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DNA barcoding sheds light on novel records in the Tunisian red algal flora

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ABSTRACT
Tunisia is located at a key position in the Mediterranean Sea where it constitutes a transitional area between the eastern and western basins benefitting from rich habitat diversity. The most recent inventory of marine macrophytes dated back to 1987 with an update in 1995, however these inventories were performed with identification based on morphological observations, which can be misleading for many seaweed species. To the best of our knowledge, this is the first study of the Tunisian macroalgal flora using DNA barcoding methods. Our target was to carry out a survey of macroalgae, to reveal cryptic species and allochthonous introductions, and to identify taxa with problematic taxonomy. COI-5P sequence analysis resolved 26 genetic species, including two new reports and three alien species. Of the remaining taxa, several displayed cryptic diversity relative to species reported from the Mediterranean versus from other geographical regions with taxonomic and nomenclature updates provided where possible. The aim of the present paper was to provide a DNA barcode-assisted floristic list of florideophycean algae collected along the Tunisian coast.

KEY WORDS
Florideophyceae, alien species, cryptic species, COI-5P, DNA barcode-assisted floristic list, Tunisian algal flora.

RÉSUMÉ
Le barcoding moléculaire met en lumière de nouveaux enregistrements dans la flore des algues rouges de Tunisie.
La Tunisie occupe une place clé dans la mer Méditerranée, car elle constitue une zone de transition entre les bassins orientaux et occidentaux et bénéficie ainsi d'une riche diversité d'habitats. L'inventaire le plus récent des macrophytes marins remonte à 1987 avec une mise à jour en 1995, cependant ces inventaires étaient limités aux observations morphologiques, ce qui peut être trompeur pour de nombreuses espèces d'algues. À notre connaissance, cette étude est la première concernant la flore macroalgale tunisienne qui utilise des méthodes de barcoding moléculaire. Notre objectif était de réaliser un inventaire des macroalgues, de révéler les espèces cryptiques et les introductions allochtones, et d'identifier les taxons présentant une taxonomie problématique. L'analyse de séquences COI-5P a résolu 26 espèces génétiques, dont deux qui n'avaient encore jamais été rapportées pour la Tunisie et trois espèces exotiques. Parmi les autres taxons, plusieurs ont montré la diversité cryptique entre les espèces de la Méditerranée et celles d'autres régions géographiques avec des mises à jour taxonomiques et de nomenclature fournies dans la mesure du possible. Le présent document visait à fournir une liste floristique assistée par barcoding moléculaire d'algues floridéophycéennes recueillies le long de la côte tunisienne.

MOTS CLÉS
Florideophyceae, espèces exotiques, espèces cryptiques, COI-5P, Liste floristique assistée par barcoding moléculaire, Flore algale tunisienne.

INTRODUCTION

Tunisia holds a key position in the Mediterranean between the eastern and western basins of the Mediterranean Sea and constitutes a transitional area with rich habitat diversity. The Strait of Sicily, the waters between Tunisia and Sicily, is the crossroads from south to north and from east to west, and is crucial in the analysis of the distribution of marine organisms in the Mediterranean Sea (Coll *et al.* 2010).

The presence of rocky or sandy coasts, deep bays, some protruding headlands, numerous islands and islets are characteristic features of the Tunisian littoral topography (Le Danois 1925; Azouz 1966, 1973; Ben Mustapha 1966; Poizat 1970; Ben Othman 1973; Blanpied *et al.* 1979a, b; Pergent & Kempf 1993). Such varied topography supports a rich marine macroalgal flora with the northern coast and the Gulf of Tunis being particularly diverse (Ben Mustapha & Afli 2007).

The first records of Tunisian marine algae were published by Piccone (1879; 1884) who reported a limited number of taxa from the northern coast, while the first inventory was compiled by Debray (1897). Subsequently, few studies were carried out until the valuable works of Meñez & Mathieson (1981) and of Ben Maiz and coworkers (Ben Maiz *et al.* 1987; Ben Maiz 1995), which have resulted in the current inventory of marine macrophytes from this country. These floristic and taxonomic studies of the Tunisian marine flora were completed only by means of classic morphological-anatomical identifications. Subsequent studies have focused on either selected taxa (Djellouli 2000; Ben Said & Ksouri 2002; Ksouri *et al.* 2002; Langar *et al.* 2002; Chebil-Ajjabi *et al.* 2005; Bouafif *et al.* 2014, 2016), limited areas (Ben Maiz & Boudouresque 1986; Ben Maiz & Shili 2007; Shili *et al.* 2007), specific ecological objectives (Ben Mustapha & Afli 2007; El Ati-Hellal *et al.* 2007), non-indigenous (alien) species (Sghaier *et al.* 2016), or dealt with the applications for which macroalgae are exploited (Ksouri *et al.* 2007; Azaza *et al.* 2008; Yaich *et al.* 2011; Kolsi *et al.* 2015). Consequently, they have had a minor impact on our understanding of species diversity in this flora.

Regrettably, morphological-anatomical identifications can be misleading in seaweed species (Saunders 2005). In this regard red algae are typical and can exhibit simple morphologies with high phenotypic plasticity and morphological convergence, which can be further complicated in species with an alternation of heteromorphic generations in their life histories. Red algal taxonomists have overcome such difficulties using molecular assisted alpha taxonomy (Saunders 2005; abbreviated MAAT in Cianciola *et al.* 2010) a "more objective technique… which uses differences in gene sequence data to delineate genetic species groups that are subsequently analysed morphologically and assigned to existing species or established as novel taxa" (Filloramo & Saunders 2016). Different barcode markers have been utilized for the different branches of the tree of life, including red macroalgal lineages (Saunders & McDevit 2012). Since the introduction of MAAT in 2005 the COI-5P region has proven effective and reliable for defining red algal species (e.g. Saunders 2005; Manghisi *et al.* 2010; Le Gall *et al.* 2015).

To achieve a comprehensive understanding of the macroalgal flora of a specific area, as well as of a specific taxonomic group, an integrated approach including MAAT, ecological and biogeographic data, together with phylogenetic analyses is necessary. However, accumulating all of these types of data is time-consuming and requires trained specialists. DNA barcoding has proved useful for facilitating rapid and effective surveys of macroalgal diversity in a specific area, uncovering cryptic species, assessing the relationships with neighbouring floras, monitoring allochthonous introductions, for the identification of problematic taxa to boost taxonomic research in a specific area, as well as to add data to the DNA library of life (Manghisi *et al.* 2010; Kress *et al.* 2015; Le Gall *et al.* 2015; Machín-Sánchez *et al.* 2016; Kogame *et al.* 2017; Le Gall *et al.* 2017). Indeed, DNA barcode surveys serve as an informed starting point to achieve that comprehensive understand-

