

A molecular and morphological study of *Corallina sensu lato* (Corallinales, Rhodophyta) in the Atlantic Iberian Peninsula

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Abstract – DNA barcodes and morphological observation were used to evaluate the number of species within the genus *Corallina sensu lato* in Atlantic Iberia and to identify a set of morphological characters that may discriminate them. Five species were detected: (i) *C. officinalis*, (ii) *C. caespitosa*, (iii) *Ellisolandia elongata*, (iv) *Corallina* sp. 1, and (v) *Corallina* sp. 2. The first three species were widespread whereas *Corallina* sp. 1 was only detected in southern Atlantic coasts of Iberia and *Corallina* sp. 2 occurred both in the Atlantic and the Mediterranean, nevertheless, with morphological and molecular differences. The widespread occurrence of the recently described *C. caespitosa* along Atlantic Iberia is substantiated with sequence data for the first time; it spans from the Bay of Biscay to Andalusia and the French Mediterranean. Molecular support is also provided for the presence of *C. officinalis* and *E. elongata* in Atlantic Iberia. Plants of *C. officinalis* were correctly identified attending to their external morphology, however two distinct species were detected under the two forms of *C. elongata* (“typical” and “elongated”) reported for Galicia in the literature (*C. caespitosa* and *Ellisolandia elongata*, respectively). Plant size, habit, branching, and shape of intergenicula seem the most informative morphological characters to discriminate among the various members of *Corallina s.l.* in Atlantic Iberia.

COI-5P / *Corallina officinalis* / *Corallina caespitosa* / coralline red algae / diversity / DNA barcodes / *Ellisolandia elongata* / network

INTRODUCTION

The genus *Corallina* Linnaeus (Corallinaceae, Rhodophyta) is characterized by erect, articulated fronds consisting in calcified intergenicula alternating with uncalcified genicula (Irvine & Johansen, 1994). The members of *Corallina* are among the commonest geniculate red algae in European coasts, thriving in intertidal and subtidal habitats (Guiry & Guiry, 2013). Their widespread occurrence is attributed to the genicular system that confers resistance to the surf, thereby allowing them to succeed in habitats where other seaweeds fail to develop (Carrington, 2013). Presently, *Corallina* contains 17 species nonetheless up to 268 species names have been reported in the literature (Guiry & Guiry, 2013). Traditionally, various species were discriminated based on their morphology. However phenotypic plasticity is common in *C. officinalis* Linnaeus

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and *C. elongata* J. Ellis & Solander (Linnaeus, 1758; Ellis & Solander, 1786; Areschoug, 1852; Hauck, 1883), and several taxa are now considered synonyms (i.e. *C. calvadosii* J.V. Lamouroux, *C. nana* Zanardini and *C. compacta* P.L. Crouan & H.M. Crouan, *C. mediterranea* Areschoug, see Guiry & Guiry, 2013). Phenotypic plasticity and environmental convergence also implies that an accurate discrimination between *C. officinalis* and *C. elongata* can be challenging (Irvine & Johansen, 1994). Fortunately, recent developments in molecular systematics have improved the ability to discriminate among the various species of *Corallina*. Thus, a new cryptic member of the genus, *Corallina caespitosa* R.H. Walker, J. Brodie & L.M. Irvine, has been recently discovered in Atlantic European coasts with the help of molecular information (Walker *et al.*, 2009). Similarly, divergence among DNA sequence data was used to propose a new genus of geniculate coralline, *Ellisolandia*, to accommodate *C. elongata* which therefore should now be named *Ellisolandia elongata* (J. Ellis & Solander) K. Hind & G.W. Saunders (Hind & Saunders, 2013a).

DNA barcodes have become a widely used tool to discriminate red algae at species level even in the absence of diagnostic morphological features (e.g. Conklin *et al.*, 2009; Kim *et al.*, 2010; Carro *et al.*, 2014). Using a short standardized fragment of the 5' end of the mitochondrial gene cytochrome oxidase I (COI-5P), the DNA barcode, allows the reliable assignment of specimens to known species (Saunders, 2005; Robba *et al.*, 2006) as well as the detection of cryptic species (e.g. Kucera & Saunders, 2012; Milstein & Saunders, 2012). COI-5P data has also been used to delineate new species of red algae, although its use for species delimitation often requires support from several independent markers (Hind & Saunders 2013a, 2013b; Pardo *et al.*, 2014) and/or other non-molecular evidence in an approach known as integrative taxonomy (Damm *et al.*, 2010; Ross *et al.*, 2010; Peña *et al.*, 2014a,b, 2015).

In the Atlantic Iberian Peninsula, *Corallina sensu lato* is currently represented by three species: *Corallina officinalis*, *Ellisolandia elongata* (known until now as *C. elongata*) and *C. caespitosa*. The latter was described as new species for the British Isles using sequence data from specimens that had been assigned to *C. officinalis* and *C. elongata* based on morphological characters (Walker *et al.*, 2009). Subsequently, *C. caespitosa* was reported for Atlantic Iberia using morphological (Pérez-Loréns *et al.*, 2012) and molecular evidence (Pardo *et al.*, 2011; Brodie *et al.*, 2013). In comparison, *C. officinalis* and *E. elongata*, are widely reported in the literature, nonetheless no molecular support were provided yet for any of these records (Ardré, 1970; Conde *et al.*, 1996; Gorostiaga *et al.*, 2004; Bárbara *et al.*, 2005; Araújo *et al.*, 2009; Cires-Rodríguez & Cuesta-Moliner, 2010). Moreover, Beltrán & Bárbara (2003) described two morphotypes of “*C. elongata*” (“typical” and “elongated”) in NW Spain that showed distinct habit and habitat preferences nevertheless, in absence of molecular data, those authors refrain from reaching any taxonomic conclusion.

In this study, we aimed: (i) to clarify the diversity of species of *Corallina sensu lato* that live in Atlantic Iberia and to detect potential cryptic species, (ii) to elucidate whether the two morphotypes of “*C. elongata*” described for Galicia may actually be distinct species, (iii) to delimitate the Atlantic Iberian range of the recently described *C. caespitosa*, and (iv) to identify a set of morphological features for discriminating species in *Corallina s.l.*

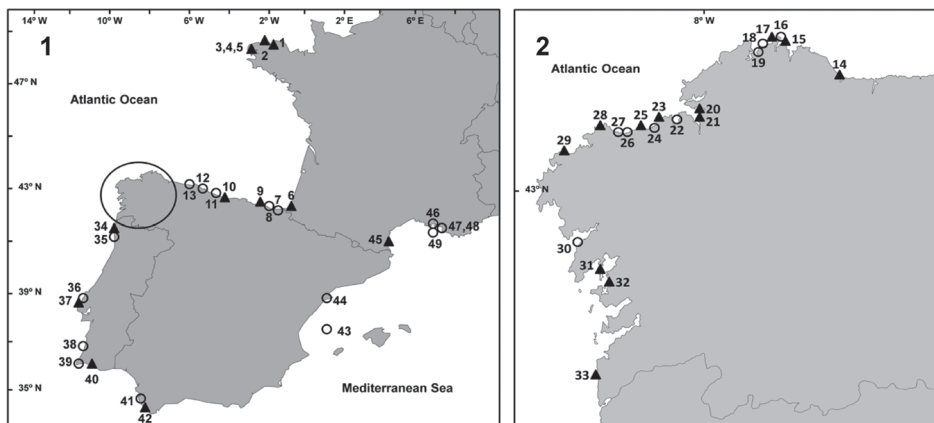
MATERIAL AND METHODS

Sampling

From 2010 to 2012, 156 plants of *Corallina sensu lato* were collected from the intertidal and subtidal of 36 sites along the Atlantic Iberian coast (Figs 1, 2). Galicia was more intensively surveyed (20 sites) to obtain larger sample sizes of the two morphotypes of “*C. elongata*” described in Beltrán & Bárbara (2003). Additional samples (27 plants) were collected in Atlantic France (six sites) and the Spanish and French Mediterranean (two and five sites, respectively). In total, we collected 183 plants. Fresh material was transported to the laboratory in sea water, air-dried, and preserved in silica. Vouchers were deposited in the Herbarium SANT of the University of Santiago de Compostela (see Table 1).

Molecular analyses

Collection and molecular information for the specimens used in the molecular-assisted identification is available in the Barcode of Life Data Systems (BOLD: www.boldsystems.org, project “Corallinaceae”); all sequence data generated in this study was uploaded to GenBank (Table 1). DNA was extracted from a portion of tissue free from epiphytes with the Wizard Magnetic 96 DNA Plant System kit (Promega, Madison, WI) following manufacture’s recommendations with minor modifications. A fragment of 664 bp of the standard DNA barcode region (COI-5P) was amplified with primers GazF1 and GazR1 (Saunders, 2005) in a Biometra TProfessional Basic thermocycler following Saunders & McDevit (2012a). Cycling protocol included an initial denaturation step at 95°C for 2’; 5 cycles of 30’’denaturation at 95°C, 30’’ anneal at 45°C and 1’ extension at 72°C; 35 cycles of 30’’ denaturation at 95°C, 30’’ anneal at 46.5°C, and extension at 72°C; and an additional 7’ at 72°C. PCRs were performed in 25 µL containing 1 µL of DNA template, 2.5 µL of 1x PCR buffer, 2.5 mM MgCl₂,



Figs 1-2. Sampling sites of *Corallina sensu lato* (circles and triangles) and distribution of *Corallina caespitosa* confirmed by DNA-barcodes (triangles). **1.** General distribution of localities. **2.** Map of localities from Galicia (NW Iberian Peninsula). Complementary information provided in Table 1.

