

The crustose red algal genus *Peyssonnelia* (Peyssonneliales, Rhodophyta) in the Azores: from five to one species

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The family Peyssonneliaceae comprises a worldwide group of non-calcified to calcified, crust-forming red algae of great ecological significance. Of the genera currently recognized in the family, *Peyssonnelia* has been widely considered to contain the largest number of species, with five members reported for the Azores. Using rbcL as a molecular marker, we here report on the taxonomic identity of recent collections of Peyssonneliaceae from the Azorean islands of São Miguel, Graciosa and Pico, and compare those specimens in a worldwide context. Only a single *Peyssonnelia* species, *P. squamaria*, is confirmed for the Azorean archipelago, with three different haplotypes. Although the populations in the Azores are genetically different from those occurring in the Mediterranean, this separation appears to be relatively recent.

Key words: Biodiversity, haplotypes, North Atlantic, phylogeny, rbcL

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INTRODUCTION

The family Peyssonneliaceae (Denizot, 1968), recently elevated to ordinal rank (Krayesky et al. 2009), comprises a worldwide group of non-calcified or calcified, crust-forming red algae that are of great ecological significance (Peña & Barbara 2013). Of the genera currently recognized in the family, *Peyssonnelia* (Decaisne, 1841) has been considered the richest in terms of species number (Pueschel & Saunders 2009). A combination of vegetative and reproductive characters are currently used to distinguish species of *Peyssonnelia*, such as location and degree of calcification, variations in crust adherence, morphology, anatomy and differences in reproductive development (Maggs & Irvine 1983). The identification of *Peyssonnelia* species is challenging, resulting in a number of species usually underestimated or overestimated (Dixon & Saunders

2013). Comparative morphology and DNA sequence analysis confirm that most species originally reported as belonging in *Peyssonnelia* in fact belong to other genera (Fredericq et al. 2014) within the Peyssonneliales (Krayesky et al. 2009).

Peyssonnelia sensu stricto (following Krayesky et al. 2009) represents species characterized by a hypothallus that cuts off additional cells forming multicellular rhizoids (Krayesky 2007). Recent studies based on worldwide collections indicate that species of *Peyssonnelia sensu stricto* have a narrow distribution and do not occur in most ocean basins, for example, the Gulf of Mexico (Krayesky et al. 2009; Fredericq et al. 2014).

Based on general flora studies, five species of *Peyssonnelia* have been reported for the Azores (Parente 2010): the generitype *P. squamaria* ((S.G. Gmelin) Decaisne, 1842) described from Italy; *P. rubra* ((Greville) J. Agardh, 1851) described from the Ionian Sea, Greece; *P. polymor-*

pha ((Zanardini) F. Schmitz in Falkenberg, 1879) described from the Adriatic Sea; *P. coriacea* (Feldmann, 1941) described from Tangier, Morocco; and *P. rosa-marina* (Boudouresque & Denizot, 1973) described from Port-Cros, Mediterranean France (see Guiry & Guiry 2015). Only one species of *Peyssonnelia*, *P. squamaria* (Fig. 1), was recognized for the Azores by Kravesky (2007) and Kravesky et al. (2009) after examination of multiple collections.

Using *rbcL* as a molecular marker, we report on the taxonomic identity of recent collections of Peyssonneliaceae from the islands of São Miguel, Graciosa and Pico in the Azores, and discuss the connection between the various Azorean haplotypes. The identity of the Azorean specimens is compared with those of *Peyssonnelia sensu stricto* in a worldwide context.