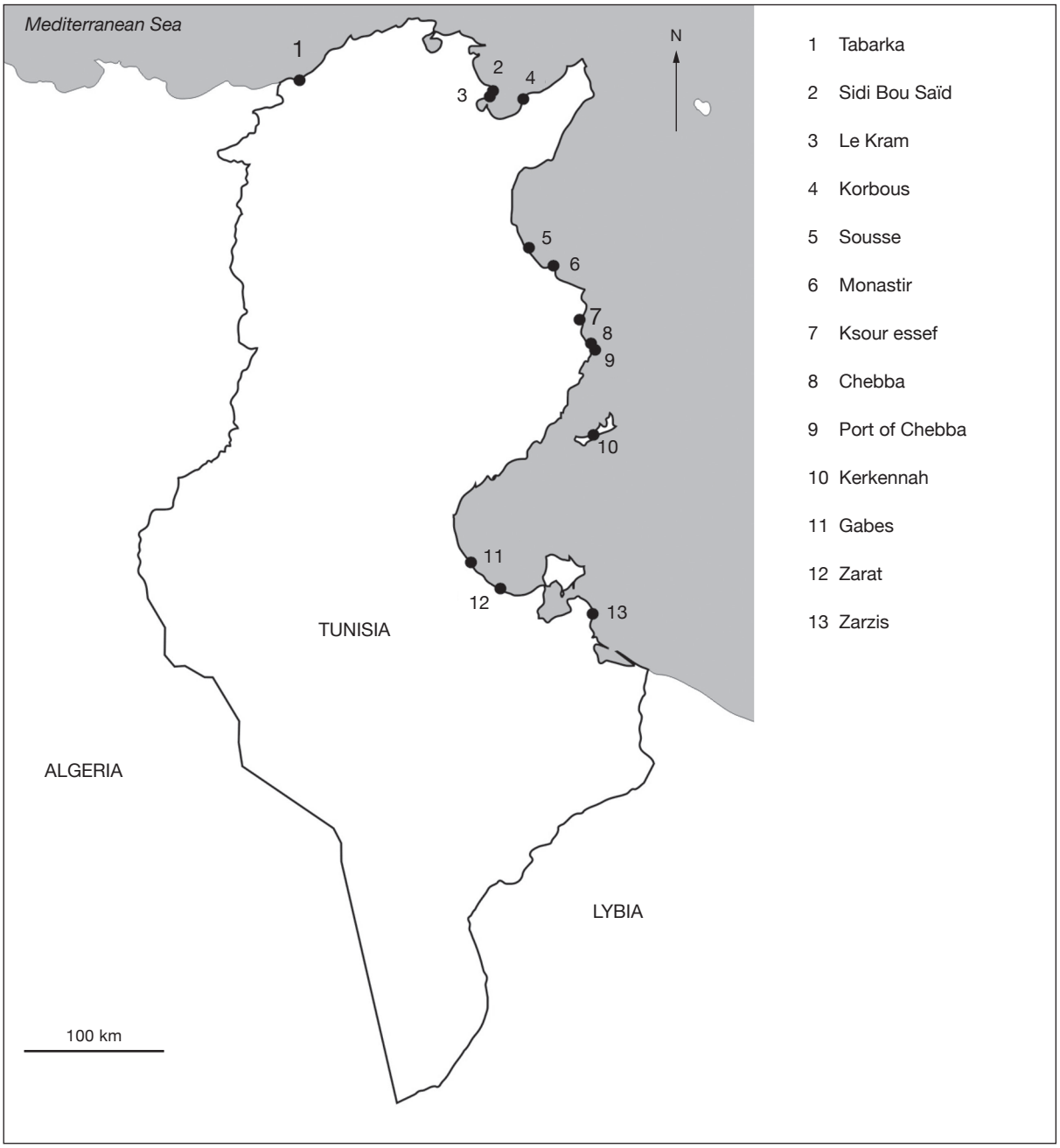

Fig. 1. — Collection sites along the Tunisian coastline. Scale bar: 100 km.

ing that we strive to achieve for macroalgal floras (Saunders 2008). The publication of DNA barcode-assisted floristic lists will further provide detailed information on the sequences generated from a definite floristic inventory, increasing the biodiversity knowledge linked to geographical information, and making them freely available for the scientific community.

The aim of the present paper is to provide a DNA barcode-assisted floristic list of florideophycean algae collected along the Tunisian coast. To the best of our knowledge, this is the first survey of the Tunisian macroalgal flora using DNA barcoding methods.

MATERIAL AND METHODS

Red macroalgae were sampled between February 2014 and August 2015 at 13 sites distributed along the Tunisian coastline (Fig. 1): Tabarka (36.9579 N, 8.7554 E); Sidi Bou Saïd (36.8664 N, 10.3501 E); Le Kram (36.8305 N, 10.3188 E); Korbous (36.8279 N, 10.5691 E); Sousse (35.8335 N, 10.6417 E); Monastir (35.7737 N, 10.8377 E); Ksour essef (35.4190 N, 11.0406 E); Chebba (35.2421 N, 11.1392 E); Port of Chebba (35.2308 N, 11.1629 E); Kerkennah (34.6622 N, 11.1222 E); Gabes (33.8918 N, 10.1188 E); Zarat (33.6901 N,

10.3819 E); Zarzis (33.5159 N, 11.1178 E). All samples were hand-collected from the intertidal and shallow subtidal zones. Samples were transported in seawater at low temperature to the laboratory, where they were washed thoroughly with seawater to remove debris. For each sample, a voucher specimen was prepared by pressing a single individual on an herbarium sheet with a subsample dried in silica gel for molecular analyses. Vouchers are housed in the Phycological Lab Herbarium (PhL) of the University of Messina, Italy. Additional samples included in the algal collections of PhL and of the Muséum national d'Histoire naturelle in Paris, France (PC) were also considered for comparison with Tunisian collections. Abbreviations of herbaria follow the online Index Herbariorum (Thiers, continuously updated).

DNA was isolated from silica dried thalli as detailed in Manghisi *et al.* (2010) and the mitochondrial COI-5P region was PCR amplified as described in Saunders & McDevit (2012). Sequencing reactions were performed by Genoscope (Centre National de Sequencage, www.genoscope.fr, Evry, France) or Macrogen Europe (The Netherlands). Specimen data, sequences and the primers used were deposited in the Barcode of Life Data Systems (BOLD, http://www.boldsystems.org) in the public dataset DS-REDTU and submitted to GenBank (GB, Benson *et al.* 2018). Collection information together with GB accession numbers are listed in Appendix 1. Forward and reverse sequence reads were assembled with the software ChromasPro (v. 1.41, Technelysium Pty Ltd) and multiple sequence alignments were constructed in Seaview (v. 4.3.3, Gouy *et al.* 2010), including sequences of related taxa downloaded from GB. Sequence alignments were subjected to distance analyses with a Neighbor-Joining algorithm under a K2P model of nucleotide substitution (Kimura 1980) as performed in PAUP* 4b10 for the Macintosh (Swofford 2002) to visualize clusters of genetic species, and pairwise distances were computed as p-distances. In addition, COI-5P sequences were searched through the BOLD "identification" interface. The closest COI-5P sequences, either from the comparison with GB records or as identified in BOLD, were recorded for each recognized genetic species uncovered in the Tunisian flora with 95% identity used as a cut-off value for recording a match.

RESULTS AND DISCUSSION

A total of 64 COI-5P sequences were generated resolving as 26 genetic species groups (Appendix 1). Of the 26 genetic species groups identified, two were newly reported for Tunisia, viz., *Ramicrusta* sp., and the alien species *Spermothamnion cymosum* (Harvey) De Toni.

Of the remaining 22 genetic species groups, two were alien species, *Hypnea cervicornis* J.Agardh and *Hypnea* "*cornuta*" (Kützing) J.Agardh and, in several instances, DNA analyses also highlighted cryptic diversity at the morphospecies level among specimens from the Mediterranean relative to other geographical regions (Appendix 1).

Eight records required a taxonomic update, viz., *Champia* sp., *Corallina caespitosa* R.H.Walker, J.Brodie & L.M.Irvine, *Ellisolandia* sp., *Gastroclonium* sp., *Halymenia* sp., *Hypnea cervicornis, Palisada tenerrima* (Cremades) D.Serio, M.Cormaci, G.Furnari & F.Boisset and *Phyllophora* sp. (Appendix 1).

CONCLUSIONS

In the present paper, we produced a DNA barcode-assisted floristic list of florideophycean algae collected along the Tunisian coast. This allowed us to reveal cryptic species, allochthonous introductions and to identify problematic taxa.

This is the first report of the presence of the alien species *Spermothamnion cymosum*, collected in Tabarka, close to a tourist harbour. We confirm the presence of two additional alien species, *Hypnea cervicornis* and *Hypnea* "*cornuta*", collected at Gabes and Zarat, close to commercial and fishing harbours. We also highlight cryptic diversity among the Mediterranean reports of these taxa. The presence of new introduced species is a crucial factor that will continue to change the biodiversity of the Mediterranean Sea (Cecere *et al.* 2016). In future, the situation could become significantly exacerbated by the recent expansion of the Suez Canal. This is currently considered one of the main vectors of species introduction into the Mediterranean Sea (Manghisi *et al.* 2011; Galil *et al.* 2015; Verlaque *et al.* 2015). Aquaculture has also been a key vector for the introduction of species from cold and temperate regions of the North Pacific Ocean (Verlaque *et al.* 2007; Kim *et al.* 2014), as have maritime and coastal development and other anthropogenic activities (Ben Mustapha & Afli 2007). Records of alien marine species from Tunisia are few because of the coastline topography and the lack of research programs or monitoring to deal with this issue (Sghaier *et al.* 2016). The Tunisian coast, in particular the Gulf of Gabes region, suffers the consequences of the above-mentioned disturbances. Hotspots for marine species introduction include harbours where human activities affect the health of ecosystems and facilitate the introduction and the secondary dispersal of alien species (Occhipinti-Ambrogi *et al.* 2011; Kim *et al.* 2014).