Table 1. *Corallina sensu lato* specimens from Southern Europe collected in this study: the numbers show the country, region, locality, geographical coordinates (MGRS, Military Grid Reference System), and collection year. For each species in their locality, numbers of herbarium sheets (SANT-Algae), BOLD, GenBank databases, and haplotype are showed in this order between parentheses; n.d. indicates there is no molecular data. Six plants were only identified to genus level: 9- Spain, Basque Country, San Juan de Gaztelugatxe, 30TWP174106, 2011-(27681, n.d., n.d.), 17- Spain, Galicia, A Coruña, Praia de Picón, 29TPJ013444, 2011-(27632, n.d., n.d.), 39- Portugal, Algarve, Ingrina, 29SNA107999, 2011- *Corallina* sp.2 -(27587, COR101-13, KF460992, hap-18), 41- Spain, Andalucía, Cala Encendida, 30SSQ555222, 2011- *Corallina* sp.1-(27794, COR079-12, KF460993, hap-17), 43-Spain, Valencia, Islas Columbretes: Columbrete Grande, 31SCE018189, 2010- *Corallina* sp.2-(27805, COR098-13, KF460991, hap-19), 46-France, Provence-Alpes-Cote d'Azur, Sausset les Pins, 31TFH709995, 2011 (25954, n.d, n.d.)

<i>Country, region, locality, MGRS, collection year</i>	<i>Corallina officinalis</i>	<i>Corallina caespitosa</i>	<i>Ellisolandia elongata</i>
1. France, Brittany, Loquémeau, 30UVU570967, 2011		(27795, COR087-12, KF460951, hap-4); (27796, COR088-12, KF460950, hap-11)	
2. France, Brittany, Île Callot, 30UVU320920, 2011		(27817, COR086-12, KF460952, hap-3); (27818, n.d., n.d.); (27820-27822, n.d., n.d.)	
3. France, Brittany, Anse du Minou, 30UUU802551, 2011	(27797, COR089-12, KF460972, hap-16)		
4. France, Brittany, Fort du Mingant, 30UUU825561, 2011	(27823, COR084-12, KF460973, hap-16); (27827, n.d., n.d.)	(27824, COR085-12, KF460953, hap-3); (27825-27826, n.d., n.d.); (27828-27829, n.d., n.d.); (27830-27836, n.d., n.d.) (28025, n.d., n.d.)	
5. France, Brittany, Pont du Diable, 30UUU845568, 2011		(25395, COR083-12, KF460955, hap-1)	
6. France, Aquitaine, Biarritz, 30TPI162155, 2011		(24453, n.d., n.d.)	(24452, COR092-13, KF461004, hap-6); (27671, COR057-12, KF461018, hap-6)
7. Spain, Basque Country, Zumaia, 30TWN608947, 2010/2011			(27811, COR058-12, KF461017, hap-6)
8. Spain, Basque Country, Itziar, 30TWN555941, 2011			
9. Spain, Basque Country, San Juan de Gaztelugatxe, 30TWP174106, 2011	(27675, COR059-12, KF460974, hap-13)	(27676, COR060-12, KF460971, hap-1)	(27678, COR061-12, KF461014, hap-6)

Table 1. *Corallina sensu lato* specimens from Southern Europe collected in this study: the numbers show the country, region, locality, geographical coordinates (MGRS, Military Grid Reference System), and collection year. For each species in their locality, numbers of herbarium sheets (SANT-Algae), BOLD, GenBank databases, and haplotype are showed in this order between parentheses; n.d. indicates there is no molecular data. Six plants were only identified to genus level: 9- Spain, Basque Country, San Juan de Gaztelugatxe, 30TWP174106, 2011-(27681, n.d., n.d.), 17- Spain, Galicia, A Coruña, Praia de Picón, 29TPJ013444, 2011-(27632, n.d., n.d.), 39- Portugal, Algarve, Ingrina, 29SNA107999, 2011- *Corallina* sp.2 -(27587, COR101-13, KF460992, hap-18), 41- Spain, Andalucía, Cala Encendida, 30SSQ555222, 2011- *Corallina* sp.1-(27794, COR079-12, KF460993, hap-17), 43- Spain, Valencia, Islas Columbretes: Columbrete Grande, 31SCE018189, 2010- *Corallina* sp.2-(27805, COR098-13, KF460991, hap-19), 46-France, Provence-Alpes-Cote d'Azur, Sausset les Pins, 31TFH709995, 2011 (25954, n.d. n.d.) (continued)

<i>Country, region, locality, MGRS, collection year</i>	<i>Corallina officinalis</i>	<i>Corallina caespitosa</i>	<i>Ellisolandia elongata</i>
10. Spain, Cantabria, Comillas, 30TUP943050, 2011		(27667, COR062-12, KF460938, hap-1); (27668, COR063-12, KF460937, hap-1)	
11. Spain, Asturias, La Franca, 30TVP727060, 2011			(27664, COR064-12, KF461015, hap-6)
12. Spain, Asturias, Sablera de San Llorenzu, 30TTP852251, 2011		(27643, n.d., n.d.)	(27641, COR065-12, KF461019, hap-6); (27642, COR066-12, KF461020, hap-6)
13. Spain, Asturias, Cadavedo, 29TQJ118255, 2011		(27636, n.d., n.d.)	(27637, COR068-12, KF461021, hap-6)
14. Spain, Galicia, Lugo, Peinzás, 29TPI389275, 2011/2012	(27764, n.d., n.d.)		(27758, n.d., n.d.); (27771, COR040-12, KF460994, hap-6); (27776, COR042-12, KF461031, hap-6)
15. Spain, Galicia, Lugo, Praia de Xilloi, 29TPJ081438, 2011		(27777, COR053-12, KF460968, hap-1); (27778, COR054-12, KF460969, hap-1)	
16. Spain, Galicia, A Coruña, Praia de Bares, 29TPJ065467, 2011	(27629, n.d., n.d.)		(27623-27624, n.d., n.d.); (27628, n.d., n.d.)
17. Spain, Galicia, A Coruña, Praia de Picón, 29TPJ013444, 2011		(27635, COR095-13Y, KF460944, hap-4)	(27630, COR051-12, KF461023, hap-6); (27631, COR052-12, KF461022, hap-6); (27634, COR100-13, KF460999, hap-6)
18. Spain, Galicia, A Coruña, Praia de Castro, 29TNI996437, 2011		(27785-27787, n.d., n.d.)	

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Country, region, locality, MGRS, collection year	<i>Corallina officinalis</i>	<i>Corallina caespitosa</i>	<i>Ellisolandia elongata</i>
19. Spain, Galicia, A Coruña, Praia de Eirón, 29TNPJ962409, 2011			(28027, COR050-12, KF461024, hap-6)
20. Spain, Galicia, A Coruña, Praia de Ber, 29TNPJ639057, 2011		(27810, COR039-12, KF460963, hap-1)	
21. Spain, Galicia, A Coruña, Praia de Perbes, 29TNPJ636021, 2011		(27694, COR026-12, KF460958, hap-1); (27697, n.d., n.d.); (27696, COR027-12, KF460959, hap-1); (27699-27702, n.d., n.d.)	
22. Spain, Galicia, A Coruña, Praia das Margaridas, 29TNPJ530017, 2011	(27788, COR049-12, KF460976, hap-5)		
23. Spain, Galicia, A Coruña, Praia de Ártabra, 29TNPJ423000, 2011/2012	(27706, n.d., n.d.); (27719, COR012-12, KF460988, hap-7); (27721, COR014-12, KF460987, hap-8); (27722, COR015-12, KF460986 hap-5); (27730, n.d., n.d.); (27735, COR019-12, KF460985, hap-5); (27736, COR020-12, KF460984, hap-7); (27737, COR021-12, KF460983, hap-10)	(27703-27705, n.d., n.d.); (27707, n.d., n.d.); (27710-27713, n.d., n.d.); (27715, COR008-12, KF460945, hap-1); (27716, COR009-12, KF460947, hap-3); (27718, COR011-12, KF460948, hap-3); (27720, COR013-12, KF460949, hap-1); (27725-27729, n.d., n.d.); (27731-27733, n.d., n.d.)	(27708-27709, n.d., n.d.); (27714, COR007-12, KF461012, hap-6); (27717, COR010-12, KF461001, hap-6); (27723, COR016-12, KF461016, hap-9); (27724, n.d., n.d.); (27734, COR018-12, KF461013, hap-6); (27738, COR022-12, KF461011, hap-6)
24. Spain, Galicia, A Coruña, Praia de Barrañán, 29TNPJH358957, 2011		(27781-27783, n.d., n.d.)	

