J (2007) *The Humboldt Current System of northern and central Chile* CRC Press, Boca Raton, FL.

Tsang LM, Chan BKK, Wu TH, Ng WC, Chatterjee T, Williams GA, Chu KH (2008) Population differentiation in the barnacle Chthamalus malayensis: postglacial colonization and recent connectivity across the Pacific and Indian Oceans. *Mar. Ecol. Prog. Ser.* **364**, 107-118.

Venegas RM, Ortiíz V, Olguín A, Navarrete SA (2000) Larval development of the intertidal barnacles *Jehlius cirratus* and *Notochthamalus scabrosus* (Cirripedia: Chthamalidae) under laboratory conditions. *J. Crust. Biol.* **20**, 495-504.

Wares JP (2002) Community genetics in the Northwestern Atlantic intertidal. *Molecular Ecology* **11**, 1131-1144.

Wares JP (2014) Why not do phylogeography on every chthamalid barnacle? The case of Jehlius cirratus. *PeerJ PrePrints* **2**, e596v592.

Wares JP (2016) Population structure and gene flow. In: *Encyclopedia of Evolutionary Biology* (ed. Kliman RM), pp. 327-331. Academic Press, Oxford.

Wares JP, Cunningham CW (2001) Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution* **55**, 2455-2469.

Wares JP, Gaines SD, Cunningham CW (2001) A comparative study of asymmetric migration events across a marine biogeographic boundary. *Evolution* **55**, 295-306.

Wares JP, Pankey MS, Pitombo FB, Gómez Daglio LE, Achituv Y (2009) A "shallow phylogeny" of shallow barnacles (*Chthamalus*). *PLos One* **4**, e5567.

Wright S (1943) Isolation by distance. *Genetics* **28**, 139-156.

Zakas C, Binford J, Navarrete SA, Wares JP (2009) Restricted gene flow in Chilean barnacles reflects an oceanographic and biogeographic transition zone. *Marine Ecology Progress Series* **394**, 165-177.