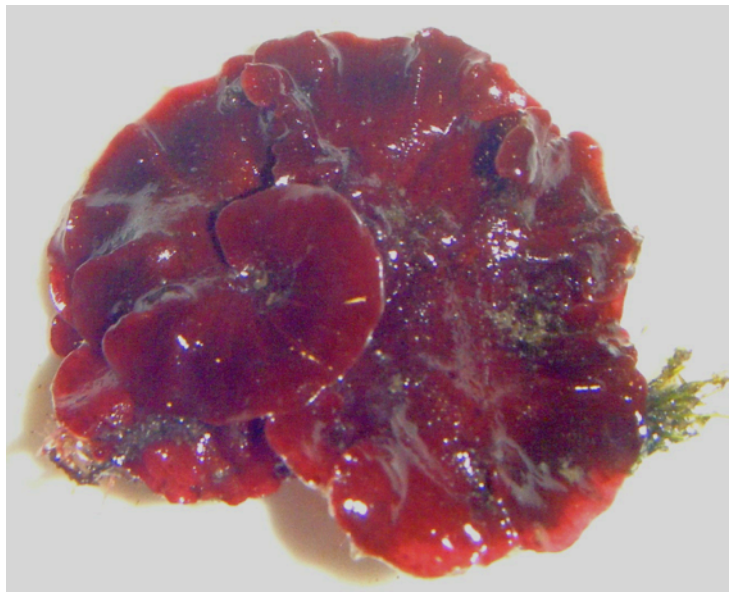


Fig. 1. Habit of *Peyssonnelia squamaria* from the Azores.

MATERIAL AND METHODS

Samples of Peyssonneliaceae were collected in the Azores and the Mediterranean during low tide or by snorkeling and SCUBA diving. Samples were kept in coolers until processed, and then dried in silica gel. Dried samples were ground with mortar and pestle, and total DNA was extracted using DNeasy Plant mini Kits (Qiagen Valencia, CA, USA). All the resulting DNA extracts were deposited in the Seaweed Lab at the University of Louisiana at Lafayette (ULL). Chloroplast-encoded *rbcL* gene sequences were amplified using PCR primers and protocols described in Lin et al. (2001) and Gabriel et al. (2010). Resulting PCR products were gel-purified

and sequenced in both directions using Bigdye terminator v 3.1 (Life Technologies Grand Island NY, USA) on the ABI 3130xl genetic analyzer at ULL and assembled with Sequencher v. 5.2 (Gene Codes Corporation). Newly acquired sequences, in addition to 14 *rbcL* sequences downloaded from GenBank, were then manually aligned in Mega v 5.2.2 (Tamura et al. 2011). The subsequent alignment was analyzed in Partitionfinder (Lanfear et al. 2012) to determine the best fitting model of evolution and data partition. The analysis resulted in the selection of the General Time Reversible model plus gamma and a proportion of invariable sites applied separately to each codon position on the basis of the three information criteria, i.e. Akaike information criteri-

on with correction (AICc), Akaike information criterion (AIC) and Bayesian information criterion (BIC). The alignment was analyzed by Maximum likelihood (ML) as implemented by RAXML v 2.4.4 (Stamatakis 2006) with the above models and partition scheme with 1000 restarts to find the tree with the lowest likelihood score and 1000 Bootstrap (BS) replications.

A Bayesian MCMC (Markov Chain Monte Carlo) was also applied to the aligned dataset using MrBayes v. 3.2.5 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The Bayesian analysis consisted of two independent runs of 5 million generations with sampling every 1,000 generations for a total of 10,002 trees. Convergence was visualized using Tracer v1.6 (Rambaut & Drummond 2007) and the first 10 percent of the trees of each run was discarded as the *burn-in*. The resulting Bayesian Posterior Probabilities derived from the consensus tree were mapped on the ML tree. A distance matrix was also resolved from the branch lengths of the ML tree using the function *cophenetic.phylo* of the APE Package in R (Paradis et al. 2004).

The resulting distance matrix was used to find species boundaries in a stand-alone version of Automatic Barcode Gap Discovery (ABGD). General Mixed Yule Coalescence (GMYC) model, as implemented by the *Splits* Package in R (Fujisawa & Barraclough 2013) with a single threshold model, was also used to determine species boundaries. The requisite ultrametric tree for the GMYC analysis was generated in Beast v 1.8.1 (Drummond et al. 2012) using a relaxed log-normal clock with a constant population coalescent as a prior and the best fitting model and partition as described above. MCMC Chains were run for 10 million generations with sampling every 1000th generation resulting in 10,000 trees. The quality of the run was assessed in Tracer v1.6 (Rambaut & Drummond 2007) to ensure that effective sample size (ESS) values were >200 with the default *burn-in* (1,000 trees). Tree annotator v 1.8.1 (Drummond et al. 2012) was used to summarize the resulting 9001 trees after burning, targeting the maximum clade credibility tree with preserved node heights. A statistical parsimony method implemented in the TCS 1.21 software

(Clement et al. 2000) was used to infer genealogical relationships among haplotypes. The maximum number of differences resulting from single substitutions among haplotypes was calculated with 95% confidence limits, treating gaps as missing data.