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Country, region, locality, MGRS, collection year	<i>Corallina officinalis</i>	<i>Corallina caespitosa</i>	<i>Ellisolandia elongata</i>
25. Spain, Galicia, A Coruña, Praia de Cañón, 29TINH318965, 2011	(27682, COR030-12, KF460981, hap-5); (27685, COR033-12, KF460980, hap-5); (27687, COR034-12, KF460979, hap-5); (27688, COR035-12, KF460978, hap-7)	(27692, COR096-13, KF460943, hap-11)	(27683, COR031-12, KF461003, hap-9); (27684, COR032-12, KF461002, hap-6); (27689, COR036-12, KF460998, hap-6); (27690, COR037-12, KF460997, hap-6); (27691, COR038-12, KF460996, hap-9); (27693, n.d., n.d.)
26. Spain, Galicia, A Coruña, Praia de Leira, 29TINH297954, 2010			(28024, COR091-13 KF461000, hap-6)
27. Spain, Galicia, A Coruña, San Martiño de Cambre, 29TINH196940, 2012	(27609, n.d., n.d.)	(27607, n.d., n.d.)	(27605, n.d., n.d.)
28. Spain, Galicia, A Coruña, Praia de Barizo, 29TINH099956, 2011		(27610, COR023-12, KF460957, hap-3)	(27612, COR024-12, KF461010, hap-6); (27613, COR025-12, KF461009, hap-6); (27614, n.d., n.d.)
29. Spain, Galicia, A Coruña, Camelle, 29TMH920819, 2011/2012	(27741, n.d., n.d.); (27749, COR046-12, KF460977, hap-7)	(27739-27740, n.d., n.d.); (27743, n.d., n.d.); (27744, COR093-13, KF460946, hap-3); (27746, n.d., n.d.); (27753, n.d., n.d.)	(27742, n.d., n.d.); (27745, COR094-13, KF461006, hap-6); (27747, COR44-12, KF461030, hap-6); (27748, COR045-12, KF461028, hap-6); (27750, COR047-12, KF461027, hap-6); (27751, COR048-12, KF461026, hap-6); (27757, COR099-13, KF461025, hap-6)
30. Spain, Galicia, A Coruña, Porto do Son, 29TMH996306, 2012		(27581-27583, n.d., n.d.)	

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Country, region, locality, MGRS, collection year	<i>Corallina officinalis</i>	<i>Corallina caespitosa</i>	<i>Ellisolandia elongata</i>
31. Spain, Galicia, Pontevedra, Punta Barbafeita, 29TNH089130, 2010	(27570, COR006-12, KF460989, hap-5); (27573, n.d., n.d.)	(27564, COR003-12, KF460940, hap-3); (27565, COR002-12, KF460939, hap-2); (27566, COR004-12, KF460941, hap-4); (27568, COR001-12, KF460954, hap-1); (27569, COR005-12, KF460942, hap-3); (27571-27572, n.d., n.d.)	
32. Spain, Galicia, Pontevedra, Tragove, 29TNH141080, 2011	(27579, COR028-12, KF460982, hap-5)	(27580, COR029-12, KF460962, hap-11)	
33. Spain, Galicia, Pontevedra, Oia, 29TNG098495, 2011	(27615, COR055-12, KF460975, hap-12); (27621-27622, n.d., n.d.)	(27616, COR056-12, KF460970, hap-1); (27620, n.d., n.d.)	(27619, n.d., n.d.)
34. Portugal, Viana do Castelo, Montedor, 29TNG100212, 2011		(27645, COR070-12, KF460966, hap-3); (27646, COR071-12, KF460965, hap-1)	(27647, COR072-12, KF461032, hap-6); (27649, COR073-12, KF460995, hap-6)
35. Portugal, Oporto, Vila do Conde, 29TNF200788, 2010		(27799, n.d., n.d.)	
36. Portugal, Estremadura, Praia de Santa Cruz, 29SMD666316, 2011		(27593, n.d., n.d.)	(27592, COR074-12; KF461005, hap-6)
37. Portugal, Estremadura, Praia Azul, 29SMD659298, 2011		(27591, COR075-12, KF460961, hap-2)	
38. Portugal, Beja, Almogrove, 29SNB176674, 2011		(27660, n.d., n.d.)	(27661, COR102-13, KF461029, hap-6)
39. Portugal, Algarve, Ingrina, 29SNA107999, 2011			(27586, n.d., n.d.)

Table 1. *Corallina sensu lato* specimens from Southern Europe collected in this study: the numbers show the country, region, locality, geographical coordinates (MGRS, Military Grid Reference System), and collection year. For each species in their locality, numbers of herbarium sheets (SANT-Algae), BOLD, GenBank databases, and haplotype are showed in this order between parentheses; n.d. indicates there is no molecular data. Six plants were only identified to genus level: 9- Spain, Basque Country, San Juan de Gaztelugatxe, 30TWP174106, 2011-(27681, n.d., n.d.), 17- Spain, Galicia, A Coruña, Praia de Picón, 29TPJ013444, 2011-(27632, n.d., n.d.), 39- Portugal, Algarve, Ingrina, 29SNA107999, 2011- *Corallina* sp.2 -(27587, COR101-13, KF460992, hap-18), 41- Spain, Andalucía, Cala Encendida, 30SSQ555222, 2011- *Corallina* sp.1-(27794, COR079-12, KF460993, hap-17), 43-Spain, Valencia, Islas Columbretes: Columbrete Grande, 31SCE018189, 2010- *Corallina* sp.2-(27805, COR098-13, KF460991, hap-19), 46-France, Provence-Alpes-Cote d'Azur, Sausset les Pins, 31TFH709995, 2011 (25954, n.d. n.d.) (continued)

<i>Country, region, locality, MGRS, collection year</i>	<i>Corallina officinalis</i>	<i>Corallina caespitosa</i>	<i>Ellisolandia elongata</i>
40. Portugal, Algarve, Olhos d'Água, 29SNB722053, 2011		(27655, COR069-12, KF460967, hap-3)	(27657, n.d., n.d.)
41. Spain, Andalucía, Cala Encendida, 30SSQ555222, 2011			(27792, COR077-12, KF461007, hap-14); (27793, COR078-12, KF461008, hap-14);
42. Spain, Andalucía, Punta Plata, 30STE456990, 2011		(27590, COR076-12, KF460960, hap-4)	
44. Spain, Cataluña, Platja de Les Delícies, 31TBE954977, 2011		(28028, n.d., n.d.)	
45. France, Languedoc-Roussillon, Étang de Salses et de Leucate, 31TEH020450, 2011		(27596, COR080-12, KF460956, hap-15)	
47. France, Bouches-du-Rhône, Anse de Malmousque, 31TFH900950, 2011		(27603, n.d., n.d.)	
48. France, Bouches-du-Rhône, Anse Batterie des Lions, 31TFH906946, 2011		(25677, n.d., n.d.)	
49. France, Bouches-du-Rhône, Au sud d'Île Rattoneau, 31TFH870947, 2011		(25802, n.d., n.d.)	

0.192 mM dNTPs, 0.1 μ M of each primer, and 0.3 U of Taq DNA Polymerase (Sigma-Aldrich). Amplification success was evaluated with agarose gels. After removing the excess of primers and nucleotides (shrimp alkaline phosphatase and exonuclease I enzymes), PCR products were bi-directionally sequenced using the Sanger method at Macrogen facilities (<http://www.macrogen.com>, Korea). Traces were checked, edited, and aligned with Geneious v. 5.6.6 (Biomatters, Auckland, New Zealand). Sequences were then partitioned into haplotypes with the help of DnaSP v. 5.10.1 (Librado & Rozas, 2009). Co-specific haplotypes were identified by comparison with epitype sequences of *Ellisolandia elongata* (Hind & Saunders, 2013a, and as *C. elongata* in Brodie *et al.*, 2013), *Corallina officinalis*, and holotype sequences of *C. caespitosa* (Walker *et al.*, 2009; Brodie *et al.*, 2013) in a Neighbor-Joining (NJ) tree with four outgroups: two Hapalidiaceae (*Phymatolithon calcareum* (Pallas) W.H. Adey & D.L. McKibbin KC861590, *Lithothamnion corallioides* (P.L. & H.M. Crouan) P.L. Crouan & H.M. Crouan KC861469) and two Corallinaceae (*Calliarthron cheilosporioides* Manza JQ615594, *Pseudolithophyllum* sp. 5 *muricatum* JQ615869). The NJ tree was generated in Mega v.6.0 (Tamura *et al.*, 2013) with the Jukes-Cantor model identified by jModelTest v. 2.1.3 (Darriba *et al.*, 2012) as the best-fitting substitution model and node support was assessed with 1,000 bootstrap replicates. The phylogenetic relationships between co-specific haplotypes were further investigated with median-joining networks generated with NETWORK v.4.6.1.2. (available at www.fluxus-engineering.com/sharenet.htm; Bandelt *et al.*, 1999) using default settings.