Zardus JD, Hadfield MG (2005) Multiple origins and incursions of the Atlantic barnacle *Chthamalus proteus* in the Pacific. *Molecular Ecology* **14**, 3719-3733.
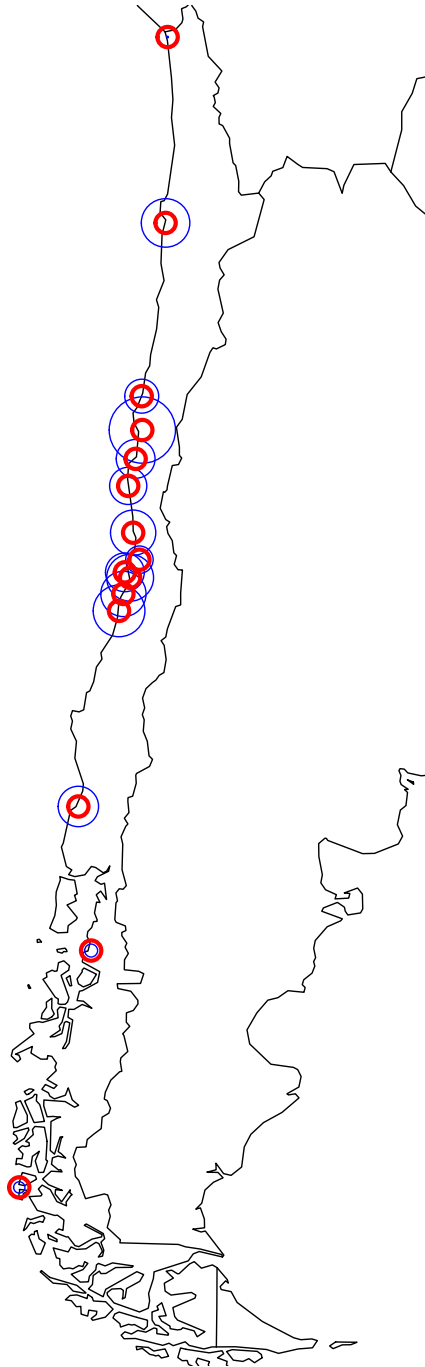
Figure 1. Sample locations (red dots) and log sample size (blue circles) indicate sampling of *J. cirratus* along the Chilean coast. Additional information in Table 1.
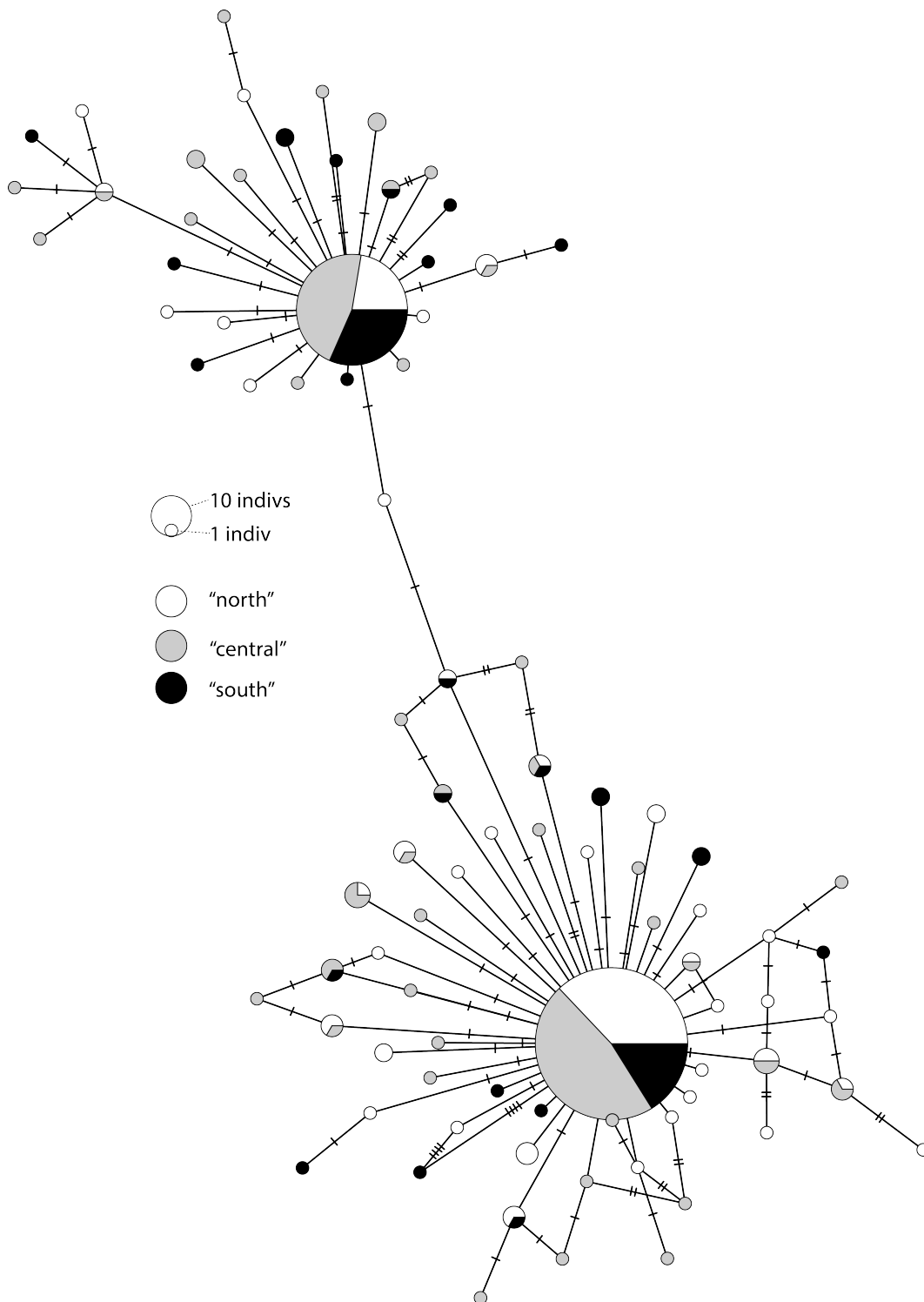
Figure 2. Minimum-spanning tree of mitochondrial COI diversity in *J. cirratus*. Regional designations are generated from maximal Φ_{CT} values along the coast.

