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Genetic affinities and biogeography of putative Levantine-endemic seaweed *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov. (Phaeophyceae)

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ABSTRACT
Cystoseira sensu lato (Ochrophyta) forests are important habitat formers in the Mediterranean Sea, but they have mostly been studied in the western basin where many species are under decline. In the eastern basin, where fewer species occur, *Cystoseira rayssiae* Ramon was described in the year 2000 as an endemic species based on morphological characteristics from herbaria samples collected on the Israeli coast. No further investigations have been conducted on this peculiar species since, but recently it has been recorded in contiguous Lebanon and outside the Mediterranean. Our work was aimed at confirming the taxonomic validity and endemic nature of this species, including its position among the recently split *Cystoseira sensu stricto, Carpodesmia* Greville and *Treptacantha* Kützing genera, by sequencing the mitochondrial COI gene and by examining morphological characteristics in samples from three different sites in northern Israel. Notwithstanding considerable morphological plasticity, molecular analyses revealed a single unique COI sequence. Phylogenetic analyses show that *Cystoseira rayssiae* belongs to the resurrected genus *Treptacantha* and hence, the new combination *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., is proposed. Unique sequences and a restricted range support its Levantine-endemic status. Intriguing extra-Mediterranean reports from the Red Sea and the Persian Gulf are probably misidentifications rather than reflecting a disjunct distribution or recent invasion.

KEY WORDS
Brown algae,
Cystoseira rayssiae,
COI,
rocky-shore assemblages,
Israel,
Mediterranean Sea,
new combination.

RÉSUMÉ
Affinités génétiques et biogéographie du peigne d'algues marines Levantine-endémique Treptacantha rayssiae *(Ramon) M.Mulas, J.Neiva et Á.Israel, comb. nov. (Phaeophyceae).*
Les forêts de *Cystoseira sensu lato* (Ochrophyta) sont d'importantes formes d'habitat dans la mer Méditerranée, mais elles ont surtout été étudiées dans le bassin occidental où de nombreuses espèces sont en déclin. Dans le bassin oriental, où moins d'espèces sont présentes, *Cystoseira rayssiae* Ramon a été décrite en l'an 2000 comme une espèce endémique sur la base des caractéristiques morphologiques d'échantillons d'herbiers prélevés sur la côte israélienne. Aucune autre enquête n'a été menée sur cette espèce particulière depuis, mais récemment, elle a été enregistrée dans le Liban contigu et en dehors de la Méditerranée. Notre travail visait à confirmer la validité taxonomique et la nature endémique de cette espèce, y compris sa position parmi les genres *Cystoseira sensu stricto, Carpodesmia* Greville et *Treptacantha* Kützing récemment divisés, en séquençant le gène mitochondrial COI et en examinant les caractéristiques morphologiques dans des échantillons provenant de trois sites différents dans le nord d'Israël. Malgré une plasticité morphologique considérable, les analyses moléculaires ont révélé une seule et unique séquence COI. Les analyses phylogénétiques montrent que *Cystoseira rayssiae* appartient au genre ressuscité *Treptacantha* et, par conséquent, la nouvelle combinaison *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., est proposée. endémie levantine. D'intrigants rapports extra-méditerranéens provenant de la mer Rouge et du golfe Persique sont probablement des erreurs de reconnaissance plutôt que le reflet d'une distribution disjointe ou d'une invasion récente.

MOTS CLÉS
Algues brunes,
Cystoseira rayssiae,
COI,
assemblages de rivages rocheux,
Israël,
mer Méditerranée,
combinaison nouvelle.

INTRODUCTION

In the Mediterranean Sea, macroalgal forests composed of species of the genus *Cystoseira sensu lato* dominate many benthic reef communities from the low intertidal down to a few tens of meters (Cormaci *et al.* 2012; Taşkin *et al.* 2012). They play crucial roles in ecosystem functioning and provide both food and shelter for many reef species (Bulleri *et al.* 2002; Chemineé *et al.* 2013). Due to direct and indirect human pressure, many *Cystoseira sensu lato* forests have gradually declined over the last decades along Atlantic and Mediterranean coasts (Vergés *et al.* 2014b; Mineur *et al.* 2015; Thibaut *et al.* 2015; Valdazo *et al.* 2017). Because of their vulnerability and ongoing degradation, *Cystoseira* species (with the exception of *C. compressa* (Esper) Gerloff & Nizamuddin) are protected by the Barcelona Convention (UNEP/MAP-RAC/SPA 2012).

In the eastern Mediterranean, the knowledge about the complex of species included in the genus *Cystoseira s.l.* is still very limited and more studies are required to clarify species pools, distributions and threats. Along the Israeli Mediterranean coast, for example, the status of this group was unclear until recently (Israel & Einav 2017). Surveys in northern Israel indicate that canopy-forming brown seaweeds are today in general a rare sight on shallow reefs, likely due to overgrazing by the two invasive rabbitfish *Siganus luridus* (Rüppell) and *S. rivulatus* Forsskål & Niebuhr (Lundberg *et al.* 1999; Bariche 2006; Rilov 2016; Rilov *et al.* 2018), and to the rising water temperature that shortens their growth season by one-two months (Rilov *et al.* 2020). A similar conclusion was inferred from field fish exclusion experiments conducted on *Cystoseira* spp. in Turkey (Sala *et al.* 2011; Vergés *et al.* 2014a, b).

Three *Cystoseira sensu lato* species have been so far reported to occur along the Israeli Mediterranean Sea coast: *C. compressa*