This is the first report of a species of *Ramicrusta* in the Mediterranean and the second in a temperate sea. It is also present along the French Riviera (Le Gall unpublished data). *Ramicrusta* is likely an overlooked genus, described at first as monospecific from South China Sea (Zhang & Zhou 1981) and later expanded with the description of other species with a potentially widespread tropical distribution, both in the Pacific Ocean and the Caribbean Sea (Pueschel & Saunders 2009; Dixon & Saunders 2013; Ballantine *et al.* 2016). The only known temperate species is *R. australica* (Dixon & Saunders 2013) the closest ally of the Tunisian entity here reported.

To the best of our knowledge this is the first study dealing with macroalgal inventory in Tunisia using a DNA approach. As opposed to morphological identifications, DNA barcodes are more useful for uniting biological specimens into genetic groups as a first step to assigning them to species and genera (Saunders 2005). This methodology is particularly useful in

organisms with simple morphologies, a high degree of phenotypic plasticity or convergence, and heteromorphic life histories, as is so common in marine macroalgae (e.g. Manghisi *et al.* 2015; Filloramo & Saunders 2016). To ensure an effective monitoring of biodiversity change over time we propose the use of DNA barcode-assisted floristic lists, which are more convenient and accurate than lists devised from traditional approaches, because associated barcode sequences are easily trackable and each specimen is unequivocally linked to a permanent genetic label regardless of any subsequent taxonomic or nomenclature variation.

DNA barcoding surveys contribute to new records to DNA libraries of life such as the BOLD catalogue, increasing our biodiversity knowledge, which is linked to geographical information and is also freely available to the scientific community. Furthermore, in the context of environmental management, the added value of DNA barcode-assisted identifications is that they give objective and verifiable data, an essential strength if we consider that floristic lists are extensively used by ecologists and environmental agencies as the basis for monitoring studies.

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APPENDICES

APPENDIX 1. — DNA barcode-assisted list of florideophycean samples analysed in this study. GenBank (GB) and BOLD systems (BOLD) accessions of sequences compared with Tunisian ones in comments are in supplementary information in Appendix 2.

Taxonomy	Vouchers (collection data)	GB accession numbers (COI-5P)	Top match	Comments	First report in Tunisia
CERAMIALES, Rhodomelaceae					
Digenea simplex (Wulfen) C.Agardh	RM0001 (Ksour Essef, 05/02/2014)	MG030761	*D. simplex*, 96.18% similarity, South Africa (BOLD# GRHOD062-10)	This genetic group lacked close matches in GB. An isolate from Western Australia is assigned to this name but is clearly distinct from the Tunisian species (95.79% sequence identity, see Schneider *et al.* 2018). The two are included in a large group with *Digenea arenahauriensis* C.W.Schneider, Hamzeh & G.W.Saunders from Bermuda, *Digenea mexicana* G.H.Boo & D.Robledo and *Digenea rafaelii* G.H.Boo, G.Andrade-Sorcia & S.M.Boo from Mexico, and *Digenea* sp. from Japan, in the past referred to *D. simplex* (Boo *et al.* 2018). The comparison with BOLD showed other specimens named as *D. simplex* that are also genetically distinct from the Tunisian species: three isolates from South Africa (Indian Ocean) and Japan (96.18-95.41% similarity), and another from Caribbean Netherlands grouping with *D. arenahauriensis* from Bermuda (96.02% similarity). The large sequence divergence and the geographical differences reflect cryptic diversity at the species level that requires further investigation. The type locality of *D. simplex* is Trieste, Italy, and therefore we retain this specific attribution for the Tunisian sample.	Pottier 1929
Palisada tenerrima (Cremades) Serio, Cormaci, G.Furnari & F.Boisset	RM0033 (Kerkennah, 20/04/2014)	MG030786	*Laurencia ceylanica* J.Agardh [currently synonym of *Chondrophycus* ceylanicus (J.Agardh) M.J.Wynne, Serio, Cormaci & G.Furnari], 95.45% similarity, Sri Lanka (BOLD# GRHOD263-10)	This genetic group lacked close matches in BOLD and GB. According to Furnari *et al.* (2002), Mediterranean entities previously identified as *Chondrophycus papillosus* (C.Agardh) Garbary & J.Harper [syn. *Laurencia papillosa*] should be attributed to *Chondrophycus tenerrimus* (J.Cremades) G.Furnari, F.Boisset, Cormaci & Serio, now *Palisada tenerrima* (J.Cremades) Serio, Cormaci, G.Furnari & F.Boisset.	Seurat 1934, as *Laurencia papillosa* (C.Agardh) Greville
	RM0070 (Kerkennah, 01/06/2014)	MG030784			
	RM0051(Port of Chebba, 27/04/2014)	MG030785			
	RM0081(Chebba, 22/06/2014)	MG030782			
	RM0140 (Monastir, 03/08/2014)	MG030783			
CERAMIALES, Wrangeliaceae					
Spermothamnion cymosum (Harvey) De Toni	RM0209 (Tabarka, 04/04/2015)	MG030803	*S. cymosum,* 99.54% similarity, Tasmania, Australia (BOLD# ABMMC7329-10)	The comparison with BOLD showed that the Tunisian specimen is conspecific with *S. cymosum* from Tasmania (Australia), the type area (BOLD# ABMMC7329-10, 99.54% similarity). This species was only known from Australia (Huisman & Walker 1990; Millar & Kraft 1993; Womersley 1998) until it was reported in Venice Lagoon based on combined morphological and molecular data (Armeli Minicante 2013). We also report the presence of the species in Brittany, France.	New report
	LLG5051 (Finistère, Brittany, France, 08/07/14)	MG030804			
	SAM390 (Isola della Certosa, Venice Lagoon, Italy, 04/10/10)	MF597664			

APPENDIX 1. — Continuation.

Taxonomy	Vouchers (collection data)	GB accession numbers (COI-5P)	Top match	Comments	First report in Tunisia
CORALLINALES, Corallinaceae					
Amphiroa "*beauvoisii*" J.V.Lamouroux	RM0254 (Monastir, 10/04/2015).	MG030753	*A. beauvoisii*, 99.54% similarity, North Carolina, United States (BOLD# MANC023-13; GB# KF367761)	This specimen is conspecific with *A. beauvoisii* from North Carolina, United States (data in GB and in BOLD) and Brazil (99.54-100% similarity). Other isolates are presently assigned to this name, but resolve as three additional genetic species, one from South Africa (Indian Ocean), with low similarity (93.96-94.58%), and the remaining two from Brazil, with 91.54-92.87% and 91.11-91.78% similarity respectively, indicating that there are different species as hypothesized by other authors (Kogame *et al.* 2017; Torrano-Silva Beatriz *et al.* 2018). Additional sequences assigned to this name are present in BOLD, but they are not closely related to each other, suggesting that two distinct groups occur, both from the Atlantic coast of Panama, one of which includes samples identified as *Amphiroa fragilissima* (Linnaeus) J.V.Lamouroux. Morphological and molecular studies of these species are required, including samples from various regions, notably the type locality (Portugal), to assess which of the many genetic species is genuine.	Meñez & Mathieson 1981
Amphiroa cryptarthrodia Zanardini	RM0050 (Port of Chebba, 27/04/2014)	MG030754	No match	This genetic group lacked close matches in BOLD and GB.	Feldmann 1961
Amphiroa rigida J.V.Lamouroux	RM0119 (Tabarka, 30/07/2014)	MG030755	*Amphiroa* sp., 95.07% similarity, Italy (BOLD# NGCOR272-16, NGCOR271-16)	There were no close matches in GB. Three sequences are recorded in BOLD with this name, which are not closely related to each other, delineating two additional genetic groups, both containing specimens collected on the Atlantic coast of Panama. The type locality of *A. rigida* is the Mediterranean Sea and, therefore, we retain the specific attribution of the Tunisian sample.	Hamel & Lemoine 1952
Corallina caespitosa R.H.Walker, J.Brodie & L.M.Irvine	RM0100 (Sousse, 01/08/2014) RM0261 (Sousse, 12/04/2015)	MG030759 MG030758	*C. caespitosa*, 100% similarity, Occitanie, France (BOLD# COR080-12)	The Tunisian specimen is conspecific with *C. caespitosa* (data in BOLD and GB, 97.37-100% similarity). *C. caespitosa* was described as a cryptic member of *Corallina* Linnaeus in the Atlantic European coasts, previously misidentified with either *C. officinalis* Linnaeus or *C. elongata* J.Ellis & Solander, now *Ellisolandia elongata* (J.Ellis & Solander) K.R.Hind & G.W.Saunders (Walker *et al.* 2009). Subsequent DNA studies revealed isolates from North Atlantic European (UK, including the holotype specimen, Ireland, Portugal, Spain, France) and American (North Carolina, United States) coasts, Atlantic Africa (Ghana), the Mediterranean (Italy, Greece, France), and the Pacific (South Korea, Japan, New South Wales-Australia, California-United States) (Brodie *et al.* 2013; Hind & Saunders 2013; Pardo *et al.* 2015; Williamson *et al.* 2015). In Tunisia and the wider Mediterranean Sea, two taxa of *Corallina* were reported (Ben Maiz *et al.* 1987; Bressan & Babbini 2003; and references therein), *C. officinalis* and *C. elongata*. Reports of *C. officinalis* and *E. elongata* in Tunisia should be attributed either to *C. caespitosa* or to *Ellisolandia* spp. (see below). The occurrence of the cool-temperate North Atlantic species *C. officinalis*, in the Mediterranean Sea requires confirmation (Brodie *et al.* 2013; Hind & Saunders 2013; Williamson *et al.* 2015).	Presumably reported in Tunisia as *C. officinalis* by Ben Alaya 1969, or as *C. elongata* by Lemoine 1924