Morphological observations

Plants identified with DNA barcodes as *Corallina officinalis*, *C. caespitosa* and *Ellisolandia elongata* were selected for morphological examination. Fragments of each plant were decalcified using acetic acid in distilled water (1:1), stained with aniline blue, washed with distilled water, and mounted in permanent slides with Karo[®] Syrup (50-80%). Morphological features were observed and photographed under stereo and optical microscopes; a few specimens were also examined by Scanning Electron Microscope (SEM, model JEOL JSM 6400). Measurements were obtained with the help of Image-Pro Plus v.4.5 (Silver Spring, MD). The terminology and morphological characters examined here (branching pattern, intergenicula and genicula shape, length, diameter, conceptacle, etc.; see Table 2) follow previous taxonomic studies of geniculate coralline red algae (Irvine & Johansen, 1994; Beltrán & Bárbara, 2003; Walker *et al.*, 2009; Brodie *et al.*, 2013).

RESULTS

Ninety-five sequences of the COI-5P DNA barcode region were obtained that ranged 555-664 bp in length. These sequences contained nineteen haplotypes (Table 1, Fig. 3) with 130 polymorphic sites: 127 parsimony informative positions plus 3 singletons. Eight haplotypes were detected in a single specimen each while eleven haplotypes (haps-1-7, hap-9, hap-11, hap-14, hap-16) were recorded in 2 to 34 specimens. In the NJ tree, sixteen haplotypes clustered with type sequences of *Corallina officinalis*, *C. caespitosa* and *Ellisolandia elongata* (Fig. 3). Three

Table 2. Habitat and morphological features of *Corallina sensu lato* in the Atlantic Iberian Peninsula. Terminology follows Irvine and Johansen (1994), Beltrán and Bárbara (2003), Walker *et al.* (2009) and Brodie *et al.* (2013)

	<i>Corallina officinalis</i>	<i>Corallina caespitosa</i>	<i>Ellisolandia elongata</i>
Habitat	Lower intertidal and subtidal on exposed to semiexposed coasts. In shady habitats. Commonly associated with <i>Gelidium cornutum</i>	Mid-littoral pools, abundant in the low intertidal and shallow subtidal on exposed to semi-exposed coasts, supporting sandy sedimentation in protected places. In illuminated environments. Commonly associated with <i>Lithophyllum incrustans</i>	Lower intertidal on exposed to semi-exposed coasts. In steep to vertical rocks and shady habitats. Commonly associated with the sponge <i>Grantia compressa</i>
Size (cm) and habit	7-11, very stiff, usually erect	3-4, stiff, usually erect	5-7, limp, feather-like fronds, usually hanging
Colour	Pink to pale pink, with a paler hue in tips of the fronds	Greyish pink to violet-pink. Pale pink, with white apex in high irradiance sites	Greyish pink, dark pink, pink, violet-pink
Branching	Simple to compound pinnate, often irregular. With large gaps among successive lateral branches	Simple to compound pinnate, in one plane. Dense and regular (occasionally irregular). With gaps among lateral branches	Simple to compound pinnate, in one plane. Very dense and regular (occasionally irregular). With inconspicuous (or absent) gaps among lateral branches
Intergenicula: shape	Cylindrical, heavily calcified. Absence lateral expansions	Cylindrical to compressed, with fan to trapezoidal shape. Often winged	Flattened. Trapezoidal. Winged. Even with lateral expansions.
Intergenicula: n° of terminal branches	(1) 2-3 (4)	3-4 (5)	3-6 (9)
Intergenicula (tips): length x diam (µm)	322-1826 × 581-844	224-1209 × 564-914	186-729 × 376-1083
Intergenicula (tips): length/diam	1.72-3.09	1-2.03	0.49-1.17
Intergenicula (tips): tiers of medullary cells	1-31	1-15	1-12
Genicula (tips): length x diam (µm)	32-201 × 52-511	31-79 × 240-441	24-69 × 204-683

Table 2. Habitat and morphological features of *Corallina sensu lato* in the Atlantic Iberian Peninsula. Terminology follows Irvine and Johansen (1994), Beltrán and Bárbara (2003), Walker *et al.* (2009) and Brodie *et al.* (2013) (*continued*)

	<i>Corallina officinalis</i>	<i>Corallina caespitosa</i>	<i>Ellisolandia elongata</i>
Intergenicula (main branches): length × diam (µm)	1477-1954 × 824-836	866-1241 × 593-991	562-803 × 646-1191
Intergenicula (main branches): length/diam	1.78-2.36	0.87-2.02	0.67-0.96
Intergenicula (main branches): tiers of medullary cells	19-25	11-16	9-12
Genicula (main branches) length × diam (µm)	60-132 × 343-485	42-75 × 222-367	61-107 × 258-401
Conceptacles: length × diam (µm)	475-538 × 642-465	307-746 × 357-706	338-282 × 203-206
Conceptacle chamber: length × diam (µm)	341-349 × 373-421	171-559 × 166-588	154-158 × 161-165

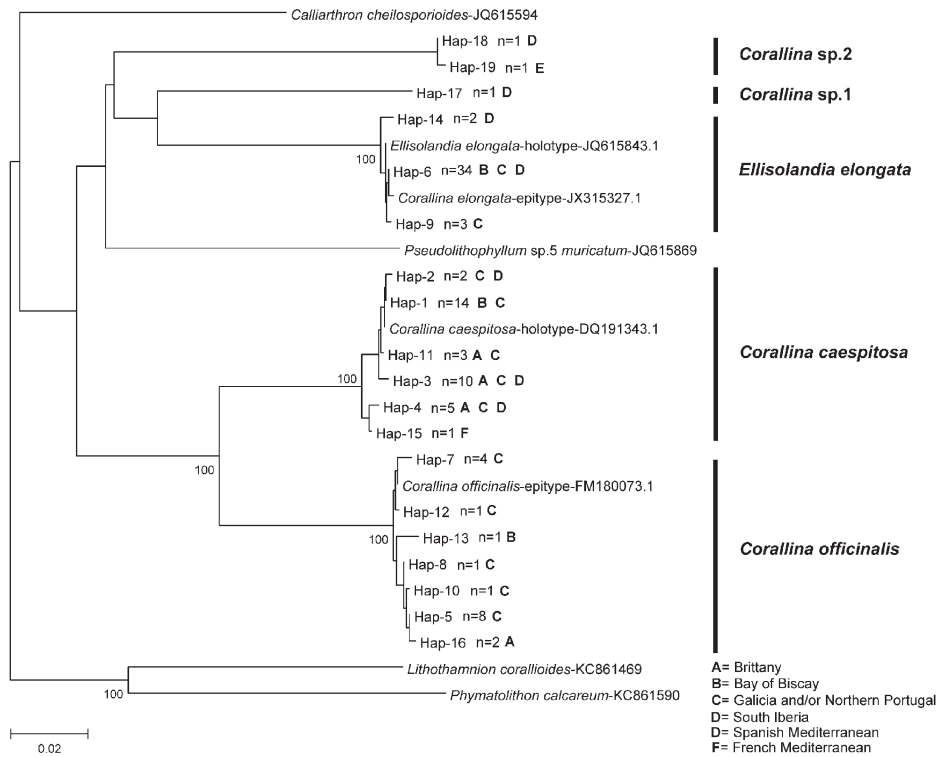
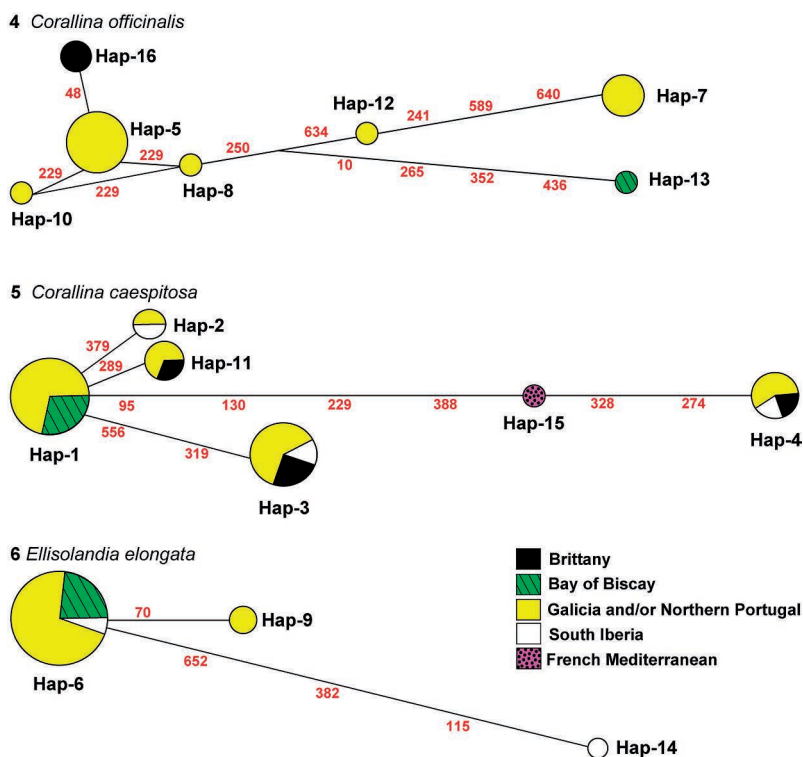


Fig. 3. Phylogram (Neighbour-Joining) generated from COI-5P data from all haplotypes included in this study. Numbers at the node are bootstrap values for support analysis. Epitypes sequences of *Ellisolandia elongata* (Hind & Saunders, 2013a, and as *C. elongata* in Brodie *et al.*, 2013), *Corallina officinalis*, and the holotype of *C. caespitosa* (Walker *et al.*, 2009; Brodie *et al.*, 2013) are also represented each by a single branch. Four outgroup taxa also were included and represented each by a single branch. For each haplotype, the number of sequences used is showed adjacently. The geographic distribution of each haplotype is showed with letters: A (Brittany), B (Bay of Biscay), C (Galicia and/or Northern Portugal), D (South Iberia), E (Spanish Mediterranean), and F (French Mediterranean). Vertical lines indicate the identity of the haplotypes generated in this study. Scale bar refers to substitutions per site.