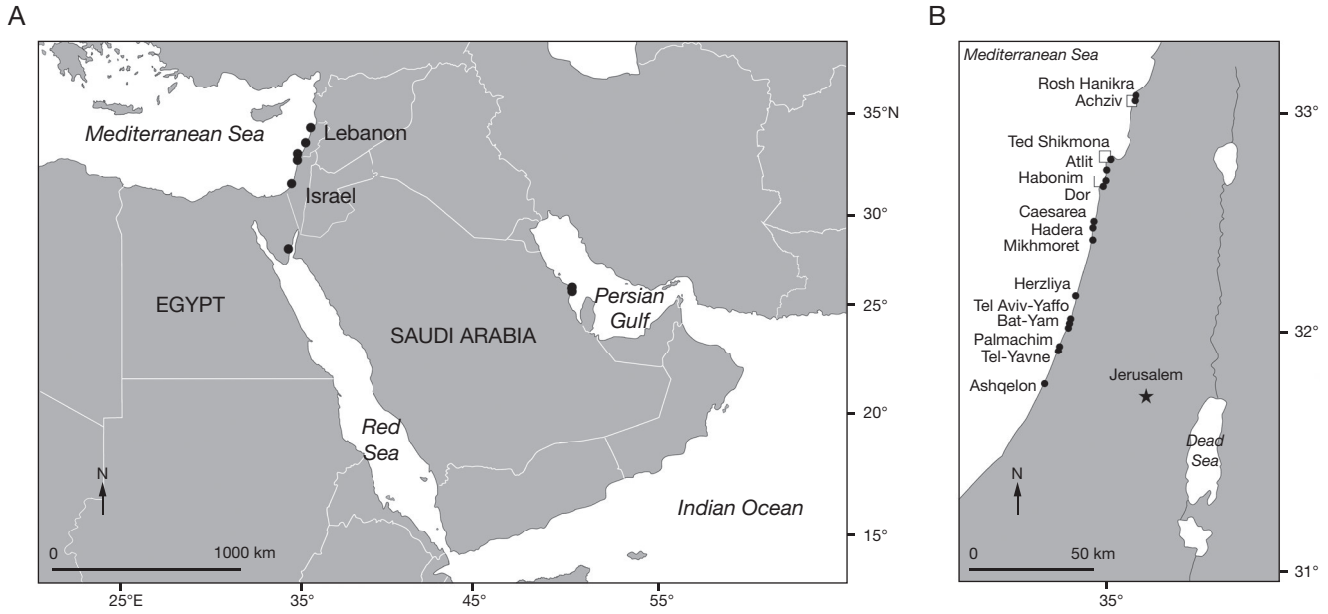


FIG. 1. —Reported distribution of *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov. (synonym of *Cystoseira rayssiae*): **A**, occurrence records based on literature records; **B**, distribution (**black dots**) in Israeli Mediterranean Sea shores as reported in Ramon (2000) based on herbaria collections. The **squares** indicate the origin of samples analyzed in this study: Achziv (Gesher Haziv), Tel Shikmona and HaBonim.

(Esper) Gerloff & Nizamuddin, *C. schiffneri* Hamel [regarded by some authors as a form of *C. foeniculacea* i.e., *C. foeniculacea* (Linnaeus) Greville f. *schiffneri* (Hamel) Gómez Garreta *et al.*] and *C. rayssiae* Ramon (Einav & Israel 2008). The first two are widely distributed in the Mediterranean Sea (Cormaci *et al.* 2012; Taskin *et al.* 2012; Bouafif *et al.* 2016). By comparison, *C. rayssiae* was described only in the year 2000 as an endemic species based on morphological characteristics from herbaria samples collected throughout the entire Israeli coast (Rosh HaNikra in the north to Ashqelon in the south) between 1906-1989 (Ramon 2000). This species was later reported also to the north of Israel, on the Lebanese coast (Badreddine *et al.* 2018), and also from the Red Sea and the Persian Gulf (Abdel-Kareem 2009; Abdel-Raouf *et al.* 2015), questioning its putative Levantine-endemic status.

Draisma *et al.* (2010) showed that *Cystoseira s.l.* was polyphyletic and composed by at least six distinct lineages, including then-resurrected *Stephanocystis* Trevisan, *Polycladia* Montagne and *Sirophysalis* Kützing. The three remaining lineages, with overlapping distributions in the North-Eastern Atlantic/Mediterranean, were maintained within *Cystoseira* until very recently, when Orellana *et al.* (2019) formalized their split. Based on morphological and DNA evidence and nomenclatural precedence, they proposed maintaining *Cystoseira sensu stricto* for the species *C. compressa* (Esper) Gerloff & Nizamuddin, *C. foeniculacea* (Linnaeus) Greville (type species) and *C. humilis* Schousboe ex Kützing; transferring *C. abies-marina* (S.G.Gmelin) C.Agardh, *C. algeriensis* Feldmann, *C. baccata* (S.G.Gmelin) P.C.Silva, *C. elegans* Sauvageau, *C. mauritanica* Sauvageau, *C. spinosa* Sauvageau, *C. nodicaulis* (Withering) M.Roberts and *C. usneoides* (Linnaeus) M.Roberts to the resurrected genus *Treptacantha* Kützing [type species *Treptacantha abies-marina* (S.G.Gmelin) Kützing] and *C. amentacea* (C.Agardh) Bory, *C. brachycarpa* J.Agardh, *C. crinita* Duby, *C. mediterranea* Sauvageau, *C. tamariscifolia* (Hudson) Papenfuss and *C. zosteroides* (Turner) C.Agardh to the resurrected genus *Carpodesmia* Greville [type species *Carpodesmia zosteroides* (Turner) Greville]. The assignment of many other *Cystoseira* species to these three genera however remains unassessed.

In this study, we investigate the genetic validity and evolutionary affinities of *Cystoseira rayssiae* from Israel, including its position within *Cystoseira sensu stricto*/*Treptacantha*/*Carpodesmia* genera. We also provide an updated morphological description, including a first assessment of its morphological plasticity, and make a critical appraisal of its distribution and potential origins based on published records and biogeography of the genus.

MATERIAL AND METHODS

SAMPLES COLLECTION AND GENETIC IDENTIFICATION

Morphologically variable samples of *Cystoseira rayssiae* were collected in 2018 and 2019 along the Northern Israeli coast. Samples for genetic analyses were collected from three representative sites – Achziv (33°3’22.23”N, 35°6’6.82”E), Tel Shikmona, Haifa (32°49’32.97”N, 34°57’19.08”E) and Dor HaBonim (32°37’48.24”N, 34°55’11.23”E), at 0-1.5 m depth (Fig. 1B). Specimens from all three sites were examined in the original species description (Appendix in Ramon 2000). Tel Shikmona is the only known dense shallow forest along the Israeli coast, but the species was observed and collected at tidal pools in Achziv and in both tidal pools and as scattered subtidal specimens in HaBonim. Samples for molecular analysis were stored in silica-gel and voucher

specimens were deposited in the Israel Oceanographic and Limnological Research Institute (Haifa, Israel) – Seaweed Herbarium "IOLR" (*Index Herbariorum*) (Appendix 2). DNA was extracted from four individuals. The two specimens collected from Tel Shikmona were extracted at CCMAR (Portugal) using the commercial Nucleospin 96 Plant II kit (Macherey-Nagel, Düren, Germany) and the other two, respectively from Achziv and HaBonim, at IOLR (Israel) according to Douek *et al.* (2002). A fragment of the mtDNA gene COI (cytochrome *c* oxidase) was amplified using the primer pairs GazF2/GazR2 (5'-CCAACCAYAAAGATATWGGTAC-3'/5'-GGATGACCAAARAACCAAAA-3') as in Silberfeld *et al.* (2010), following the PCR temperature profile: 95°C, 5 min; (95°C, 30 s; 45°C, 45 s; 72°C, 1 min) × 35 cycles; 72°C, 10 min. Fragments were sequenced in an ABI PRISM 3130xl automated capillary sequencer (Applied Biosystems) at CCMAR (Portugal), and commercially (Macrogen Inc., South Korea). The newly generated sequences were deposited in GenBank (Benson *et al.* 2017).