APPENDIX 1. — Continuation.

Taxonomy	Vouchers (collection data)	GB accession numbers (COI-5P)	Top match	Comments	First report in Tunisia
Ellisolandia sp.	RM0231 (Korbous, 08/04/2015)	MG030762	*Corallina* sp., 99.30% similarity, Portugal (BOLD# COR101-13; GB# KF460992)	This specimen is conspecific with an undetermined species of *Corallina* (data in GB and in BOLD, 99.12-99.3% similarity) reported both along the Mediterranean coast of Spain and in Portugal (Pardo *et al.* 2015). According to Pardo *et al.* (2015), this species does not match any of the species previously recorded from the Iberian Peninsula, and the Atlantic and Mediterranean isolates display morphological differences with the former resembling *E. elongata* and the latter morphologically similar to *C. officinalis.* Additional collections are required to ascertain this species' identity, but the considerable divergence of its DNA barcode indicates that this entity may actually belong to the recently proposed genus *Ellisolandia* K.R.Hind & G.W.Saunders. Further we cannot conclude whether *Ellisolandia* sp. recorded here is new for Tunisia or corresponds to previous reports misidentified as either *E. elongata* or *Corallina officinalis*.	Presumably reported in Tunisia as *C. officinalis* by Ben Alaya 1969, or as *C. elongata* by Lemoine 1924
Jania adhaerens J.V.Lamouroux	RM0074 (Chebba, 22/06/2014) RM0169 (Zarzis, 30/08/2014)	MG030778 MG030779	*J. adhaerens*, 100% similarity, Bermuda (BOLD# BERMR281-10)	The Tunisian samples are conspecific with an undetermined species of *Jania* from Sicily (Italy), Malta and the Indian Ocean coast of South Africa (data in GB, 99.81-100% sequence similarity). Comparison with data in BOLD showed that Tunisian samples are conspecific with *J. adhaerens* from Western Australia and Bermuda (99.49-100% sequence similarity). Other sequences are present in BOLD assigned to this name, which are not closely related to each other delineating two additional genetic groups, one from Japan and South Africa, and another from Western Australia. The specific attribution of the Tunisian sample is strengthened by its alliance with sequences from the type locality, which is the Mediterranean Sea.	Piccone 1879
Jania virgata (Zanardini) Montagne	RM0202 (Tabarka, 04/04/2015) RM0232 (Korbous, 08/04/2015) PhL257 (Ragusa, Italy)	MG030780 MG030781 MG191361	Uncultured Corallinales, 99.67% similarity, Provence-Alpes-Cote d'Azur, France (GB# GQ917514)	The Tunisian samples and a specimen of *J. virgata* from Ragusa, Italy (type locality: Adriatic Sea), are conspecific with an undetermined species from the French Riviera (GB# GQ917514).	Piccone 1884, as *Corallina virgata* Zanardini

Appendix 1. — Continuation.

Taxonomy	Vouchers (collection data)	GB accession numbers (COI-5P)	Top match	Comments	First report in Tunisia
GELIDIALES, Pterocladiaceae					
Pterocladiella capillacea (S.G.Gmelin) Santelices & Hommersand	RM0005 (Le Kram, 01/03/2014)	MG030794	*P. capillacea*, 99.82% similarity, South Korea (GB# HM629885)	The Tunisian specimens are conspecific with *P. capillacea* (98.32-100% similarity, data from BOLD and GB) from different localities in the Indo-Pacific region (South Korea, Australia), Mexico (as *Pterocladia mexicana* W.R.Taylor, isotype specimen), Ecuador (as *Pterocladia robusta* W.R.Taylor, holotype specimen) and Brazil. Tunisian samples allied also with unpublished sequences of Mediterranean samples from France (99.65-100%, data not shown). *P. capillacea* is present along all Mediterranean coasts (type locality) and unpublished molecular analyses suggest that COI-5P sequences are useful to separate populations with a distinct geographical origin since Mediterranean samples (both Tunisian and unpublished isolates) are different from Asian and other samples (Ga Hun Boo, personal communication). At present, it is not possible to decide whether these differences will affect the taxonomy at the specific or intraspecific levels. Supplementary sequences and other molecular markers would be useful.	Piccone 1879, as *Gelidium corneum* var. *pinnatum* Greville
	RM0043 (Sidi Bou Said, 25/04/2014)	MG030795			
	RM0099 (Sousse, 01/08/2014)	MG030796			
	RM0125 (Tabarka, 30/07/2014)	MG030797			
	RM0198 (Tabarka, 04/04/2015)	MG030793			
	RM0139 (Monastir, 03/08/2014)	MG030798			
GIGARTINALES, Cystocloniaceae					
Hypnea cervicornis J. Agardh	RM0218 (Tabarka, 04/04/2015)	MG030767	*Hypnea flexicaulis* Y.Yamagishi & M.Masuda (currently regarded as a synonym of *H. cervicornis*), 100% similarity, Western Australia, Australia (GB# KP708661, KP708660, KP708659, KP708658); *Hypnea pannosa* [non *H. pannosa* J.Agardh], 100% similarity, Western Australia, Australia (BOLD# OZSEA1018-10, OZSEA1029-10)	The Tunisian specimen had 100% identity with COI-5P sequences for *H. cervicornis* (as *H. flexicaulis,* type locality: Japan) in GB from Western Australia, but a lower similarity (96.77-96.98%) with *H. cervicornis* from Brazil (type locality). Additional sequences are present in GB attributed to *H. cervicornis* (also as *H. flexicaulis*, *H. boergesenii* T.Tanaka and *H. valentiae* (Turner) Montagne) from several localities in the Indo-Pacific, the Atlantic and the Mediterranean seas (95.70-99.18% similarity). The large sequence variation indicates a cluster of cryptic species rather than a single species. The comparison with BOLD showed that the Tunisian sample is conspecific with unpublished COI-5P sequences for *H. pannosa* from Tasmania and Western Australia, but a lower identity (96.77-100%) with additional sequences of *H. pannosa* from Pacific Mexico (type area), New South Wales, Western Australia and South Korea, and an unnamed sample from Brazil. Further investigations are needed to clarify this cluster of cryptic species. In Tunisia, the species had been listed previously as either *H. cervicornis* (Djellouli *et al.* 2000; Zerzeri *et al.* 2010) or as *H. spinella* (C.Agardh) Kützing (Ben Maiz & Boudouresque 1986; Sghaier *et al.* 2016) on the basis of the synonymy proposed by Haroun & Prud'Homme van Reine (1993). However, the latter synonymy was recently rejected by Jesus *et al.* (2016; and references therein).	Ben Maiz & Boudouresque 1986, as *Hypnea* cf. *spinella*

APPENDIX 1. — Continuation.

