Population diversity and structure in *Lipophrys pholis* (Blenniidae, Teleostei) in Central Portugal, variation across a decade

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Objective: To evaluate the genetic composition and diversity of the shanny *Lipophrys pholis* in a Central Portugal location (S. Pedro do Estoril), and their variation over a decade.

***Lipophrys pholis**:*
- typical benthic rocky intertidal fish
- dispersion limited to long larval stage (29 days in the plankton)

Methods:
- Fish collected in S. Pedro do Estoril (Central Portugal) in 2003 (N=30), 2013 (N=97) and 2014 (N=99)
- DNA extraction from dorsal fin and amplification of a fragment from the mitochondrial control region.
- Genealogy analyses (haplotype network).
- Genetic composition and diversity indices.
- Demographic analysis.

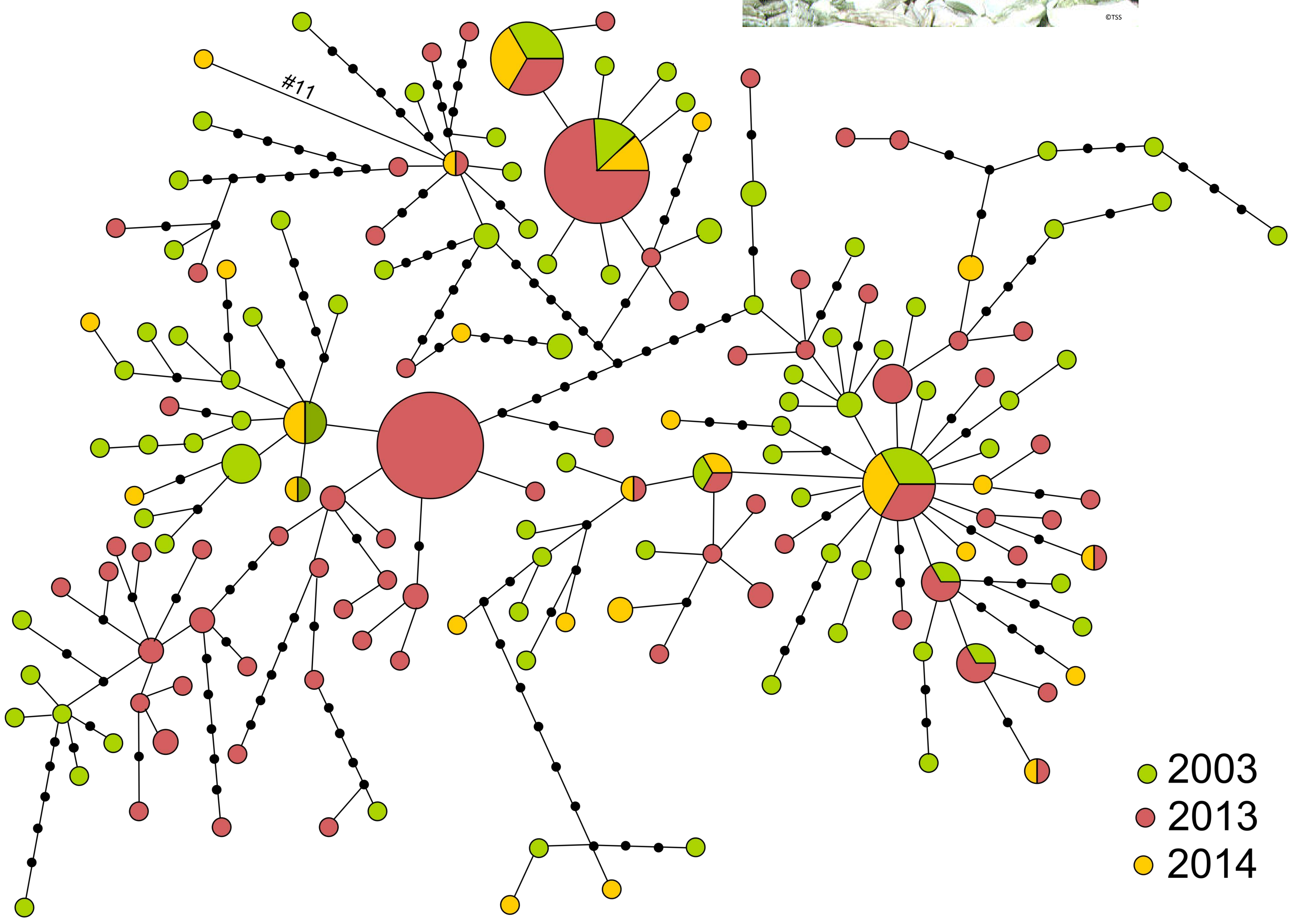

Fig.1 - Minimum spanning network showing relationships among haplotypes of *L. pholis* samples.

Table 1. Diversity measures for the three temporal samplings of *L. pholis* : number of sequences (N), number of haplotypes (Nh), percentage of private haplotypes (%Ph), number of polymorphic sites (S), haplotype diversity (h), nucleotide diversity (π) and mean number of pairwise differences (PD).

Sampling site	N	N_h	%Ph	S	h	π	*PD*
2014	99	88	93.18	84	0.997	0.031	11.97
2013	97	73	87.61	69	0.988	0.028	10.84
2003	30	26	69.23	57	0.991	0.031	11.86
All	226	171		108	0.995	0.030	11.50

Table 2. Demographic parameters of *L. pholis*. F_s (Fu's), D (Tajima's), t (time in years), N_0 and N_1 (female effective population size before and after the expansion), N_f (female effective population size), g (growth rate), $N_{1\%}$ (age of population, accessed as the age at which N_f drops below 1%)and t_{MRCA} (time to most recent common ancestor). * significant at p<0.05

		2003	**2013**	**2014**
Neutrality tests	Fu's F_s	-11.081*	-24.393*	-19.997*
	Tajima's D	-0.914	-0.786	-0.808
Mismatch distribution	t (ky)	376	363	394
Demographic model	N_0	0	0	46
	N_1	1,269,765	1,013,786	1,272,885
Lamarc	g	181	211	127
	N_f	3,431,965	1,461,855	1,441,615
	$N_{1\%}$ (ky)	509	435	726
BEAST	t_{MRCA} (ky)	497	554	641

The network represents a **deep genealogy** with **multiple levels of diversification**, with some haplotypes being separated by more than fifty mutational steps (Figure 1). Only 12 haplotypes (out of 171) were shared between sampled periods. It is interesting to notice that in recent years some of the previously inferred missing haplotypes were sampled.

The genetic diversity indices showed little variation among sampling periods and were generally high (Table 1). There is no significant differences between the 3 sampling periods (AMOVA: $F_{ST} = 0.008$; *P*-value = 0.074). Nevertheless, significant differences were found between 2013 and 2014 ($F_{ST} = 0.013$; *P*-value = 0.028) - **temporal changes in the gene pool composition need to be considered in population structuring of *L. pholis*** (>99% of variation within temporal samples)

Demographic parameters (neutrality tests, effective population size, growth rate, age of population, and time to most recent common ancestor) revealed **little variation** between sampling periods (Table 2).

The genealogy of this shanny population showed a **large expansion in the Pleistocene** (363-394Ky), clearly older than the last glacial maximum.

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