Sequences were aligned in Geneious v.11.03 with published (retrieved from GenBank; https://www.ncbi.nlm.nih.gov/genbank/) and new sequences (amplification as above) of other *Cystoseira sensu lato* (including *Cystoseira s.s.*, *Treptacantha* and *Carpodesmia*) and the related genera *Bifurcaria* Stackhouse, *Stephanocystis* Trevisan and *Sargassum* C.Agardh (Table 1; Appendix 1). Two data-sets were analyzed separately for model selection, implementation and analyses (Bayesian, ML) – a smaller dataset restricted to *Treptacantha* species and another one including multiple Sargassaceae genera.

Nucleotide substitution models (three substitution schemes, unique sequences only) were assessed with jModelTest v.2.6.5 (Darriba *et al.* 2012) using ML optimized tree for likelihood calculations and Best tree search, and best-fit models were selected as the best-ranked model selected by both the Akaike and Bayesian information criterion. Bayesian phylogenetic analyses were run with MrBayes v.3.2.6 (Ronquist *et al.* 2012). Two parallel MCMC Monte Carlo searches were run for 10^6 generations, sampling trees and parameters every 100 generations. For each data set, the number of substitution rates, among-site rate variation and base frequency priors were set according to the substitution model selected, leaving the remaining options as default. Run length sufficiency was confirmed by inspecting the average standard deviation of split frequencies between runs (ASDSF <0.02). 10^5 generations (1000 trees in each run) were discarded as burn-in, and the remaining 18000 sampled trees were used to produce consensus trees and to calculate branch posterior probabilities. Maximum likelihood analyses were performed with PhyML v.3.0 using the same substitution models in the ATGC bioinformatics platform (http://www.atgc-montpellier.fr/phyml/). Nodal support was calculated using 1000 bootstrap replicates. Trees were rooted using the *Stephanocystis/C. compressa/Sargassum* branch as outgroup (Draisma *et al.* 2010). The same analyses were run for a smaller subset of species closely related to *Cystoseira rayssiae* (genus *Treptacantha*, see below). Genealogic relationships were further illustrated with a network calculated with PopART v.1.7 (http://popart.otago.ac.nz) using the Median-Joining (MJ) algorithm (Bandelt *et al.* 1999). The original alignments and the phylogenetic tree are deposited in the Dryad repository and public available (https://doi.org/10.5061/dryad.sj3tx9617).

Thalli morphology

Morphological descriptions were conducted on the vouchers deposited in the "IOLR" Seaweed Herbarium, in the "HUJ" National Herbarium of the Hebrew University, and on additional 20 samples collected from Tel Shikmona, Achziv and HaBonim. The size, shape and diameter of the holdfast and main axis were measured, and branch characteristics were annotated. Photo-images were captured using a DS-Fi3 camera installed on a SMZ745T Nikon stereomicroscope and digital camera Olympus Tough TG-620. Fragments of primary branches were selected from fresh and frozen thalli (respectively, *Cystoseira rayssiae* (Ramon) from Tel Shikmona and from Achziv) and crossed sections were prepared using a CM 1850 Leica cryostat. Microscopic characteristics such as medullary, cortical and meristoderm cells were observed and photographed with a SZX16 Olympus stereomicroscope and/or under a 20 µm magnification under an Olympus BX50 microscope, both connected to a SC-180 Olympus camera.

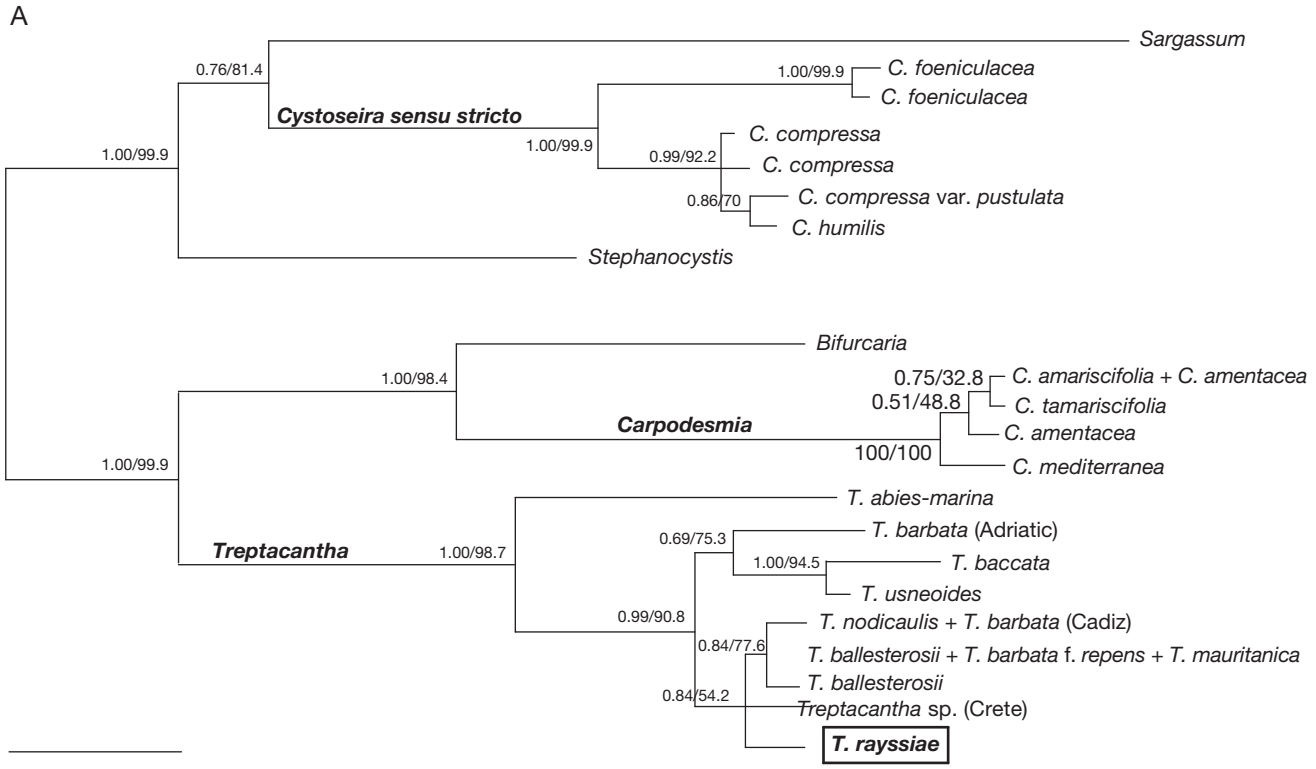
Species distribution

Google Scholar, Scopus and GBIF were used as queries to collate all available reported occurrence records of *C. rayssiae*. These data, our field observations and monitoring along the Israel coast were used to produce a map of its apparent global distribution (Fig. 1A).