RESULTS

The final dataset was composed of 34 *rbcL* sequences of Peyssonneliaceae, 20 of which were newly generated, with 31 sequences representing hitherto confirmed *Peyssonnelia* species and three sequences of *Sonderopelta capensis* ((Montagne) Kraysky, 2009) and *S. coriacea* (Womersley & Sinkora, 1981) (Table 1). *Sonderopelta* (Womersley & Sinkora, 1981) was selected as an outgroup based on previous studies that established the genus as a sister taxon of *Peyssonnelia* (Kato et al. 2006; Kraysky et al. 2009; Dixon & Saunders 2013). [Note: *Sonderopelta* has been viewed to be an illegitimate name by Wynne (2011); however, *Sonderophycus* (Denizot, 1968) is not a valid name (Womersley & Sinkora 1981) in agreement with Article 41.5 of the International Code of Botanical Nomenclature (2012, Melbourne Code)].

The results of the ABGD and GMYC analyses showed the existence of six species of *Peyssonnelia* within the dataset: *P. replicata* (Kützing, 1847), *P. bornetii* (Boudouresque & Denizot, 1973), *P. rubra* and three closely related species initially identified as *P. squamaria* (Fig. 2). All Azorean collections belonged to *P. squamaria*, along with Mediterranean representatives from Catalonia (Spain), Sicily (Italy), Malta and Greece (not shown). Its two sister clades were only observed in the Mediterranean, and are here referred to as *P. coriacea* from Malta and *P. polymorpha* from Sicily. Sequence JX969797, referred to as *P. squamaria* by Dixon & Saunders (2013), corresponds to material of Sicily that has a unistratose hypothallus layer in contrast to the 2-layered hypothallus of *P. squamaria* (Boudouresque & Denizot, 1975).

Besides the generitype, four species that are also true *Peyssonnelia* were only collected in the

Table 1. Summary of specimens included in the present study. Asterisk marks newly generated sequences. Letters within brackets refer to the haplotypes of *Peyssonnelia squamaria* in Fig. 3.

Extraction	Collection number	Taxa	Locality	Depth	Collection date	Collector	Accession number
K163	SMG-05-242	<i>Peyssonnelia squamaria</i> (A1)	São Miguel, Azores	intertidal	09-jul-2005		EU349177
K164	SMG-05-152	<i>Peyssonnelia squamaria</i> (A1)	São Miguel, Azores	8 m	08-oct-2005		EU349178
LAF4197	MD0002162	<i>Peyssonnelia squamaria</i> (A1)	Pico, Azores	26 m	10-aug-2011	D. Gabriel, J. Micael	KR732897*
LAF6390	MD0002066	<i>Peyssonnelia squamaria</i> (A1)	São Miguel, Azores	intertidal	10-jul-2011	M.I. Parente, D. Gabriel	KR732900*
LAF6393	MD0001927	<i>Peyssonnelia squamaria</i> (A1)	São Miguel, Azores	15 m	29-oct-2010	A. Botelho	KR732899*
LAF6395	MD0001933	<i>Peyssonnelia squamaria</i> (A1)	São Miguel, Azores	12 m	05-jul-2011	A. Botelho, M. Dionísio, C. Lopes	KR732898*
K229	SMG-06-88	<i>Peyssonnelia squamaria</i> (A2)	São Miguel, Azores	16 m	11-aug-2006		EU349174
K231	GRW-06-100	<i>Peyssonnelia squamaria</i> (A3)	Graciosa, Azores	15 m	22-jun-2006		EU349176
K230	CAT-06-10	<i>Peyssonnelia squamaria</i> (B)	Catalonia, Spain	intertidal	16-jul-2006		EU349175
LAF5459	PG-08-1353	<i>Peyssonnelia squamaria</i> (C)	Malta	10 m	06-aug-2008	M.I. Parente, J. Micael	KR732910*
LAF5357	PG-08-1210	<i>Peyssonnelia squamaria</i> (D)	Malta	15 m	02-aug-2008	M.I. Parente	KR732905*
LAF5360	PG-08-1146	<i>Peyssonnelia squamaria</i> (D)	Malta	30 m	02-aug-2008	M.I. Parente, J. Micael	KR732909*
LAF5362	PG-08-1248	<i>Peyssonnelia squamaria</i> (D)	Malta	26 m	05-aug-2008	M.I. Parente, J. Micael	KR732901*
LAF5363	PG-08-1088	<i>Peyssonnelia squamaria</i> (D)	Malta	30 m	02-aug-2008	M.I. Parente, J. Micael	KR732902*
LAF5455	PG-08-1100	<i>Peyssonnelia squamaria</i> (D)	Malta	30 m	02-aug-2008	M.I. Parente, J. Micael	KR732907*
LAF5458	PG-08-1153	<i>Peyssonnelia squamaria</i> (D)	Malta	30 m	02-aug-2008	M.I. Parente, J. Micael	KR732904*
LAF5463	PG-08-795	<i>Peyssonnelia squamaria</i> (D)	Sicily, Italy	intertidal	12-mar-2008	M.I. Parente, R. Sousa, J. Matzen	KR732903*