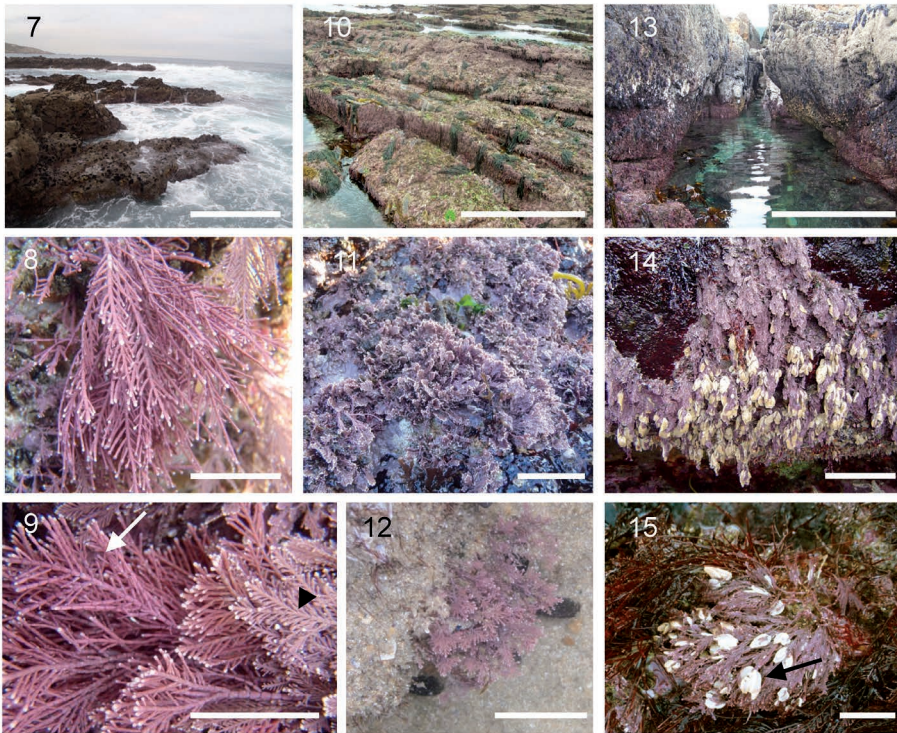
haplotypes that did not cluster with any of the species already described for the Iberian Peninsula were designated as *Corallina* sp.1 (hap-17) and *Corallina* sp.2 (hap-18 and hap-19 separated by 1 bp) after GenBank and BOLD searches failed to find any co-specific hit. Sequence distance between the three species already described for Atlantic Iberia ranged 8.1-15.5% while intraspecific variation ranged 0.2-1.2% in *C. officinalis* and *C. caespitosa*, and 0.2-0.6% in *E. elongata*. Interspecific distances for the two unnamed *Corallina* (spp. 1-2) were in the range of those observed among named entities: 11.5-16.1% with either *C. officinalis*, *C. caespitosa*, and *E. elongata*, and 14.7-14.8% between the two unnamed entities. The five species were found in Atlantic Iberia; however, only *Corallina* sp. 2 was also detected among our Mediterranean samples.

Eighteen specimens from Galicia (NW Spain), Bay of Biscay and Brittany (France) corresponding to seven haplotypes clustered with type COI-5P sequences of *Corallina officinalis* (Fig. 3). Most of the haplotypes were detected



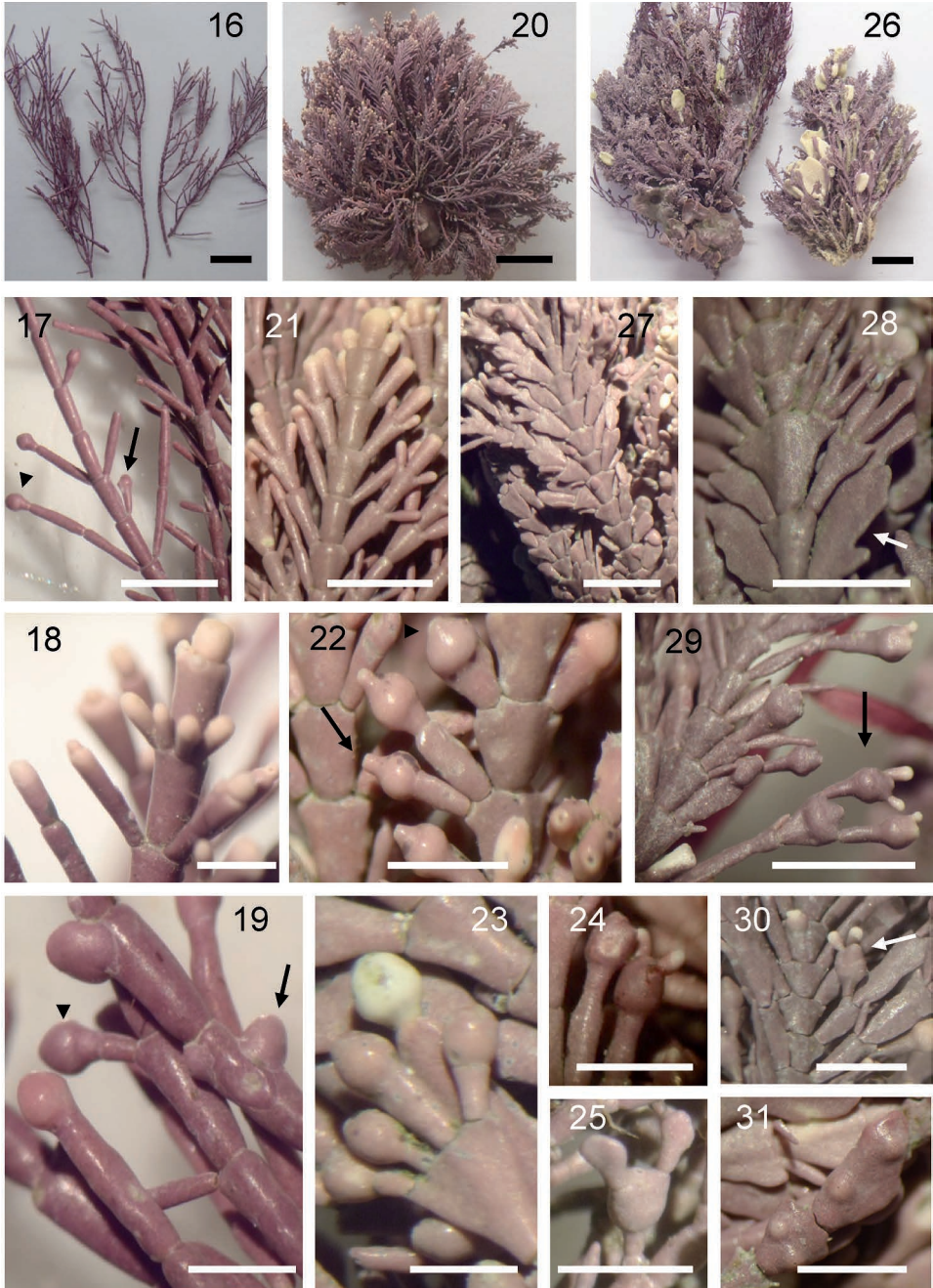
Figs 4-6. Haplotype networks of *Corallina sensu lato*. **4.** Network of *C. officinalis*. **5.** Network of *C. caespitosa*. **6.** Network of *Ellisolandia elongata*. The size of each circle is proportional to the corresponding haplotype frequency. Colours indicate the collecting region (black: Brittany; green striped: Bay of Biscay; yellow: Galicia and/or Northern Portugal; white: South Iberia; pink dotted: French Mediterranean). Red numbers indicate the mutation position.

in Galicia where two of them (hap-7 and hap-5) were particularly common; Galician hap-12 was 100% identical to the epitype of *C. officinalis* (FM180073) designated by Brodie *et al.* (2013). In comparison, only two haplotypes were detected in the Bay of Biscay (hap-13) and Brittany (hap-16). The median-joining network (Fig. 4) revealed that the haplotypes widely distributed along Galicia (hap-5 and hap-7) were clearly separated by six mutational steps. Similarly, the only haplotype found in Bay of Biscay (hap-13) was 6-8 mutations away from either hap-5 or hap-7. However, the haplotype detected in Brittany (hap-16) was closely related to the widespread Galician hap-5. In our collections, *C. officinalis* normally occurred in the lower intertidal and subtidal of exposed to semi-exposed coasts, particularly in shady habitats and commonly associated with *Gelidium corneum* (Hudson) J. V. Lamouroux (Table 2, Figs 7-9). Our plants matched the description provided by Brodie *et al.* (2013) (Figs 16, 17). However, rather than just three terminal branches, we often observed 2-3, sometimes a single undivided intergeniculum, and rarely four or more branches (Figs 18, 32). Our observations also revealed that intergenicula were made of up to 31 tiers of medullary cells (Figs 32, 33). Conceptacles were ovoid to pyriform in shape, sometimes with *cornicula*, but never beaked (Figs 17, 19, 34). Sessile conceptacles were rarely observed (Fig. 19, arrow).