RESULTS AND DISCUSSION

Genetic affinities and general morphology

The final COI alignment was 627 base-pair long, with no indels, and included 65 sequences corresponding to 18 species-level taxa (Fig. 2, see Table 1 and Appendix 1 for new and previously published GenBank accession records). The sequence of all samples of *C. rayssiae* were identical (GenBank accession MK370729), despite clear morphological differences among them. The best substitution model for the final dataset (22 unique sequences) was HKY + I + G. Genetic data confirmed that *C. rayssiae* belongs to the well supported *Treptacantha* genus (*sensu* Orellana *et al.* 2019; synonym of *Cystoseira*-6 *sensu* Draisma *et al.* 2010; synonym of *Cystoseira*-II Bruno de Sousa *et al.* 2019), together with *T. baccata* (S.G.Gmelin) Orellana and Sansón, *T. usneoides* (Linnaeus) Orellana and Sansón, *T. barbata* (Stackhouse) Orellana and Sansón, *T. nodicaulis* (Withering) Orellana and Sansón, *T. ballesterosii* Orellana and Sansón, *T. mauritanica* (Sauvageau) Orellana and Sansón, and another tophulose (i.e., with characteristic reserve structures called tophules) *Treptacantha* sp. from the eastern Mediterranean (Crete) (Fig. 2). When the sequences were analyzed with *Treptacantha* spp. (HKY + I, seven unique sequences), the closer relationship of *C. rayssiae* with the latter four species became more apparent (Appendix 1).



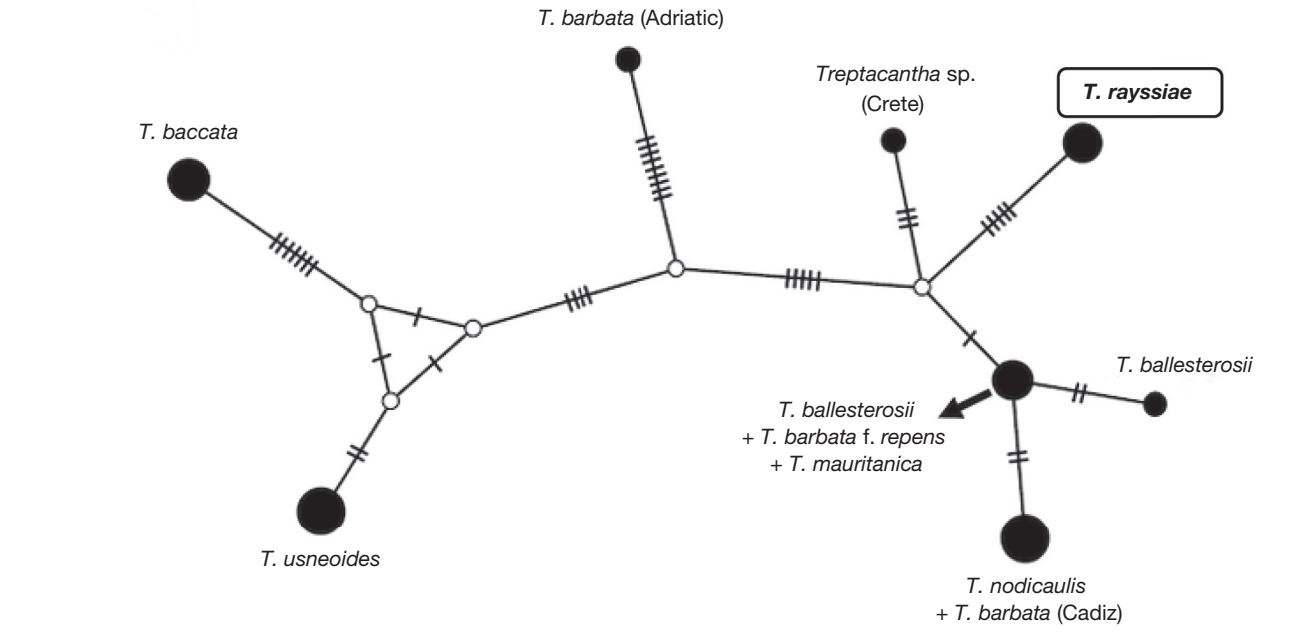


Fig. 2. — Phylogenetic affinities of *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov. (synonym of *C. rayssiae*): **A**, bayesian 50% majority-rule consensus COI tree of selected species of *Cystoseira sensu lato* (including *Cystoseira sensu stricto*, *Carpodesmia* Greville and *Treptacantha* Kützing) and related genera, showing the phylogenetic position of *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov. Numbers above the branches are Bayesian posterior probabilities (>0.50) and maximum likelihood bootstrap support values, respectively. The outgroup used was represented by *Stephanocystis* Trevisan, *C. compressa* (Esper) Gerloff & Nizamuddin and *Sargassum* C.Agardh; **B**, neighbour-joining network of COI sequences of *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., and related taxa. Terminal **black circles** correspond to unique sequences/taxa and are sized to their frequency. **Small white circles** represent internal nodes and perpendicular dashes along branches represent unique base-pair mutations between sequences/taxa. Branch lengths not to scale. Scale bar: A, 0.02 BIPP (Bayesian Inference Posterior Probability).

TABLE 1. — Geographic origin (decimal degrees), collection details, accession number and sequence quality of samples processed in this study.

Species	Collection site	Latitude /Longitude	Collector	Date	GenBank code	Herbarium accession	# sequences	% bases high quality
Sargassum sp.	Tel Shikmona, Haifa, Israel	32°49'32.97"N /34°57'19.08"E	M. Mulas	V.2018	MK370733	IOLR-MM00630	1	97.80%
Cystoseira compressa	Tel Shikmona, Haifa, Israel	32°49'32.97"N /34°57'19.08"E	M.Mulas	IV.2018	MK388673	IOLR-MM00640	1	97.80%
C. foeniculacea	Mades, Crete, Greece	35°23'57.42"N /14°2'1.89"E	J. Neiva	V.2018	MK370732	João Neiva, Personal Herbarium Faro, Algarve, Portugal	1	94.50%
Treptacantha rayssiae, comb. nov.	Tel Shikmona, Haifa, Israel	32°49'32.97"N /34°57'19.08"E	M. Mulas	IV.2018	MK370729	IOLR-MM00641 MM00642	2	95.9%, 97.5%
T. rayssiae, comb. nov.	Achziv, Israel	33°3'22.23"N /35°6'6.82"E	S. Sadogurska	V.2019	MK370729	IOLR - AmR1652019	1	88%
T. rayssiae, comb. nov.	HaBonim, Israel	32°37'48.24"N /34°55'11.23"E	S. Sadogurska	IV.2019	MK370729	IOLR - BS5942019	1	73.70%
T. ballesterosii	Cabrera, Baleares, Spain	39°12'23.82"N /2°58'45.47"E	E. Ballesteros	XI.2017	MK370731	–	1	98%
Treptacantha sp.	Mades, Crete, Greece	35°23'57.42"N /14°2'1.89"E	J. Neiva	V.2018	MK370730	João Neiva, Personal Herbarium Faro, Algarve, Portugal	1	97.30%

COI sequences of *C. rayssiae* were, however, unique and divergent (six-eight substitutions) from these other related species (Fig. 2A, B), much more than other *Treptacantha* (e.g. *T. nodicaulis/T. ballesterosii*, two substitutions, see Fig. 2B) and *Sargassum* (e.g. Amaral-Zettler *et al.* 2017) species complexes. These results indicate that *C. rayssiae* is unique and belongs unequivocally to the resurrected genus *Treptacantha*, and thus we propose the new combination:

Treptacantha rayssiae (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov.