Extraction	Collection number	Taxa	Locality	Depth	Collection date	Collector	Accession number
LAF5464	PG-08-756	<i>Peyssonnelia squamaria</i> (D)	Sicily, Italy	intertidal	12-mar-2008	M.I. Parente, R. Sousa, J. Matzen	KR732906*
LAF5465	PG-08-747	<i>Peyssonnelia squamaria</i> (D)	Sicily, Italy	intertidal	12-mar-2008	M.I. Parente	KR732908*
LAF5355	PG-08-1268	<i>Peyssonnelia coriacea</i>	Malta	26 m	05-aug-2008	M.I. Parente, J. Micael	KR732911*
LAF5361	PG-08-1173	<i>Peyssonnelia coriacea</i>	Malta	30 m	02-aug-2008	M.I. Parente, J. Micael	KR732912*
LAF5457	PG-08-1291	<i>Peyssonnelia coriacea</i>	Malta	26 m	05-aug-2008	M.I. Parente, J. Micael	KR732913*
LAF5461	PG-08-804	<i>Peyssonnelia polymorpha</i>	Sicily, Italy	intertidal	12-mar-2008	M.I. Parente, R. Sousa, J. Matzen	KR732915*
LAF5462	PG-08-790	<i>Peyssonnelia polymorpha</i>	Sicily, Italy	intertidal	12-mar-2008	M.I. Parente, R. Sousa, J. Matzen	KR732916*
-	GWS018179	<i>Peyssonnelia polymorpha</i>	Sicily, Italy	-	10-apr-2010	G. Furnari	JX969797
LAF4199	PG-08-551	<i>Peyssonnelia polymorpha</i>	Sicily, Italy	intertidal	09-mar-2008	M.I. Parente	KR732914*
K166	LAF-8-2-1-1-12	<i>Peyssonnelia rubra</i>	Liguria, Italy	3 m	02-aug-2001	B. Gavio	EU349179
K217	LAF-7-30-1-1-2	<i>Peyssonnelia bornetii</i>	Liguria, Italy	3 m	30-jul-2001	B. Gavio	EU349180
K218	LAF-7-28-1-1-2	<i>Peyssonnelia bornetii</i>	Liguria, Italy	2-20 m	28-jul-2001	B. Gavio	EU349181
K241	LAF-2-6-01-2-2	<i>Peyssonnelia replicata</i>	KwaZulu-Natal, South Africa	intertidal	06-feb-2001	T. Schils	EU349182
K243	LAF-7-23-93-1-1	<i>Peyssonnelia replicata</i>	KwaZulu-Natal, South Africa	Drift (beach)	23-jul-1993	M. Hommersand	EU349183
K214	LAF-2-6-01-1-15	<i>Sonderopelta capensis</i>	KwaZulu-Natal, South Africa	30 m	06-feb-2001	S. Fredericq & O. De Clerck	EU349186
K215	LAF-2-6-01-1-1	<i>Sonderopelta capensis</i>	KwaZulu-Natal, South Africa	30 m	06-feb-2001	S. Fredericq & O. De Clerck	EU349187
K220	LAF-7-13-95-1-1	<i>Sonderopelta coriacea</i>	Victoria, Australia	-	13-jul-1995	M. Hommersand	EU349190