Taxonomy	Vouchers (collection data)	GB accession numbers (COI-5P)	Top match	Comments	First report in Tunisia
Hypnea "*cornuta*" (Kützing) J.Agardh	RM0155 (Gabes, 10/08/2014)	MG030768	*H. cornuta*, 100% similarity, Italy (BOLD# ITRED091-13, ITRED090-13, ITRED089-13, ITRED088-13, ITRED087-13, ITRED086-13, ITRED085-13, ITRED084-13, ITRED083-13, ITRED081-13, ITRED080-13; GB# KF714860, KF714861, KF714862, KF714863, KF714865, KF714867, KF714866, KF714864, KF714859, KF714858, KF714868)	The Tunisian specimens are conspecific with *H.* "*cornuta*" from Sicily (Lake Ganzirri and Torre Faro), Italy (100% similarity, data from BOLD and GB). It is noteworthy that two additional samples named as *H.* "*cornuta*" are present in GB, which are not closely related to the entity from Tunisia, one from Korea (83.84% similarity), and another from Western Australia (92.39% similarity). The comparison with the type specimen or with samples from the type locality (Guinea) is needed to draw conclusions on their taxonomic affinities.	Sghaier *et al.* 2016
	RM0288 (Gabes, 10/08/2015)	MG030769			
	RM0289 (Gabes, 10/08/2015)	MG030770			
	RM0292 (Gabes, 10/08/2015)	MG030771			
	RM0167 (Zarat, 15/08/2014)	MG030772			
Hypnea musciformis (Wulfen) J.V.Lamouroux	RM0007 (Le Kram, 01/03/2014)	MG030774	*Hypnea musciformis*, 100% similarity, Italy (BOLD# ITRED082-13, GB# KF714869), Croatia (BOLD# REDEU143-11, REDEU142-11), Provence-Alpes-Cote d'Azur, France (BOLD# REDEU188-11), Occitanie, France (BOLD# REDEU747-11, REDEU737-11), Malaga, Spain (GB# KT428779)	The Tunisian specimens are conspecific with *H. musciformis* from the Mediterranean (Spain, France and Italy including Venice lagoon, which is in the type area) and the Atlantic Ocean (Namibia, North Carolina and Florida-United States) (0.98-100% similarity, data from BOLD and GB). Other sequences from tropical central America, both Atlantic and Pacific, are present in both BOLD and GB assigned to this name but, based on the observed low similarity (96.93-0.98%), they represent a complex of closely related species rather than a single cosmopolitan taxon.	Piccone 1879
	RM0097 (Sousse, 01/08/2014)	MG030775			
	RM0098 (Sousse, 01/08/2014)	MG030776			
	RM0271 (Sousse, 12/04/2015)	MG030773			
	RM0295 (Sousse, 13/08/2015)	MG030777			

Appendix 1. — Continuation.

Taxonomy	Vouchers (collection data)	GB accession numbers (COI-5P)	Top match	Comments	First report in Tunisia
GIGARTINALES, Gigartinaceae					
Chondracanthus acicularis (Roth) Fredericq *in* Hommersand, Guiry, Fredericq & Leister	RM0233 (Korbous, 08/04/2015)	MG030757	*C. acicularis*, 100% similarity, Brazil (BOLD# GIGSP005-10), Brittany, France REDEU161-11)	The Tunisian specimen is conspecific with *C. acicularis* (type area: Adriatic Sea) from various localities along the Mediterranean coast of France and Spain, the Atlantic (France, North Carolina, Florida and Brazil) and Indian Oceans (Western and South Australia) (99.21-100% similarity, data from BOLD and GB). Other sequences are present in BOLD assigned to this name (as *C. acicularis* and *Chondracanthus* sp. "2acicularis"), but they are conspecific with *Chondracanthus saundersii* C.W.Schneider & C.E.Lane, which was described from the western Atlantic (Schneider & Lane 2005).	Ben Alaya 1969, as *Gigartina acicularis* (Roth) J.V.Lamouroux
GIGARTINALES, Phyllophoraceae					
Phyllophora sp.	RM0184 (Tabarka, 04/04/2015)	MG030792	*Phyllophora* sp., 99.39% identity, Catalonia, Spain (BOLD# ABMMC1750-07; GB# GQ380359)	The Tunisian specimen is conspecific with an undetermined species of *Phyllophora* from Catalonia, Spain (99.39% similarity, data from BOLD and GB) already identified as a cryptic species segregated from *Phyllophora crispa* (Hudson) P.S.Dixon (type locality: England) (Le Gall & Saunders 2010), possibly *Phyllophora nervosa* (A.P.De Candolle) Greville (type locality: Mediterranean Sea and Cap of Good Hope), which is currently considered as a synonym of *P. crispa*.	Debray 1897, as *P. nervosa*
Schottera nicaeensis (J.V.Lamouroux *ex* Duby) Guiry & Hollenberg	RM0230 (Korbous, 08/04/2015) RM0236 (Korbous, 08/04/2015)	MG030801 MG030802	*S. nicaeensis*, 99.85% similarity, Provence-Alpes-Cote d'Azur, France (BOLD# REDEU463-11, REDEU555-11, REDEU579-11, REDEU613-11)	The Tunisian specimens are conspecific with *S. nicaeensis* from the Mediterranean sea (French Riviera, France, close to the type locality, and Catalonia, Spain, 99.54-99.85% similarity, data from BOLD and GB). Other sequences are present in both databases also identified as *S. nicaeensis* from Brittany (Atlantic France) (98.11-98.22%), and from Victoria and Tasmania, Australia (92.05-92.20%), which represent two distinct genetic species.	Molinier & Picard 1954, as *Gymnogongrus nicaeense* (J.V.Lamouroux ex Duby) Ardissone & Straforello
Sphaerococcus sp.	RM0113 (Tabarka, 30/07/2014) RM0182 (Tabarka, 04/04/2015) RM0212 (Tabarka, 04/04/2015)	MG030807 MG030805 MG030806	*Sphaerococcus coronopifolius* Stackhouse, 98.66% similarity, Brittany, France (BOLD# PNMIA648-14, PNMIA683-14; GB# KJ961098, KJ961100)	The comparison with the BOLD database showed that Tunisian samples are close to *S. coronopifolius* from the Atlantic French coast (type area), and from the Mediterranean, namely from France, Spain and Croatia (97.40-98.66% similarity). However, based on the observed sequence variation the assemblage might represent a cluster of cryptic species, with the Tunisian samples distinct from all others. More data are needed to draw taxonomic conclusions.	Pottier 1929
HALYMENIALES, Halymeniaceae					
Grateloupia filicina (J.V.Lamouroux) C.Agardh	RM0054 (Port of Chebba, 27/04/2014)	MG030764	*Grateloupia* sp., 99.67% similarity, Brittany, France (BOLD# REDEU254-11)	The Tunisian sample is conspecific with *G. filicina* from Asturias, Spain (99.62% similarity, data from GB), while the comparison with BOLD showed it allying with an unidentified species from Brittany, France (98.85-99.67%). Other sequences are present in BOLD and in GB assigned to this name, which are not closely related to the Tunisian one, delineating two additional genetic species from South Africa (89.98%) and from Hawaii (89.60%). The specific attribution of the Tunisian sample is strengthened by its *rbc*L sequence (GB# MH670459) showing it is conspecific with *Grateloupia filicina* from Italy (type area, GB# JX070629, 100% similarity).	Djellouli *et al.* 2000

APPENDIX 1. — Continuation.