Cystoseira rayssiae Ramon, *Israel Journal of Plant Sciences* 48: 59 (English), 61 (Latin); figs 1-5 (fig. 1: holotype) (1970) (basionym).

TYPE MATERIAL. — **Holotype. HUJ** (Ashqelon, Israel; 23.V. 1953).

DESCRIPTION

The morphological characteristics of this species along the Israeli coast are well in accordance with the features of the genus as described by Orellana *et al.* (2019), although Ramon herself noted that the species exhibits considerable morphological plasticity (Figs 3; 4). *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., shows a tophulose, non-caespitose habit, growing up to *c.* 30 cm high (Figs 3A; 4A). The thallus is attached to the substrate by a basal disc from which a cylindrical simple or branched axis grows (Figs 3D; 4C). Tophules are present (albeit in young thalli they are poorly distinguishable) and can be of different shapes (from obovate, ovate, to club-shaped, spherical and oblong), are 3-8 mm long and up to 4 mm broad (Figs 3B; 4B), and often concentrated in the tip of the main axes. Holdfast, main axes, and tophules are perennial. Primary branches are seasonal (March-June) (Mulas *et al.* 2019), typically smooth in the basal region and occasionally with small and widely spaced spiny appendages. Branches of higher order are more robust, show lateral spines, and their inner aerocysts are inconspicuous. Transformed ends of last order branches show spiny laterals containing receptacles (Fig. 3C). Differences in the morphology among specimens seem to be environmentally driven. For instance, subtidal specimens display shorter and robust branches densely covered with spine-like appendages and without pronounced receptacles compared to tide pool specimens that have less pronounced spines and correspond to Ramon's description (Fig. 4).

Anatomical characteristics of *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., cross sections corresponded to the genus *Treptacantha* as recently described by Orellana *et al.* (2019). *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., shows medullary cells which form a central mass, while the cortical ones having a bigger size, globose shape and thickened walls and the meristoderm is composed by a single layer of square-shaped cells (Fig. 5).

This is in accordance with the description of the genus (Orellana *et al.* 2019): *Treptacantha* often displays significant polymorphism attributable to regional, seasonal and habitat differences (e.g. genetically – confirmed Atlantic *Treptacantha nodicaulis, Treptacantha* sp. from Crete, *T. baccata*, *T. barbata*, *T. abies-marina*, *T. ballesterosii* and *T. mauritanica*) stressing that morphological differences must be supported by additional evidence, such as molecular data, before describing additional species.

Species distribution

Literature and database records suggest a disjunct geographical distribution for *T. rayssiae* (Ramon) M.Mulas, J.Neiva &

Fig. 3. — Typical morphology of *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., at Achziv, Israel (May 2019, voucher IOLR - AmR1652019): **A**, habitus of specimens; **B**, apex with smooth tophules; **C**, detail of apical part of slightly spiny branches with receptacles; **D**, holdfast. Scale bars: A, C, 2 cm; B, D, 1 cm.

Á.Israel, comb. nov., as shown in Figure 1A. *Treptacantha rayssiae* can be found in tide pools associated with vermetid reefs (abrasion platforms) in north Israel (Rilov *et al.* 2020), and as scattered individuals (in several locations), or as an extensive forest (only in one location, in Haifa) on horizontal subtidal bedrocks down to 5 m depth (Fig. 1B, based on Ramon 2000, Mulas *et al.* 2019, Peleg *et al.* 2019 and personal observations). In addition to Israel (Ramon 2000; Einav & Israel 2008), *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., has been also recently reported from several sites in Lebanon (Nakoura, Adloun, Barbara, Ras-Chekaa) (Badreddine *et al.* 2018). Surprisingly, extra-Mediterranean records were also reported from one site (Dahab) in the Red Sea along the Egyptian coast of the Gulf of Aqaba (Abdel-Raouf *et al.* 2015) and from six sites in the Persian Gulf (Ras Tanura, Saftwah, Al Qatif, Sayhat, Ad Dammam and Al Azizayah) in Saudi Arabia (Abdel-Kareem 2009).

These extra-Mediterranean records have not been genetically confirmed. In case they are not misidentifications, three exclusive scenarios can be postulated. In the first scenario, *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., may be a palaeoendemic species that was formerly widespread (before the closure of the Mediterranean passages to the Indian Ocean, some 20 MYA), and is now restricted to several very small "relict" areas of its past distribution. In the second scenario, *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., may be a Lessepsian migrant first detected in the invaded region, the Levantine basin, and later in the origin region, the Red Sea and the Persian Gulf. These two scenarios seem unlikely because *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., and *T. nodicaulis* are more closely related than *T. nodicaulis* and *T. baccata*, and the two latter are estimated to have diverged around 10 MYA (Silberfeld *et al.* 2010), i.e., already after the closure of the

Fig. 4. — Morphology of *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., at Tel Shikmona, Israel (May 2018, voucher IOLR-MM00641): **A**, habitus of specimen; **B**, holdfast; **C**, detail of apical part of branches with receptacles, packed, cylindrical and spiny. Scale bars: A, 4 cm; B, C, 2 cm.

eastern Tethyan seaway (Bialik *et al.* 2019). Under the third scenario, *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., is an eastern Mediterranean endemic seaweed that has migrated to the Indo-Pacific, a rare case of anti-Lessepsian migration. It should be noted that there are only a handful examples of anti-Lessepsian species, making this last scenario also unlikely (Golani *et al.* 2002). Among the very few marine organisms that have moved from the Mediterranean into the Red Sea, are the fish *Solea aegyptiaca* Chabanaud, and six species of polychaetes (Golani *et al.* 2002; Faiza 2009; Chanet *et al.* 2012), but no macroalgal species recorded so far. In contrast, a large number of Lessepsian macroalgae migrants have been recorded in the Mediterranean Sea along the years (Por 1971, 1978; Galil & Zenetos 2002; Rilov & Galil 2009; Otero *et al.* 2013; Boudouresque *et al.* 2016; Galil *et al.* 2017; Israel & Einav 2017), where the last update has counted 119 alien macrophytes introduced by different sources out of a total of 613 confirmed marine organisms (Verlaque *et al.* 2015; Zenetos *et al.* 2017). All possible hypothetical scenarios to explain the extra-Mediterranean records are not supported by evidence. The main growing/reproductive season of *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., is winter-