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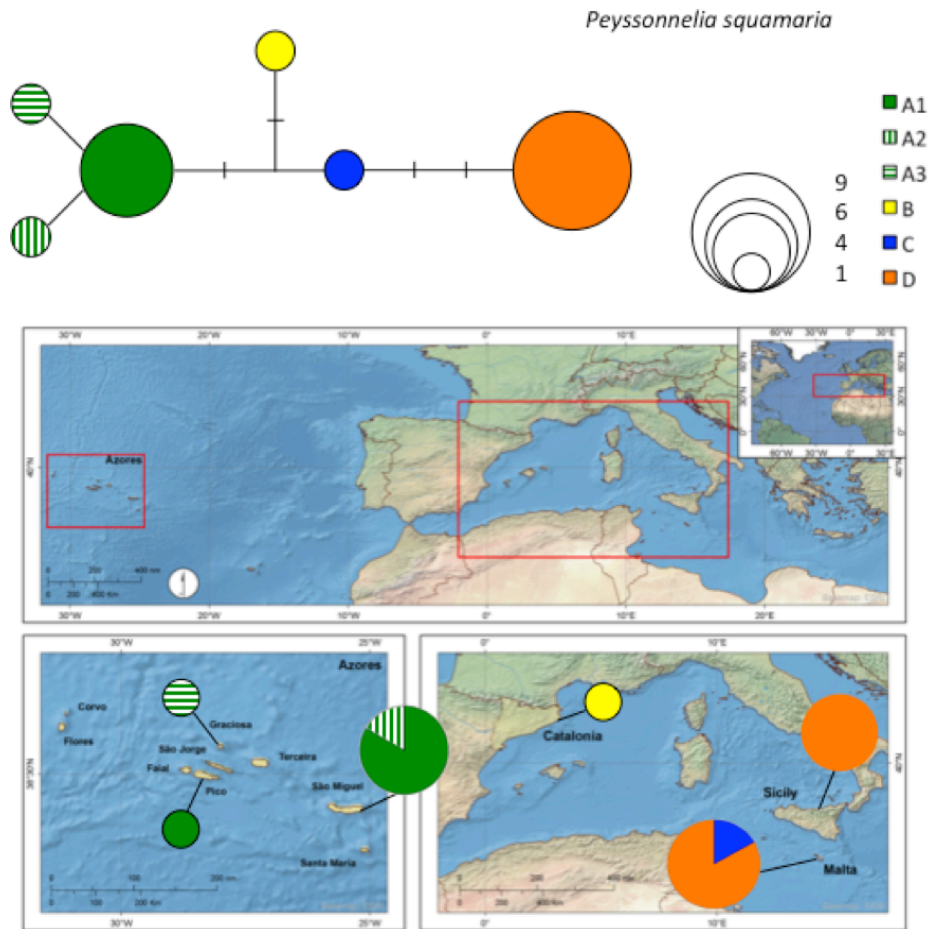


Fig. 3. RbcL haplotype network of *Peyssonnelia squamaria* and its spatial distribution in the North Atlantic. Haplotype and population sizes are proportional to the number of individuals; each haplotype is represented by a color and a letter (A1 to D).

CONCLUSION

Only a single, true *Peyssonnelia* species is confirmed for the Azorean Archipelago, in contrast to the five previously reported (Parente 2010). Although the populations in the Azores are genetically different from those occurring in the Mediterranean, this separation might be relatively recent, since the archipelago emerged about 8 My ago (Rumeu et al. 2011). Further studies including more islands and more samples are necessary to assess the variability of the species within the archipelago and the connection between its populations (Gabriel et al. 2014).

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