Taxonomy	Vouchers (collection data)	GB accession numbers (COI-5P)	Top match	Comments	First report in Tunisia
Halymenia sp.	RM0062 (Kerkennah, 01/06/2014)	MG030765	*Halymenia floresii* [non *H. floresii* (Clemente) C.Agardh], 100% similarity, Occitanie, France (BOLD# REDEU726-11, REDEU709-11)	The Tunisian specimens have 100% similarity with *Halymenia* sp. (as *H. floresii*) from Occitanie, Mediterranean coast of France (data in BOLD). Other sequences are present in BOLD assigned to this name which represent two genetic species: one including the above mentioned specimens from Tunisia and France (99.67-100% similarity), the other with specimens from the Provence-Alpes-Cote d'Azur, Mediterranean coast of France, and Portugal, close to type locality (Cadiz, Spain) (97.57-97.59 similarity with the Tunisian specimens). The taxonomic status of the former genetic species should be further investigated. Other isolates from Brazil were present as *H.* cf. *floresii* in BOLD and GB, but they represent two newly described species, *H. ignifera* C.A.A.Azevedo, Cassano & M.C.Oliveira and *H. pinnatifida* C.A.A.Azevedo, Cassano & M.C.Oliveira. In Tunisia, Boudouresque (1970) reported an unidentified *Halymenia* species without any description, making it impossible to assess whether it is conspecific to *Halymenia* sp. reported here. Furthermore, an unidentified *Halymenia* species was also recorded by Ben Mustapha and Afli (2007) described as having a "*thalle cartilagineux à surface « glissante »*" (cartilagineous thallus with a slippery surface) and, therefore, clearly different from the Tunisian specimens reported here.	Presumably new report
	RM0281 (Gabes, 10/08/2015)	MG030766			
PEYSSONNELIALES, Peyssonneliaceae					
Peyssonnelia rubra (Greville) J.Agardh	RM0052 (Chebba, 27/04/2014)	MG030787	*P. rubra*, 100% similarity, Provence-Alpes-Cote d'Azur, France (BOLD# REDEU538-11), Croatia (BOLD# REDEU149-11), Sicily, Italy (BOLD# ABMMC11219-10).	The comparison with BOLD showed that Tunisian samples are conspecific with *P. rubra* (type locality: Ionian Islands, Greece) (99.85-100%) from other Mediterranean site, viz. Greece, France, Italy and Croatia.	Piccone 1879
	RM0116 (Tabarka 30/07/2014)	MG030788			
Peyssonnelia squamaria (S.G.Gmelin) Decaisne *ex* J.Agardh	RM0123 (Tabarka, 30/07/2014)	MG030789	*P. squamaria,* 99.85% similarity, Provence-Alpes-Cote d'Azur, France (BOLD# REDEU189-11, REDEU473-11, REDEU588-11)	The Tunisian specimens are conspecific with *P. squamaria* (98.77-99.85%) from other Mediterranean (type locality) location including Croatia, Italy and France (data from BOLD and GB).	Debray 1897
	RM0180 (Tabarka, 04/04/2015)	MG030790			
	RM0137 (Monastir, 03/08/2014)	MG030791			

Appendix 1. — Continuation.

Taxonomy	Vouchers (collection data)	GB accession numbers (COI-5P)	Top match	Comments	First report in Tunisia
Ramicrusta sp.	RM0115 (Tabarka, 30/07/2014)	MG030799	No match	There were no close matches in either in BOLD or in GB. However, in both databases, the Tunisian specimen was allied with the holotype specimen of *R. australica* K.R.Dixon (91.13% similarity) and other species of *R.* D.R.Zhang and J.H.Zhou from the Pacific Ocean (Vanuatu, China, Philippines, Australia) and the Caribbean Sea (Bonaire, Jamaica and Puerto Rico). Whether the Tunisian taxon is a new species or a Mediterranean species already described in another genus that should be transferred to the genus *Ramicrusta* will be better resolved after a larger sampling of Peyssonneliaceae in the Mediterranean.	New report
RHODYMENIALES, Champiaceae					
Champia sp.	RM0282 (Gabes, 10/08/2015)	MG030756	No match	There were no close matches in either in BOLD or GB. In Tunisia the genus *Champia* Desvaux is represented only by *C. parvula* (C.Agardh) Harvey (Meñez & Mathieson 1981). A COI-5P sequence of a specimen of *C. parvula* from the type locality (Cadiz, Spain, GB KF356103) is distantly related to the Tunisian ones (89.50% similarity). Specimens identified as *C. parvula* but not related to topotype sequence, both in GB (Hawaii) and in BOLD (Mediterranean France), highlighted the presence of cryptic diversity at the species level under this taxon name that should be further investigated.	Feldmann 1961, as *C. parvula*
Gastroclonium sp.	RM0223 (Korbous, 08/04/2015)	MG030763	*Gastroclonium reflexum* (Chauvin) Kützing, 98.92% similarity, Provence-Alpes-Cote d'Azur, France (BOLD# REDEU618-11)	The comparison with BOLD showed that the Tunisian sample is conspecific with *Gastroclonium* sp. (as *G. reflexum*) from the Mediterranean French coast (98.92% similarity). In both BOLD and GB, another entity is represented under the name *G. reflexum*, including samples from the Atlantic coasts of France (type area) and Spain. This is likely the genuine species and is only distantly related to the Tunisian taxon (86.56-86.59%). Further sampling and morphological and molecular observations, including *G. clavatum* (Roth) Ardissone, a taxon described from the Mediterranean Sea, are necessary to assess the actual specific identity of the Tunisian sample.	Possibly reported in Tunisia as *G. clavatum* by Feldmann 1961
Rhodymenia ardissonei (Kuntze) Feldmann	RM0165 (Zarat, 15/08/2014)	MG030800	*R. ardissonei*, 99.84% similarity, Occitanie, France (BOLD# REDEU734-11), Provence-Alpes-Cote d'Azur, France (BOLD# REDEU447-11, REDEU571-11, REDEU531-11)	The comparison with BOLD showed that the Tunisian sample is conspecific with *R. ardissonei* from the French Riviera (type area) (99.52-99.84% similarity).	Boudouresque 1970

Appendix 2. — GenBank (GB) and BOLD systems (BOLD) accessions of sequences compared with Tunisian ones in Appendix 1.

Taxonomy	GB	BOLD
Digenea simplex (Wulfen) C. Agardh	*D. simplex* Western Australia (Australia): MF093979 *D. arenahauriensis* Bermuda: MG648076, MG648075, MG648074, MG648073, MG648072, MG648071, MG648070, MG648069 *D. mexicana* Mexico (Atlantic Ocean): MH514878, MH514877, MH514876, MH514875, MH514874 *D. rafaelii* Mexico (Pacific Ocean): MH514883, MH514882, MH514881, MH514880, MH514879 *Digenea* sp. Japan: MH514885, MH514884	*D. simplex* South Africa (Indian Ocean): GRHOD062-10 *D. simplex* Japan: GUAMR357-10, GUAMR351-10 *D. simplex* Caribbean Netherlands: ALSEU311-16 *D. arenahauriensis* Bermuda: BERMR302-10, ABMMC23182-17, BERMR327-17, BERMR325-17, BERMR196-10, BERMR328-17, BERMR326-17, BERMR197-10, BERMR322-17, BERMR324-17, BERMR323-17
Spermothamnion cymosum (Harvey) De Toni		*S. cymosum* Tasmania (Australia): ABMMC7329-10
Amphiroa "*beauvoisii*" J.V. Lamouroux	*A. beauvoisii*, North Carolina (United States): KF367761 Brazil: MG521348, MG521337, MG521324, MG521319 South Africa (Indian Ocean): LC071728, LC071727, LC071726, LC071729 Brazil: MG521318, MG521309, MG521340, MG521327, MG521342	*A. beauvoisii* North Carolina (United States): MANC023-13 Panama (Atlantic Ocean): ABBAD184-16, ABBAD183-16, ABBAD113-15 *A. fragilissima* Panama (Atlantic Ocean): ABBAD163-16, ABBAD160-16
Amphiroa rigida J.V. Lamouroux		*A. rigida* Panama (Atlantic Ocean): ABBAD082-15, ABBAD081-15, ABBAD117-16

Appendix 2. — Continuation.