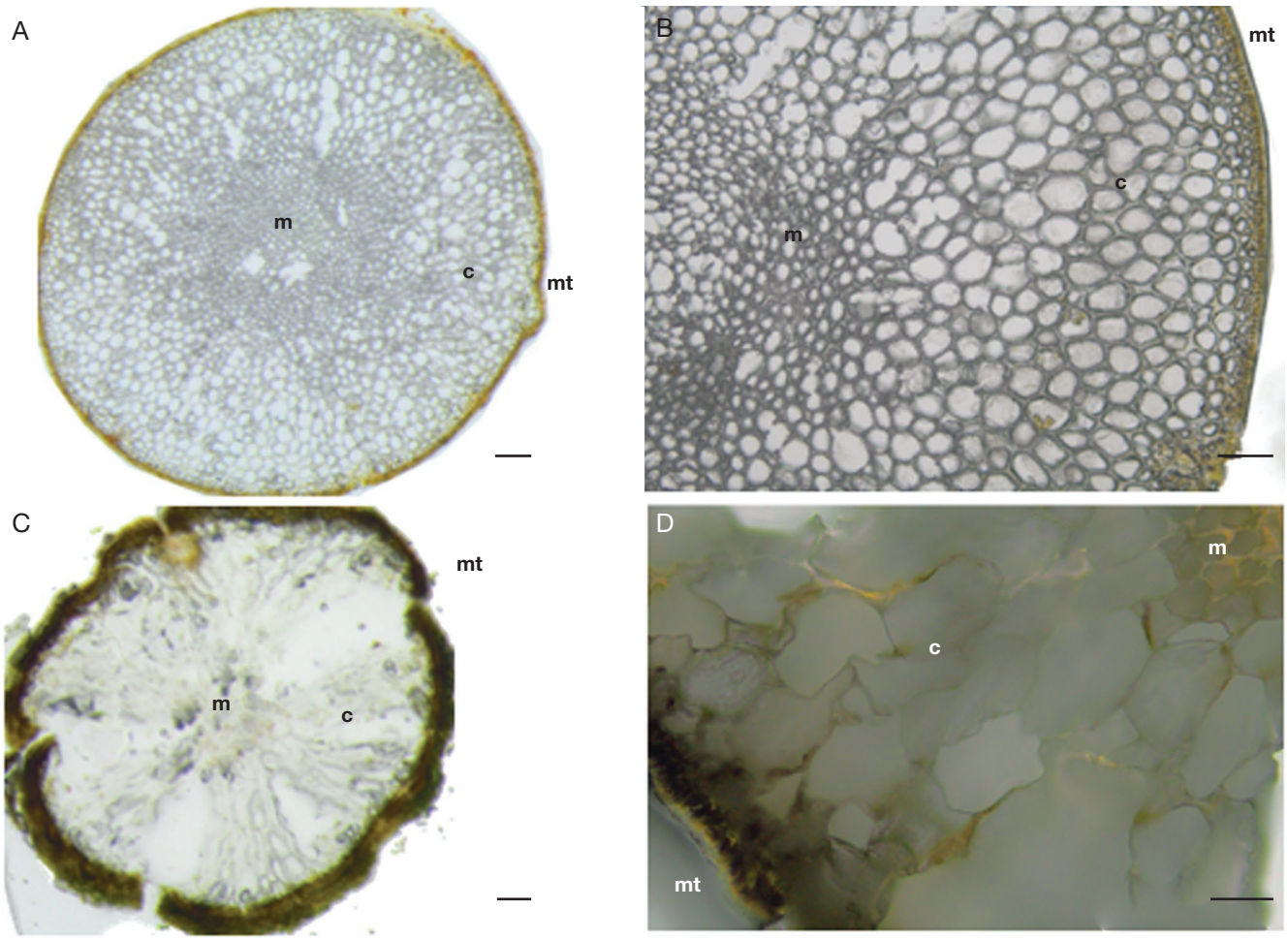


FIG. 5. — Cross sections of the thallus of *Treptacantha rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov.: **A**, primary branch; **B**, detail of the three vegetative tissues at Tel Shikmona; **C**, primary branch; **D**, detail of the three vegetative tissues from Achziv. Scale bars: A, 50 µm; B, 200 µm; C, 100 µm; D, 20 µm. Abbreviations: **mt**, meristoderm; **c**, cortex; **m**, medulla.

spring and not warmer summer months when the fronds are shed, and the basal perennial parts enter a dormancy period (Mulas *et al.* 2019). A temperate growth and reproductive window do not support a tropical origin, but seasonal shifts are also observed among tropical species. Because all three scenarios are highly unlikely, we suspect that the records from the Persian Gulf and the Red Sea (Abdel-Kareem 2009; Abdel-Raouf *et al.* 2015) are based on misidentifications. In the study of Abdel-Kareem (2009), the photographic record has poor quality but does not resemble *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov. In fact, in both studies of Abdel-Kareem (2009) and Abdel-Raouf *et al.* (2015), the taxonomical identification of *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov. (as *Cystoseira rayssiae*) was based on the reference check-list of the Red Sea of Lipkin & Silva (2002) which, however, never mentions *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., but rather the common fucoid *Polycladia myrica* (as *Cystoseira myrica*). This species and *Syrophysalis trinodis* (as *Cystoseira trinodis*) have also been reported from the Red Sea in other studies (e.g. Ibraheem *et al.* 2014). Many seaweed groups are notoriously difficult to identify, and this applies also to *Cystoseira sensu lato* and related genera. Thus, we conclude that the most plausible explanation is that *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov., was misidentified in these studies, and the species is a unique example of a Levantine Basin endemism. If what we claim is confirmed by further analyses of Mediterranean samples, conclusively excluding it from nearby areas (Cyprus, southern Turkey and farther away), the protection of this species emerges as a priority in the Mediterranean Sea, because of the restricted local distribution and increasing pressures such as rabbitfish and sea urchin overgrazing, pollution, ocean warming and urbanization.

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REFERENCES

Abdel-Kareem M. S. M. 2009. — New algal records from the Arabian Gulf coast of Saudi Arabia. *Botany Research International* 2 (4): 268-276.