Figs 7-15. *Corallina sensu lato* in the field. **7.** *C. officinalis* in lower intertidal exposed rocks (site 23). **8.** Close-up of *C. officinalis* (site 23). **9.** *C. officinalis* (arrow) and *C. caespitosa* (arrowhead) growing together in the low intertidal (site 29). **10.** *C. caespitosa* covering middle intertidal rocks (site 14). **11.** *C. caespitosa* with *Lithophyllum incrustans* (site 23). **12.** *C. caespitosa* growing in sand covered rock habitat (site 21). **13.** *Ellisolandia elongata* growing in vertical lower intertidal (site 23). **14.** *E. elongata* hanging from rocks in shady habitats and growing associated with the sponge *Grantia compressa* (site 25). **15.** A specimen of *E. elongata* associated with the sponge *Grantia compressa* (arrow) growing in a rock of lower intertidal (site 25). Scale bars – 7: 10 mm; 8, and 9: 2 cm; 10: 4 mm; 11, and 14: 10 cm; 12, and 15: 4 cm; 13: 2 mm.

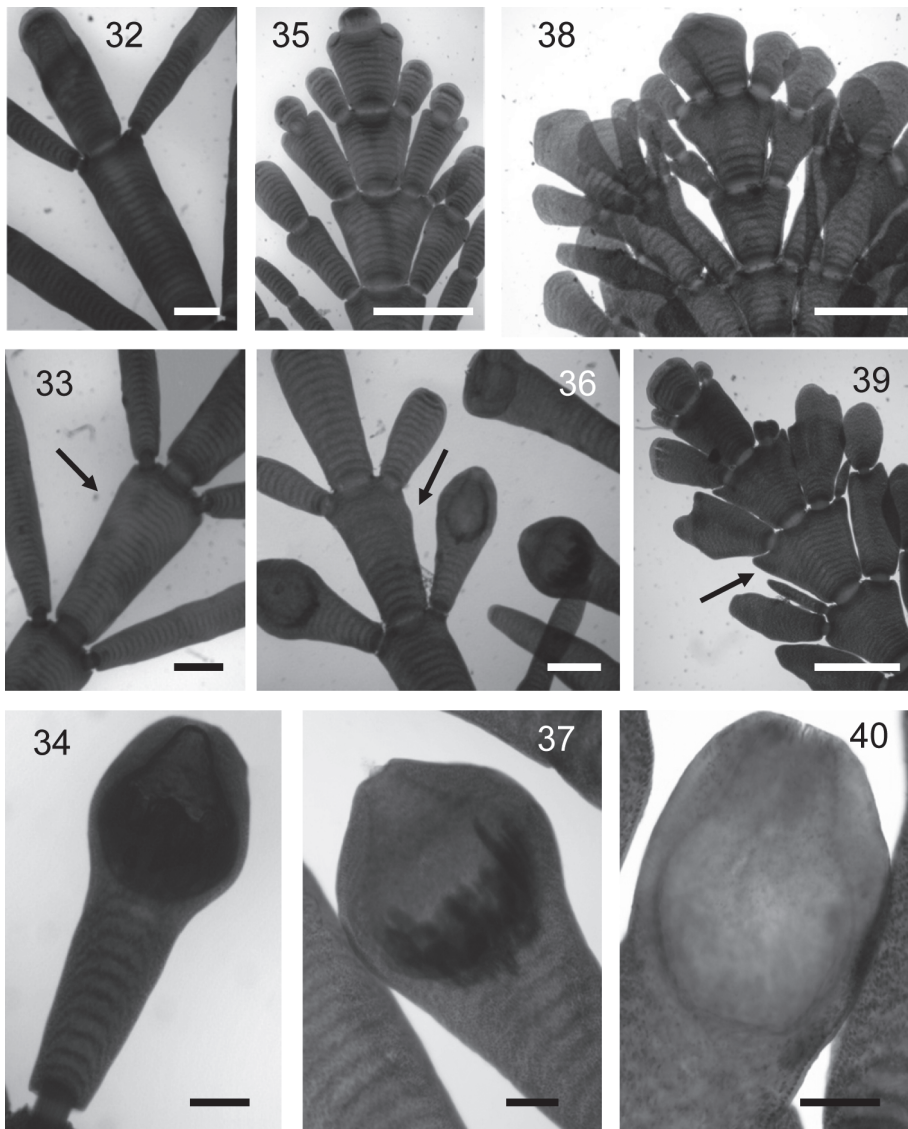
Six haplotypes (35 sequences) that included material from the French Mediterranean clustered with sequences of the type material of *Corallina caespitosa* (Fig. 3). Unlike *C. officinalis* where each haplotype seemed restricted to a particular region, most of the haplotypes found in our Atlantic collections of *C. caespitosa* were widely distributed across regions. Thus, nearly half of our specimens belonged to one haplotype (hap-1) that seemed widespread in the Bay of Biscay and Galicia-North Portugal (see Table 1 for further details); this haplotype was 100% similar to the holotype of *C. caespitosa* (DQ191343) from Devon (South England). Likewise, two haplotypes (hap-3 and hap-4) ranged from Brittany to South Iberia (but went unrecorded in the Bay of Biscay), one haplotype (hap-11) was detected in collections from Brittany and Galicia, and another one (hap-2) was recorded in Galicia and South Iberia. Our collection from the French Mediterranean produced a distinctive haplotype (hap-15). The median-joining network (Fig. 5) revealed that the COI-5P sequences of most of our Atlantic collections of *C. caespitosa* were closely related (1-2 mutational steps) to the widespread hap-1; only hap-4



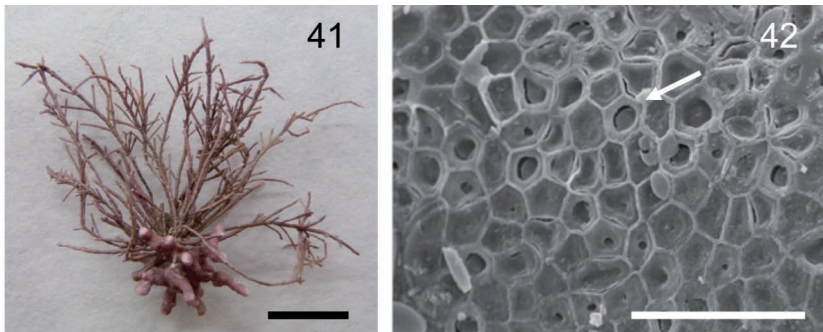
represented a distinct lineage separated by 6 mutational steps from hap-1. Surprisingly, the haplotype generated by our collection from the Mediterranean (hap-15) occupied an intermediate position between the Atlantic lineages. Our collections of *C. caespitosa* were epilithic plants growing in mid-littoral pools as well as in the lower intertidal and shallow subtidal of exposed to semi-exposed habitats (Fig. 10, Table 2). *Corallina caespitosa* was commonly associated with *Lithophyllum incrustans* Philippi (Fig. 11) and supported sandy sedimentation in some protected places (Fig. 12). Our collections matched the description of Brodie *et al.* (2013) and they were small plants (3-4 cm long, Fig. 20) characterized by a regular branching and lateral branchlets separated by conspicuous gaps (Fig. 21). Intergenicula were fan-shaped to trapezoidal (Figs 21, 22), often winged (Fig. 22). Terminal intergenicula were mainly trifurcate, occasionally 4-5 branched; however we failed to observe the up to 7 times branched structures described by Brodie *et al.* (2013). Under the microscope, the intergenicula were made of up to 15 tiers of medullary cells (Figs 35, 36). Also in our plants, pseudolateral conceptacles were as common as axial ones. Regardless of their position, conceptacles were mostly pedicellate, ovoid to pyriform in shape, and occasionally beaked (Figs 22, 23, 36, 37). Some conceptacles bore cylindrical to compressed *cornicula* (Figs 24, 25); sessile conceptacles were scarce. It is worth to mention that several collections of *C. caespitosa* from a maerl bed in Galicia (site 31, Table 1) resembled the habit of *C. officinalis* (Fig. 41). These plants also showed trichocytes with a slightly swollen base that have been described for *C. officinalis* in the literature (Fig. 42; Economou-Amilli *et al.*, 1990; Irvine & Johansen, 1994; Bressan & Babbini, 2003); nonetheless our COI-5P sequences confirmed that they were co-specific with *C. caespitosa*.