Taxonomy	GB	BOLD
Corallina caespitosa R.H. Walker, J. Brodie & L.M. Irvine *in* Walker *et al.* 2009	*C. caespitosa* England (UK): DQ191342, DQ191343, DQ191344, FM180072 Portugal: HQ919502, HQ919503, HQ919504, HQ919505, HQ919506, HQ919507, HQ919508, HQ919509, HQ919510, HQ919511, KP834356, KP834357, KF460961, KF460965, KF460966, KF460967, KP834373 France (Atlantic Ocean): GQ917248, KF460950, KF460951, KF460952, KF460953, KF460955, KJ960596, KP834360, KP834361, KP834371, KP834372 Spain (Atlantic Ocean): , KF460937, KF460938, KF460939, KF460940, KF460941, KF460942, KF460943, KF460944, KF460945, KF460946, KF460947, KF460948, KF460949, KF460954, KF460957, KF460958, KF460959, KF460960, KF460962, KF460963, KF460964, KF460968, KF460969, KF460970, KF460971, KP834363, KP834367, KP834370 North Carolina (United States): KJ202079 Ghana: KP834368, KP834369 Italy: KP834366 Greece: FM180066, FM180067, KP834358 France (Mediterranean Sea): KF460956 South Korea: HQ544043, HQ544048, JQ615599, JQ615600 Japan: HM918980, JQ615602, JQ615603, LC035644, LC035645, LC035648, LC035649, LC035651, LC035652, LC035654, LC035656, LC035657, LC035658, LC035659, LC035713, LC035714, LC035719, LC035720, LC035721, LC035723, LC035725, LC035727, LC035728, LC035732, LC035735, LC035737, LC035738, LC035740, LC035741, LC035742, LC035743, LC035744, LC035749, LC035827, LC035830, LC035831, LC035832, LC035884, LC035885, LC035896, LC035897, LC035898, LC035899, LC035900, LC035901, LC035902, LC035903, LC035904, LC035905, LC035906, LC035907, LC035908, LC035909, LC035910, LC035911, LC035912, LC035913, LC035914, LC035915, LC035916, LC035917, LC035918, LC035919, LC035920, LC035921, LC035922, LC035923, LC035924, LC035925, LC035926, LC035927, LC035936, LC035938, LC035939, LC035940, LC035943, LC035953, LC035954, LC035955, LC035956, LC035959, LC035960, LC035961, LC035962, LC035963, LC035964, LC035965, LC035967, LC035968, LC035969, LC035975, LC035998, LC035999, LC036000, LC036001, LC036002, LC036003, LC036004, LC036006, LC036007, LC036008, LC036012, LC036013, LC036016, LC036025, LC036029, LC036038, LC036043, LC036066, LC036067, LC036069, LC036084, LC036086, LC036087, LC036088, LC036089, LC036090, LC036092, LC036093, LC036096, LC036100, LC036102, LC036103, LC036105, LC036107, LC036109, LC036110, LC036111, LC036112, LC036113, LC036114, LC036115, LC036116, LC036117, LC036118, LC036119, LC036120, LC036122, LC036124, LC036125, LC036126, LC036127, LC036128, LC036129, LC036130, LC036131, LC036132, LC036133, LC036134, LC036135, LC036136, LC036137, LC036139, LC036140, LC036141, LC036142, LC036143, LC036144, LC036145, LC036147, LC036148, LC036153, LC036158, LC036162, LC036165, LC036166, LC036170, LC036174, LC036183, LC036184, LC036185, LC036186, LC036187, LC036188, LC036189, LC036190, LC036191, LC036192, LC036193 New South Wales (Australia): KP834362 California (United States): JQ615601, KP834359	*C. caespitosa* Ireland: ABMMC14831-11, ABMMC14830-11 Portugal: COR075-12, COR069-12, ABMMC14519-10, ABMMC14521-10, ABMMC14522-10, ABMMC14524-10, ABMMC14529-10, ABMMC14528-10, ABMMC14527-10, ABMMC14518-10, ABMMC14525-10, ABMMC14526-10, COR071-12, COR070-12 France (Atlantic Ocean): COR088-12, COR087-12, COR086-12, COR085-12, PNMIA379-14, COR083-12 Spain (Atlantic Ocean): COR060-12, COR076-12, COR062-12, COR063-12, COR029-12, COR096-13, COR056-12, COR053-12, COR054-12, COR039-12, COR027-12, COR026-12, COR013-12, COR008-12, COR001-12, COR002-12, COR041-12, COR004-12, COR095-13, COR023-12, COR011-12, COR009-12, COR005-12, COR003-12, COR093-13 North Carolina (United States): MANC052-13 France (Mediterranean Sea): COR080-12 South Korea: ABMMC11268-10, ABMMC11262-10, ABMMC11813-10, ABMMC11793-10 Japan: ABMMC9900-10, ABMMC8527-10, HOKUP167-10, ABMMC8600-10 California (United States): ABMMC11452-10
Ellisolandia sp.	*Corallina* sp. Spain (Mediterranean Sea): KF460991 Portugal: KF460992	*Corallina* sp. Spain (Mediterranean Sea): COR098-13 Portugal: COR101-13

Appendix 2. — Continuation.

Taxonomy	GB	BOLD
Jania adhaerens J.V. Lamouroux	*Jania* sp. Italy: KP834351 Malta: KP834350 South Africa (Indian Ocean): LC071780	*J. adhaerens* Western Australia (Australia): HUWAR195-11, OZSEA054-10, OZSEA053-10, OZSEA123-10 Bermuda: BERMR281-10 Japan: ABMMC8603-10, ABMMC11109-10 South Africa: GRHOD145-10
Jania virgata (Zanardini) Montagne	*Jania* sp. (as Uncultured Corallinales) France (Mediterranean Sea): GQ917514	
Pterocladiella capillacea (S.G. Gmelin) Santelices & Hommersand	*P. capillacea* South Korea: HM629885 Australia: KU382033 P. capillacea (as *P.mexicana*) Mexico: KX427235 *P. capillacea* (as *P. robusta*) Ecuador: KX427237	*P. capillacea* South Korea: ABMMC11843-10, ABMMC11263-10 Australia: OZSEA3147-13, OZSEA2511-11, ABMMC1269-07, ABMMC8216-10, OZSEA2747-11, OZSEA906-10, OZSEA933-10, OZSEA934-10, OZSEA929-10, OZSEA945-10, OZSEA935-10, OZSEA936-10
Hypnea cervicornis J. Agardh	*H. cervicornis* (as *H. flexicaulis*) Western Australia (Australia): KP708658, KP708659, KP708660, KP708661 Mexico (Pacific Ocean): KP708647 Hawaii: HQ422674 South Korea: EF136592, EF136593, EF136594, EF136595, EF136596, EF136597, EF136598, EF136599, EF136600, EF136601, EF136602, EF136603, EF136604, EF136605, EF136606, EF136607, EF136609, EF136610, EF136611, KP708607, KP708608, KP708609, KP708610, KP708611, KP708612, KP708613, KP708614, KP708615, KP708616, KP708617, KP708618, KP708619, KP708620, KP708621, KP708622, KP708623, KP708624, KP708625, KP708626, KP708627, KP708628, KP708629, KP708630, KP708631, KP708632, KP708633, KP708634, KP708635, KP708636, KP708637, KP708638, KP708639, KP708640, KP708641, KP708642 Hong Kong: KP708646 Taiwan: EF136608 Indonesia: KP708648, KP708649, KP708650, KP708651, KP708652, KP708653, KP708654 Philippines: EF136591, KP708655, KP708656, KP708657 Maldives: KU905089, KU905102, KU905115 India: KP708643, KP708644, KP708645 Italy: FN823052 *H. cervicornis* (as *H. boergesenii*) Brazil: KM233169, KM214568, KM214567, KM214566, KM214565, KM214564, KM214563, KM214562, KM214561, KM214530 South Korea: EU345983 *H. cervicornis* (as *H. valentiae*) Hawaii: HQ422901 *H. cervicornis* Brazil: KU905081, KU905082, KU905088, KU905095, KU905096, KU905099, KU905101, KU905103, KU905106, KU905109, KU905114, KU905116, KU905120, KU905126, KU905131, KU905134, KU905139, KU905142, KU905143, KU905149, KU905150, KU905151, KU905153, KU905154 Panama (Atlantic Ocean): KU905147	*H. pannosa* Mexico: GRHOD438-10 New South Wales (Australia): ABMMC8464-10, OZSEA1006-10, OZSEA1007-10, OZSEA977-10, OZSEA1008-10, 89 Tasmania (Australia): ABMMC7275-10 Western Australia (Australia): OZSEA1029-10, OZSEA1018-10, OZSEA1032-10, OZSEA1031-10, OZSEA1030-10, OZSEA1028-10, HUWAR060-10 South Korea: ABMMC11715-10, ABMMC11702-10, ABMMC11924-10 *Hypnea* sp. Brazil: GIGSP065-11
Hypnea "*cornuta*" (Kützing) J. Agardh	*H.* "*cornuta*" Italy: KF714858, KF714859, KF714860, KF714861, KF714862, KF714863, KF714864, KF714865, KF714866, KF714867, KF714868 Korea: GQ141878	*H.* "*cornuta*" Italy: ITRED091-13, ITRED090-13, ITRED089-13, ITRED088-13, ITRED087-13, ITRED086-13, ITRED085-13, ITRED084-13, ITRED083-13, ITRED081-13, ITRED080-13 Western Australia (Australia): HUWAR240-11

Appendix 2. — Continuation.