Abdel-Raouf N., Al-Enazi N. M., Al-Homaidan A. A., Ibrahee, I. B. M., Al-Othman M. R. & Hatamieh A. A. 2015. — Antibacterial β-amyrin isolated from *Laurencia microcladia*. *Arabian Journal of Chemistry* 8 (1): 32-37. https://doi.org/10.1016/j.arabjc.2013.09.033

Amaral-Zettler L. A., Dragone N. B., Schell J., Slikas B., Murphyl.G., Morral C. E. & Zettler E. R. 2017. — Comparative mitochondrial and chloroplast genomics of a genetically distinct form of *Sargassum* contributing to recent "Golden Tides" in the Western Atlantic. *Ecology and Evolution* 7 (2): 516-525. https://doi.org/10.1002/ece3.2630

Badreddine A., Abboud-Abi Saab M., Gianni F., Ballesteros E. & Mangialajo L. 2018. — First assessment of the ecological status in the Levant Basin: Application of the CARLIT index along the Lebanese coastline. *Ecological Indicators* 85: 37-47. https://doi.org/10.1016/j.ecolind.2017.10.006

Bandelt H. J., Forster P. & Röhl A. 1999. — Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16 (1): 37-48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Bariche M. 2006. — Diet of the Lessepsian fishes, *Siganus rivulatus* and *S. luridus* (Siganidae) in the eastern Mediterranean: a bibliographic analysis. *Cybium* 30 (1): 41-49.

Benson D. A., Cavanaugh M., Clark K., Karsch-Mizrachi I., Lipman D. J., Ostell J. & Sayers E. W. 2017. — GenBank. *Nucleic Acids Research* 45: D37-D42. https://doi.org/10.1093/nar/gkw1070

Bialik O. M., Frank M., Betzler C., Zammit R. & Waldmann N. D. 2019. — Two-step closure of the Miocene Indian Ocean Gateway to the Mediterranean. *Scientific Reports-UK* 9 (1): 1-10. https://doi.org/10.1038/s41598-019-45308-7

Bouafif C., Verlaque M. & Langar H. 2016. — New contribution to the knowledge of the genus *Cystoseira* C. Agardh in the Mediterranean Sea, with the reinstatement of species rank for *C. schiffneri* Hamel. *Cryptogamie Algologie* 37 (2): 133-154. https://doi.org/10.7872/crya/v37.iss2.2016.133

Boudouresque C. F., Perret-Boudouresque M. & Verlaque M. 2016. — Donor and recipient regions for exotic species of marine macrophytes: a case of unidirectional flow, the Mediterranean Sea, *in 41th CIESM Congress Proceeding: Rapport de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée*. CIESM, Monaco: 426.

Bruno de Sousa C., Cox C. J., Brito L., Pavão M. M., Pereira H., Ferreira A., Ginja C., Campino L., Bermejo R., Parente M. & Varela J. 2019. — Improved phylogeny of brown algae *Cystoseira* (Fucales) from the Atlantic-Mediterranean region based on mitochondrial sequences. *PLoS ONE* 14 (1): e0210143. https://doi.org/10.1371/journal.pone.0210143

Bulleri F., Benedetti-Cecchi L., Acunto S., Cinelli F. & Hawkins S. J. 2002. — The influence of canopy algae on vertical patterns of distribution of low-shore assemblages on rocky coasts in the northwest Mediterranean. *Journal of Experimental Marine Biology and Ecology* 267: 89-106. https://doi.org/10.1016/S0022-0981(01)00361-6

Chanet B., Desoutter-Meniger M. & Bogorodsky S. V. 2012. — Range extension of Egyptian sole *Solea aegyptiaca* (Soleidae: Pleuronectiformes), in the Red Sea. *Cybium* 36 (4): 581-584. https://doi.org/10.26028/cybium/2012-364-012

Chemineé A., Sala E., Pastor J., Bodilis P., Thiriet P., Mangialajo L., Cottalorda J.-M. & Francour P. 2013. — Nursery value of *Cystoseira* forests for Mediterranean rocky reef fishes. *Journal of Experimental Marine Bioogy and Ecology* 442: 70-79. https://doi.org/10.1016/j.jembe.2013.02.003

Cormaci M., Furnari G., Catra M., Alongi G. & Giaccone G. 2012. — Flora marina bentonica del Mediterraneo: Phaeophyceae. *Bollettino dell'Accademia Gioenia Scienze Naturale* 45: 1-508.

Darriba D., Taboada G. L., Doallo R. & Posada D. 2012. — jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Douek J., Barki Y., Gateno D. & Rinkevich B. 2002. —Possible cryptic speciation within the sea anemone *Actinia equina* complex detected by AFLP markers. *Zoological Journal of the Linnean Society-London* 136 (3): 315-320. https://doi.org/10.1046/j.1096-3642.2002.00034.x

Draisma S. G. A., Ballesteros E., Rousseau F. & Thibaut T. 2010. — DNA sequence data demonstrate the polyphyly of the genus *Cystoseira* and other Sargassaceae genera (Phaeophyceae). *Journal of Phycology* 46 (6): 1329-1345. https://doi.org/10.1111/j.1529-8817.2010.00891.x

Einav R. & Israel A. 2008. — Checklist of seaweeds from the Israeli Mediterranean: Taxonomical and ecological approaches. *Israel Journal of Plant Sciences* 56 (1): 127-191.

Faiza A. A.-E. 2009. — New records of polychaetes from the south part of Suez Canal, Egypt. *World Journal of Fish and Marine Sciences* 1 (1): 7-19.

Galil B. S. & Zenetos A. 2002. — A Sea Change - Exotics in the Eastern Mediterranean Sea, *in* Leppakoski E., Gollash S. & Olenin S. (eds), *Invasive Aquatic species of Europe – distribution impacts and management.* Kluwer Academic Publishers, London: 1-19. https://doi.org/10.1007/978-94-015-9956-6_33

Galil B., Marchini A., Occhipinti-Ambrogi A. & Ojaveer H. 2017. — The enlargement of the Suez Canal - Erythraean introductions and management challenges. *Management of Biological Invasions* 8 (2): 141-152. https://doi.org/10.3391/mbi.2017.8.2.02

Golani D., Orsi-Relini L., Massuti E. & Quignard J. P. 2002. — CIESM. *Atlas of Exotic Species in the Mediterranean. Vol. 1. Fishes*. CIESM, Monaco, 256 p.

Ibraheem I. B. M., Alharbi R. M., Abdel-Raouf N. & Al-Enazi N. M. 2014. — Contributions to the study of marine algae inhabiting Umluj Seashore, Red Sea. *Beni-Suef University Journal of Basic and Applied Sciences* 3 (4): 278-285. https://doi.org/10.1016/j.bjbas.2014.11.001

Israel A. & Einav R. 2017. — Alien seaweeds from the Levant basin (Eastern Mediterranean Sea), with emphasis to the Israeli shores. *Israel Journal of Plant Sciences* 64: 99-110.

Lipkin Y. & Silva P. 2002. — Marine algae and seagrasses of the Dahlak Archipelago, southern Red Sea. *Nova Hedwigia* 75 (1-2): 1-90. https://doi.org/10.1127/0029-5035/2002/0075-0001

Lundberg B., Ofgorek R., Galil B. & Goren M. 1999. — Algal food of siganid fishes in Shiqmona, Israel. *Israel Journal of Zoology* 45: 314.