Three haplotypes (39 specimens) were conspecific with *Ellisolandia elongata* (Fig. 3). The hap-6 was overwhelmingly dominant (34 specimens) and widespread as it ranged from the Bay of Biscay to South Iberia (see Table 1 for more details). Besides, hap-6 was identical to the DNA barcodes of the epitype of *Corallina elongata* (JX315327) designated by Brodie *et al.* (2013) from material

- ◀ Figs 16-31. *Corallina sensu lato* under stereomicroscope. **16.** Habit of *C. officinalis* (SANT-Algae 27687). **17.** Fronds of *C. officinalis* (SANT-Algae 27687) with simple branching and gaps between successive lateral branches, and axial (arrow) to pseudolateral (arrowhead) conceptacles, pedicellate with ovoid to pyriform shape. **18.** Apex of *C. officinalis* (SANT-Algae 27615) with branching pattern in more than one plane and with four terminal intergenicula. **19.** *C. officinalis* (SANT-Algae 27719) with axial sessile (arrow) and pedicellate (arrowhead) conceptacles, and pedicellate pseudolateral conceptacles. **20.** Habit of *C. caespitosa* (SANT-Algae 27716). **21.** Frond of *C. caespitosa* (SANT-Algae 27716) with typical branching pattern, and three terminal intergenicula. **22.** Frond of *C. caespitosa* (SANT-Algae 27716) with axial conceptacles, pedicellate, beaked (arrow) and not beaked (arrowhead), and pyriform in shape. **23.** *C. caespitosa* (SANT-Algae 27610) with axial conceptacles, pedicellate, not beaked and with ovoid to pyriform shape. **24.** *C. caespitosa* (SANT-Algae 27716) with pseudolateral conceptacles, pedicellate, one of them with *cornicula* cylindrical. **25.** *C. caespitosa* (SANT-Algae 27569) with pseudolateral conceptacles, pedicellate, with *cornicula* flattened. **26.** Habit of *Ellisolandia elongata* associated with the sponge *Grantia compressa* (SANT-Algae 27689). **27.** Frond of *E. elongata* (SANT-Algae 27717) with branching dense and with flattened, trapezoidal intergenicula. **28.** Frond of *E. elongata* (SANT-Algae 27771) with lateral expansions (arrow). **29.** Frond of *E. elongata* (SANT-Algae 27689) with axial and pseudolateral conceptacles, pedicellate, ovoid to pyriform in shape; some conceptacles bearing *cornicula*. Besides, the arrow show a conceptacle where the *cornicula* turned into new conceptacles. **30.** *E. elongata* (SANT-Algae 27689) with a pseudolateral conceptacle, pedicellate pyriform in shaped, with *cornicula* (arrow). **31.** *E. elongata* (SANT-Algae 27612) with flattened intergenicula with sessile conceptacles. Scale bars – 16, and 26: 1 cm, and; 17: 3 mm; 20: 7 mm; 21: 2 mm; 27, 29, and 30: 1.6 mm; 18, 19, 22, 23, 24, 25, 28, and 31: 1 mm.



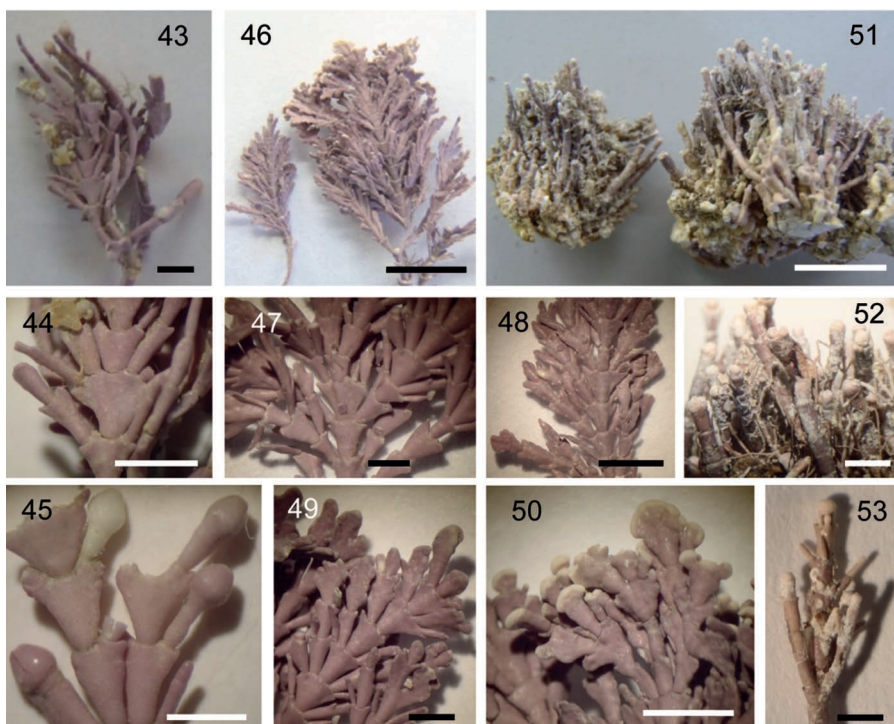
Figs 32-40. *Corallina sensu lato* under optical microscope, after decalcification and staining. **32.** Terminal branch of *C. officinalis* (SANT-Algae 27687) with three terminal intergenicula. **33.** Intergenicula with 21 tiers of medullary cells (arrow) of the apical zone of *C. officinalis* (SANT-Algae 27719). **34.** Axial conceptacle, pedicellate and ovoid in *C. officinalis* (SANT-Algae 27687). **35.** Terminal branch of *C. caespitosa* (SANT-Algae 27716) with three terminal intergenicula. **36.** Intergenicula with 12 tiers of medullary cells (arrow) in the apical zone of *C. caespitosa* (SANT-Algae 27610). **37.** Conceptacle axial, pedicellate and pyriform in shape of *C. caespitosa* (SANT-Algae 27610). **38.** Terminal branch of *Ellisolandia elongata* (SANT-Algae 27771) with up to four terminal intergenicula. **39.** Intergenicula with 10 tiers of medullary cells (arrow) of the apical zone of *E. elongata* (SANT-Algae 27689). **40.** Conceptacle axial, pedicellate, and ovoid in shape of *E. elongata* (SANT-Algae 27689). Scale bars – 32: 300 μm ; 33: 380 μm ; 34: 140 μm ; 35: 1100 μm ; 36: 400 μm ; 37: 100 μm ; 38, and 39: 700 μm ; 40: 50 μm .



Figs 41-42. Details of *Corallina caespitosa* (SANT-Algae 27564) growing on maerl. **41.** Habit of a specimen from the Galician site 31. **42.** Surface view under SEM of intergenicula showing epithallial cells and trichocytes (arrow). Scale bars – 41: 1 cm; 42: 50 μ m.

collected in South England and to the *E. elongata* (JQ615843) described by Hind & Saunders (2013a) using collections from Ireland. Our three haplotypes were closely related to each other (Fig. 6). *Ellisolandia elongata* was usually found hanging from rocks in the lower intertidal of exposed to semi-exposed coasts where it showed a clear preference for shady habitats with steep slopes and/or vertical rocks (Figs 13, 14). This plant was often associated with the sponge *Grantia compressa* Fabricius, 1780 (Figs 13, 14, 15; Table 2). Our specimens displayed the feather-like fronds described by Brodie *et al.* (2013) and were up to 5-7 cm long (Fig. 26). Branching typically was very dense. Intergenicula were more flattened and trapezoidal than in either *C. officinalis* or *C. caespitosa* (Figs 27, 28). The flattened appearance is further enhanced because the intergenicula are usually winged and they even show lateral expansions sometimes (Figs 27, 28, 38, 39). Under the microscope, the intergenicula were made of up to 12 tiers of medullary cells (Figs 38, 39). Brodie *et al.* (2013) mentioned that conceptacles were axial and never pseudolateral. However, we also often observed pseudolateral conceptacles in our plants (Fig. 30). Regardless of their placement, conceptacles were mostly pedicellate, ovoid to pyriform in shape, and commonly beaked (Figs 29, 30, 40); sessile conceptacles were rarely observed (Fig. 31). Interestingly, some conceptacles bore *cornicula* that occasionally turned into new conceptacles (Fig. 29).

Three haplotypes generated by samples collected in the Atlantic (hap-17) and the Iberian Mediterranean (hap-18 and hap-19, 1 bp difference) did not cluster with any of the *Corallina* described for the Iberian Peninsula (Fig. 3, Table 1) and were designated as *Corallina* sp. 1 (hap-17) and *Corallina* sp. 2 (hap-18 and hap-19). Interestingly, both *Corallina* sp. 1 and the hap-18 of *Corallina* sp. 2 resembled *E. elongata* in their habit (Figs 43, 46) while hap-19 of *Corallina* sp. 2 was morphologically similar to *C. officinalis* (Fig. 51). *Corallina* sp. 1 had violet-pink fronds that were stiff, erect, and 6 mm long (Fig. 43). Branching usually was in one plane, simple to compound pinnate, dense along the frond, somewhat irregular, with successive lateral branchlets typically separated by gaps (Figs 44, 45). Fronds were cylindrical to compressed; the latter had fan to trapezoidal intergenicula, somewhat winged (Figs 43-45). The number of terminal branches usually was three. Conceptacles were axial and pseudolateral, terminal, pedicellate, and ovoid to pyriform shape (Fig. 45). The hap-18 of *Corallina* sp. 2 had stiff and erect fronds, 25 mm long, with a violet-pink colour and occasional white apices (Fig. 46).