Taxonomy	GB	BOLD
Hypnea musciformis (Wulfen) J.V. Lamouroux	*H. musciformis* Italy: KF714869, KM523207 Spain (Mediterranean Sea): KT428779 France (Mediterranean Sea): GQ141881 Namibia: KM523208 North Carolina (United States): KJ202077, KM523205 Florida (United States): KM523206 Dominican Republic: KM523204 Mexico (Atlantic Ocean): KT428772, KT428773, KT428774, KT428775, KT428776, KT428777, KT428778 Colombia (Atlantic Ocean): KP725275 Barbados: KM523203 Caribbean Netherlands: Hawaii: HQ422612, HQ422630, HQ422646, HQ422876	*H. musciformis* Spain (Mediterranean Sea): 99 France (Mediterranean Sea): REDEU747-11, REDEU737-11, REDEU188-11, REDEU143-11, REDEU142-11 Italy: ITRED082-13 North Carolina (United States): MANC059-13 Colombia (Atlantic Ocean): ALGBA007-14 Caribbean Netherlands: ALSEU111-16, ALSEU031-16 Texas (United States): ABMMC1572-07
Chondracanthus acicularis (Roth) Fredericq *in* Hommersand, Guiry, Fredericq & Leister	*C. acicularis* France (Atlantic Ocean): KJ960534, KJ960535 North Carolina (United States): KJ202081 Florida (United States): KR909521, KR909520, KR909519	*C. acicularis* France (Mediterranean Sea): REDEU468-11, REDEU591-11 Spain (Mediterranean Sea): REDEU135-11, REDEU131-11 France (Atlantic Ocean): REDEU080-09, PNMIA1406-14, PNMIA071-14, REDEU269-11, REDEU184-11, REDEU305-11, REDEU676-11, REDEU161-11, REDEU341-11, REDEU383-11, REDEU414-11, North Carolina (United States): MANC042-13 Brazil: GIGSP005-10 Western Australia (Australia): OZSEA960-10, OZSEA961-10, OZSEA923-10, HUWAR077-10, ABMMC23442-17, HUWAR090-10, OZSEA1100-10 South Australia (Australia): OZSEA4212-13, OZSEA4211-13 *Chondracanthus* sp. New South Wales (Australia): OZSEA946-10 *C. saundersii* Brazil: GIGSP007-10 *C. saundersii* (as *C. acicularis*) Sri Lanka: GRHOD238-10
Phyllophora sp.	*Phyllophora* sp. Spain (Mediterranean Sea): GQ380359	*Phyllophora* sp. Catalonia, Spain: ABMMC1750-07
Schottera nicaeensis (J.V. Lamouroux ex Duby) Guiry & Hollenberg	*S. nicaeensis* France (Mediterranean Sea): KU640333, KU640332 France (Atlantic Ocean): KJ961097, KJ961096, KJ961095 Victoria (Australia): GQ380372	*S. nicaeensis* France (Mediterranean Sea): REDEU463-11, REDEU555-11, REDEU579-11, REDEU613-11 Spain (Mediterranean Sea): REDEU516-11, REDEU524-11 France (Atlantic Ocean): PNMIA1164-14, PNMIA685-14, PNMIA1194-14 Victoria (Australia): ABMMC19559-14, ABMMC19560-14, ABMMC208-06, ABMMC5017-09 Tasmania (Australia): ABMMC7173-10, ABMMC7183-10, ABMMC8229-10, ABMMC8247-10, ABMMC8264-10, ABMMC8270-10, ABMMC7287-10, ABMMC7292-10, ABMMC7295-10, ABMMC7315-10, ABMMC7179-10, ABMMC7180-10, ABMMC7182-10, ABMMC7208-10, ABMMC7217-10, ABMMC7248-10, ABMMC7166-10, ABMMC7169-10, ABMMC4997-09, ABMMC7146-10

APPENDIX 2. — Continuation.

Taxonomy	GB	BOLD
Sphaerococcus sp.		*S. coronopifolius* France (Atlantic Ocean): PNMIA013-14, PNMIA060-14, PNMIA1052-14, PNMIA1079-14, PNMIA1098-14, PNMIA1152-14, PNMIA1166-14, PNMIA1167-14, PNMIA1196-14, PNMIA1213-14, PNMIA1227-14, PNMIA505-14, PNMIA648-14, PNMIA683-14, REDEU169-11, REDEU258-11, REDEU287-11, REDEU314-11, REDEU316-11, REDEU333-11, REDEU351-11, REDEU691-11, REDEU227-11, REDEU216-11, REDEU204-11, REDEU363-11, REDEU377-11, REDEU379-11, REDEU406-11 France (Mediterranean Sea): REDEU729-11, REDEU719-11, REDEU139-11, REDEU532-11, REDEU505-11, REDEU432-11, REDEU478-11, REDEU499-11, REDEU059-09 Spain (Mediterranean Sea): REDEU157-11, REDEU132-11 Croatia: REDEU038-09, REDEU150-11
Grateloupia filicina (J.V. Lamouroux) C.Agardh	*G. filicina* Spain (Atlantic Ocean): KP271165 Hawaii: HQ422590	*Grateloupia* sp. France (Atlantic Ocean): REDEU296-11, REDEU343-11, REDEU254-11 *G. filicina* South Africa: ABMMC20760-15, ABMMC20752-15, ABMMC20743-15
Halymenia sp.	*Halymenia floresii* Portugal: GQ862071	*Halymenia* sp. (as *H. floresii*) France (Mediterranean Sea): REDEU726-11, REDEU709-11, REDEU491-11 *H. floresii* Portugal: ABMMC3751-09
Peyssonnelia rubra (Greville) J. Agardh		*P. rubra* Greece: ABMMC4960-09 France (Mediterranean Sea): REDEU538-11, REDEU441-11 Italy: ABMMC11219-10 Croatia: REDEU149-11
Peyssonnelia squamaria (S.G. Gmelin) Decaisne *ex* J. Agardh	*P. squamaria* Italy: JX969741	*P. squamaria* Croatia: REDEU146-11, REDEU140-11, REDEU145-11 Italy: ABMMC11218-10, ABMMC11217-10 France (Mediterranean Sea): REDEU506-11, REDEU533-11, REDEU563-11, REDEU587-11, REDEU189-11, REDEU473-11, REDEU588-11

Appendix 2. — Continuation.

Taxonomy	GB	BOLD
Ramicrusta sp.	*R. australica* Australia: JX969724 *Ramicrusta lateralis* Vanuatu: JX969734, JX969721 *Ramicrusta trichaurea* Vanuatu: JX969719 *Ramicrusta aranea* Vanuatu: JX969701 *Ramicrusta appressa* Philippines: JX969707 Vanuatu: JX969695 *Ramicrusta nanhaiensis* China: JX969713 *Ramicrusta bonairensis* Bonaire: KX417374 *Ramicrusta textilis* Jamaica: JX969749 Vanuatu: JX969704, JX969690 Puerto Rico: KX417373 *Ramicrusta monensis* Bonaire: KX417375	*Ramicrusta australica* Australia: ABMMC1975-07, ABMMC8190-10, ABMMC8858-10, ABMMC7509-10, OZSEA2210-11, OZSEA2213-11 *Ramicrusta appressa* Australia: ABMMC8642-10, CREEF044-12 Philippines: ABMMC14602-11 Vanuatu: ABMMC1876-07 *Ramicrusta trichaurea* Vanuatu: ABMMC1885-07 *Ramicrusta* spp. Australia: ABMMC19098-13, ABMMC19416-13, CREEF069-12 Tanzania: ABMMC5201-09
Champia sp.	*C. parvula* Spain (Atlantic Ocean): KF356103 Hawaii: HQ422763, HQ422819, HQ422822, HQ422864	*C. parvula* France (Mediterranean Sea): REDEU717-11, REDEU487-11
Gastroclonium sp.	*G. reflexum* France (Atlantic Ocean): KJ179926 Spain (Atlantic Ocean): KJ168046, KJ168048, KJ168050	*Gastroclonium* sp. (as *G. reflexum*) France (Mediterranean Sea): REDEU618-11 *G. reflexum* Spain (Atlantic Ocean): SERSP040-10, SERSP041-10, SERSP042-10
Rhodymenia ardissonei (Kuntze) Feldmann		*R. ardissonei* France (Mediterranean Sea): REDEU734-11, REDEU447-11, REDEU571-11, REDEU531-11, REDEU074-09, REDEU023-09