Table 1 Collection sites, number of individuals per sampling site (n) and summary statistics of genetic variability for *Jehlius cirratus*.

Site (South Latitude)	sampled	haplotypes	haplotype diversity	nucleotide diversity (π)
Antofagasta/Arica (18.49°)	31	27	0.978±0.020	0.012±0.009
Huasco (28.46°)	41	25	0.945±0.022	0.009±0.003
Temblador (29.40°)	21	16	0.948±0.040	0.009±0.006
Guanaqueros (30.20°)	24	18	0.942±0.040	0.011±0.006
Punta Talca (30.95°)	23	14	0.893±0.052	0.008±0.004
Los Molles (32.25°)	28	23	0.971±0.024	0.011±0.007
Monte Mar (32.95°)	28	24	0.987±0.014	0.011±0.006
El Quisco (33.45°)	29	25	0.988±0.013	0.010±0.006
Las Cruces (33.49°)	17	16	0.993±0.023	0.012±0.006
Matanzas (33.95°)	24	20	0.975±0.024	0.011±0.006
Pichilemu (34.42°)	32	24	0.958±0.025	0.010±0.008
Niebla (39.85°)	25	17	0.957±0.024	0.014±0.008
Añihue (43.85°)	8	7	0.964±0.077	0.016±0.009
Isla Madre de Dios (50.42°)	7	3	0.667±0.160	0.009±0.004

Table 2 Pairwise Φ_{ST} values among sites (indicated as header) for mitochondrial COI sequence data in *J. cirratus*. Statistically significant (p<0.01) comparisons are bolded and in blue.

Antofagasta	Huasco	Temblador	Guanaqueros	Punta Talca	Los Molles	Monte Mar	El Quisco	Las Cruces	Matanzas	Pichilemu	Niebla	Añihue
-0.10721												
-0.02397	-0.10075											
-0.06007	0.00344	-0.09836										
-0.00797	-0.07271	0.01272	-0.01539									
-0.01641	-0.09486	-0.01873	-0.07157	0.00493								
-0.07084	0.01909	-0.06296	**0.05349**	-0.0808	-0.03693							
-0.17547	-0.01582	-0.18666	0.02576	-0.1819	-0.15953	-0.03391						
-0.00509	-0.06798	0.00201	-0.02185	-0.02005	0.01097	-0.08597	-0.16477					
-0.07137	0.01015	-0.05613	**0.04841**	-0.0811	-0.04482	-0.0131	-0.02592	-0.07314				
0.06509	0.01927	**0.10959**	**0.10642**	0.01976	**0.085**	-0.01377	-0.10077	**0.04336**	-0.02223			
-0.03313	-0.0885	0.01678	-0.04187	-0.04029	-0.02781	-0.09641	-0.21442	-0.03887	-0.10159	-0.01699		
-0.01175	0.02556	-0.00176	0.07232	-0.03869	0.00933	-0.03799	-0.02988	-0.04939	0.00464	0.02127	-0.05271	
-0.0777	0.01877	-0.04544	0.08615	-0.11043	-0.08512	0.04286	-0.00793	-0.07119	0.03113	-0.09806	-0.13056	0.04426

Table 3 Iterative AMOVA for K=2 regions of sequence diversity. Site is listed as dividing *that location and all sites to the north* from all locations to the south. The northernmost 2 sites (Arica, Antofagasta) were pooled for analysis as were the southernmost 2 sites (Añihue, Madre de Dios). Strongest values of Φ_{CT} (by magnitude and p-value) indicated in bold. Similar value of Φ_{CT} (0.0366, p<0.001) is obtained with K=3 and the regions separated as in Figure 2.

Site	Φ_{CT}	p-value
Huasco	0.01406	0.16
Temblador	0.01977	0.11
Guanaqueros	**0.03679**	**<0.001**
Punta Talca	0.02623	0.03
Los Molles	0.03215	<0.01
Monte Mar	0.02998	0.01
El Quisco	0.02896	<0.01
Las Cruces	0.03463	<0.01
Matanzas	**0.03615**	**<0.005**
Pichilemu	0.00076	0.55
Valdivia	0.00635	0.64