Figs 43-53. *Corallina* spp. under stereomicroscope. **43.** Habit of *Corallina* sp.1-hap-17 (SANT-Algae 27794). **44.** *Corallina* sp.1 (SANT-Algae 27794) with branching pattern in one plane, simple to compound pinnate with compressed, fan to trapezoidal intergenicula. **45.** *Corallina* sp.1 (SANT-Algae 27794) with axial conceptacles, pedicellate, with ovoid to pyriform shape. **46.** Habit of *Corallina* sp.2-hap-18 (SANT-Algae 27587). **47.** *Corallina* sp.2-hap-18 (SANT-Algae 27587) with branching pattern in one plane, simple to compound pinnate, with successive lateral branchlets, typically separated by inconspicuous gaps. **48.** *Corallina* sp.2-hap-18 (SANT-Algae 27587) with branching pattern in one plane, simple to compound pinnate, with successive lateral branchlets, typically separated by inconspicuous gaps, and with intergenicula trapezoidal, flattened and winged. **49.** *Corallina* sp.2-hap-18 (SANT-Algae 27587) with three terminal branches. Note that intergenicula are trapezoidal, flattened, winged, and they are welded. **50.** *Corallina* sp.2-hap-18 (SANT-Algae 27587) with intergenicula trapezoidal, flattened and winged, with lobed apex and white tips. **51.** Habit of *Corallina* sp.2-hap-19 (SANT-Algae 27805). **52.** *Corallina* sp.2-hap-19 (SANT-Algae 27805) unbranched, with stiff cylindrical intergenicula. **53.** *Corallina* sp.2-hap-19 (SANT-Algae 27805) with cylindrical intergenicula and branching pattern in more than plane, irregular. Scale bars – 43, 44, and 53: 750 μ m; 45, 46, 47, 49, and 52: 500 μ m; 48, and 50: 1 mm, 51: 1 cm.

Branching pattern was in one plane, simple to compound pinnate, dense along the thallus, sometimes irregular, with successive lateral branchlets typically separated by inconspicuous (or absent) gaps (Figs 47, 48). Intergenicula were trapezoidal, flattened and winged, sometimes welded, forming lobed intergenicula toward the apex (Figs 49, 50); some intergenicula were cylindrical at the basal part. The number of terminal branches usually was three (Figs 49, 50). On the other hand, the hap-19 of *Corallina* sp. 2 had stiff, erect fronds up to 12 mm long, pale greyish pink in colour (Figs 51, 52). Middle to basal sections of the fronds were

branched in more than one plane, simple to compound pinnate, irregular, with gaps among successive lateral branches (Fig. 53). Intergenicula were cylindrical, without lateral expansions and heavily calcified. No conceptacles were observed.

DISCUSSION

Corallina officinalis and *Ellisolandia elongata* (as *C. elongata*) were traditionally regarded as the only members of *Corallina sensu lato* in Atlantic Iberian Peninsula (Ardré, 1970; Conde *et al.*, 1996; Gorostiaga *et al.*, 2004; Bárbara *et al.*, 2005; Araújo *et al.*, 2009; Cires-Rodríguez & Cuesta-Moliner, 2010), although *E. elongata* was previously known displaying two conspicuously distinct morphotypes (Beltrán & Bárbara, 2003). Unfortunately, Beltrán & Bárbara (2003) lacked the molecular information necessary to corroborate whether these morphotypes were co-specific or not. Using sequence data, Walker *et al.* (2009) later found that both *C. officinalis* and *E. elongata* contained “pseudocryptic” diversity in the Northeastern Atlantic and described *C. caespitosa* as a new member of the tribe Corallineae. More recently, Brodie *et al.* (2013) and Hind & Saunders (2013) showed with DNA sequences that *C. caespitosa* is widely distributed and it occurs in the European Atlantic (Ireland, Britain, France, Portugal), Mediterranean (Italy, Greece), Macaronesia (Azores, Canary Islands), Africa (Ghana), North America (California), Asia (Japan), and Australia (New South Wales). Our results show that *C. caespitosa* is likewise widespread and common along the Atlantic coasts of Iberia. In fact, our results indicate that *C. caespitosa* possibly is the commonest *Corallina* in middle intertidal of rocky shores of the Atlantic Iberian coasts. In comparison, *E. elongata* is restricted to lower intertidal and mainly to shady habitats with steep slope while *C. officinalis* prefers locations under stronger wave action (exposed to semi-exposed) where it is frequent from the lower intertidal to the subtidal (Irvine & Johansen, 1994; Brodie *et al.*, 2013; Guiry & Guiry, 2013).

Despite their important biological similarity, we still find some potentially interesting differences in the pattern of COI-5P variation found in each species. Genetic variation was lower in *E. elongata* even though this species is widely distributed along Atlantic Iberia. Thus, we detected just three haplotypes in *E. elongata* while similar or even lower sampling efforts in the other two *Corallina* resulted in 6-7 haplotypes. Whether this lower diversity is due to its preference to a very restricted habitat or results of historical factors is still uncertain and warrants further investigation. Similarly, both *E. elongata* and *C. caespitosa* had one or a few geographically widespread haplotypes while the various haplotypes detected in *C. officinalis* seemed region-specific. The latter suggests a stronger phylogeographic structure in *C. officinalis* that deserves further study.

Most (94%) of our collections of *C. officinalis* were identified correctly using morphological traits, indicating that this species has a distinctive morphology, at least in Atlantic Iberia. Additionally, our results showed that the partition of *E. elongata* into the morphotypes proposed by Beltrán & Bárbara (2003) was loosely related to the pseudocryptic diversity revealed by Walker *et al.* (2009). Thus, 80% of the specimens assigned to the “typical” morphology were *C. caespitosa* while a majority (82%) of those assigned to the “elongated” morphotype belonged to *E. elongata*. Therefore, morphology alone could be of some use to re-identify voucher specimens originally assigned to *E. elongata*/*C. elongata* that are not amenable to molecular identification (Saunders & McDevit, 2012b). However, the considerable fraction of misidentifications detected in our study (approx. 20%) indicates that morphology must be used with caution.

Size, habit, branching, and intergenicula shape seem useful morphological attributes to discriminate among *Corallina officinalis*, *C. caespitosa*, and *Ellisolandia elongata* in the Atlantic Iberian Peninsula (Table 2). Thus, *C. officinalis* had large and robust thalli with cylindrical intergenicula while *C. caespitosa* consisted of smaller plants with compressed, trapezoidal intergenicula. It is noteworthy that *E. elongata* resembled, in general appearance, to *C. caespitosa* but with larger fronds and smaller intergenicula. Moreover, intergenicula in *E. elongata* were flatter, mainly trapezoidal, and more winged than in *C. caespitosa*; they even showed occasional lateral expansions that we never observed in *C. caespitosa*. As a result, *E. elongata* had a compact appearance while branchlets in *C. caespitosa* were typically separated by conspicuous gaps. Additionally, *C. caespitosa* displayed stiff, usually erect fronds while *E. elongata* had limp, feather-like fronds that usually hung from vertical rocks.

Two entities detected by our DNA barcodes could be identified only at genus level and were left unnamed. Further collections will be required to ascertain their actual identity, but the considerable divergence of their DNA barcodes indicates that these taxa must be cryptic members of *Corallina sensu lato* in Europe. In this regard, our results provide further support to the notion that *Corallina s. l.* possibly has larger diversity in Europe than previously thought (Brodie *et al.*, 2013). It is worth to note that the COI-5P sequences of these unnamed entities were slightly closer to *Ellisolandia* than to entities currently ascribed to genus *Corallina*. Thus, it cannot be disregarded that our unnamed entities might actually belong to the recently proposed genus *Ellisolandia* (Hind & Saunders, 2013a). Likewise interesting, the two haplotypes detected in *Corallina* sp.2 showed clear morphological differences and occurred on different seas (Atlantic, Mediterranean). It could be speculated that *Corallina* sp.2 might also show some phylogeographic structure or, alternatively, the variation observed in this study may reflect morphological plasticity in response to local ambient conditions. Further collections are warranted to resolve the magnitude, if any, of its population structure.

In conclusion, we have shown that the recently described *Corallina caespitosa* is widely distributed along Atlantic Iberia where it seems a common inhabitant of the rocky intertidal. Using morphology, we successfully assigned specimens to *Corallina officinalis*. However, discrimination between *C. caespitosa* and *E. elongata* has been more challenging. In the field, assignment of specimens to *E. elongata* can benefit from the fact that this plant shows a preference for shady, steep microenvironments. Likewise, some of the morphological traits identified by Beltrán & Bárbara (2003) allow a reasonably successful (80%) discrimination between these two species. However, a completely reliable assignment of specimens to either *C. caespitosa* or *E. elongata* requires sequence data.

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