Mcdevit D. C. & Saunders G. W. 2009. — On the utility of DNA barcoding for species differentiation among brown macroalgae (Phaeophyceae) including a novel extraction protocol. *Phycological Resarch* 57 (2): 131-141. https://doi.org/10.1111/j.1440-1835.2009.00530.x

Mineur F., Arenas F., Assis J., Davies A., Engelen A. H., Fernandes F., Malta E. J., Thibaut T., Van Nguyen T., Vaz-Pinto F., Vranken S., Serrão E. A. & de Clerk O. 2015. — European seaweeds under pressure: Consequences for communities and ecosystem functioning. *Journal of Sea Research* 98: 91-108. https://doi.org/10.1016/j.seares.2014.11.004

Mulas M., Silverman J., Israel A., Golomb D. & Rilov G. 2019. — Marine algal forests in the Levantine Basin: the case of *Cystoseira rayssiae* along the Israeli coast, *in Proceedings of the 6th Mediterranean Symposium on Marine Vegetation*. UNEP/MAP-RAC/SPA, Antalya, 155 p.

Orellana S., Hernandez M. & Sansón M. 2019. — Diversity of *Cystoseira sensu lato* (Fucales, Phaeophyceae) in the eastern Atlantic and Mediterranean based on morphological and DNA evidence, including *Carpodesmia* gen. emend. and *Treptacantha* gen. emend. *European Journal of Phycology* 54 (3): 447-465. *https://doi.org/10.1080/09670262.2019.1590862*

Otero M., Cebrian E., Francour P., Galil B. & Savini D. 2013. — Monitoring marine invasive species in Mediterranean Marine Protected Areas (MPAs): A strategy and practical guide for managers. *IUCN*. Malaga, Spain, 136 p.

Peleg O., Guy-Haim T., Yeruham E., Silverman J., Rilov G. 2019. — Tropicalization may invert trophic state and carbon budget of shallow temperate rocky reefs. *Journal of Ecology,* 00: 1-11. https://doi.org/10.1111/1365-2745.13329

Por F. D. 1971. — One hundred years of Suez Canal - A century of Lessepsian migration: Retrospect and viewpoints. *Systematic Biology* 20 (2): 138-159. https://doi.org/10.2307/2412054

Por F. D. 1978. — *Lessepsian Migration. The Influx of Red Sea Biota into the Mediterranean by way of the Suez Canal.* Ecological Studies. Berlin. https://doi.org/10.1007/978-3-642-66728-2

Ramon E. 2000. — *Cystoseira rayssiae* - a new *Cystoseira* (Cystoseiraceae, Fucophyceae) from the shores of Israel, Eastern Mediterranean Sea. *Israel Journal of Plant Sciences* 48 (1): 59-65.

Rilov G. & Galil B. 2009. — Marine bioinvasions in the Mediterranean Sea - History, distribution and ecology, *in* Rilov G. & Crooks J. A. (eds), *Biological Invasions in Marine Ecosystems: Ecological, Management, and Geographic Perspectives*. Springer, Berlin Heidelberg: 549-575. https://doi.org/10.1007/978-3-540-79236-9

Rilov G. 2016. — Multi-species collapses at the warm edge of a warming sea. *Scientific Reports* 6: 36897. https://doi.org/10.1038/srep36897

Rilov G., Peleg O., Yeruham E., Levy G. & Rave O. 2018. — Alien turf: Overfishing, overgrazing and invader domination in south-eastern Levant reef ecosystems. *Aquatic Conservation* 28 (2): 351-369. https://doi.org/10.1002/aqc.2862

Rilov G., Peleg O., Guy-Haim T. & Yeruham H. 2020. — Community dynamics and ecological shifts on Mediterranean vermetid reefs. *Mediterranean Marine Research*, 105045. https://doi.org/10.1016/j.marenvres.2020.105045

Ronquist F., Teslenko M., Van Der Mark P., Ayres D. L., Darling A., Höhna S., Larget B., Liu L., Suchard M. A. & Huelsenbeck J. P. 2012. — MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539-542. https://doi.org/10.1093/sysbio/sys029

Sala E., Kizilkaya Z., Yildirim D. & Ballesteros E. 2011. — Alien marine fishes deplete algal biomass in the Eastern Mediterranean. *PLoS ONE* 6 (2): e17356. https://doi.org/10.1371/journal.pone.0017356

Silberfeld T., Leigh J. W., Verbruggen H., Cruand C., de Reviers B. & Rousseau F. 2010. — A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the 'brown algal crown radiation'. *Molecular Phylogenetics and Evolution* 56 (2): 659-674. https://doi.org/10.1016/j.ympev.2010.04.020

Taşkin E., Jahn R., Öztürk M., Furnari G. & Cormaci M. 2012. — *The Mediterranean Cystoseira (with photographs)*. Celar Bayar University, Manisa: 1-75.

Thibaut T., Blanfuné A., Boudouresque C. F. & Verlaque M. 2015. — Decline and local extinction of Fucales in the French Riviera: The harbinger of future extinctions. *Mediterranean Marine Science* 16 (1): 206-224. https://doi.org/10.12681/mms.1032

UNEP/MAP-RAC/SPA 2012. — *Annex II. Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean. List of Endangered or Threatened Species*.

Valdazo J., Rodrìguez-Rodrìguez M. A., Espino F., Haroun E. & Tuya F. 2017. — Massive decline of *Cystoseira abies-marina* forests in Gran Canaria Island (Canary Islands, eastern Atlantic). *Scientia Marina* 81 (4): 499-507. https://doi.org/10.3989/scimar.04655.23A

Vergés A., Steinberg P. D., Hay M. E., Poore A. G. B., Campbell A. H., Ballesteros E., Heck K. L. J., Booth D. J., Coleman M. A., Feary D. A., Figuera W., Langlois T., Marzinelli E. M., Mizerek T., Mumby P. J., Nakamura Y., Roughan M., Van Sebille E., Sen Gupta A., Smale D. A., Tomas F., Wernberg T. & Wilson S. K. 2014a. — The tropicalization of temperate marine ecosystems: Climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society B: Biological Sciences* 281 (1789): 20140846. https://doi.org/10.1098/rspb.2014.0846

Vergés A., Tomas F., Cebrian E., Ballesteros E., Kizilkaya Z., Dendrinos P., Karamanlidis A. A., Spiegel D. & Sala E. 2014b. — Tropical rabbitfish and the deforestation of a warming temperate sea. *Journal of Ecology* 102 (6): 1518-1527. https://doi.org/10.1111/1365-2745.12324

Verlaque M., Ruitton S., Mineur F. & Boudouresque C.-F. 2015. — *CIESM. Atlas of Exotic Species in the Mediterranean Sea. Vol. 4 Macrophytes.* CIESM, Monaco, 364 p.

Zenetos A., Çinar M.E., Crocetta F., Golani D., Rosso A., Servello G., Shenkar N., Turon X. & Verlaque M. 2017. — Uncertainties and validation of alien species catalogues: The Mediterranean as an example. *Estuarine Coastal and Shelf Science* 191: 171-187. https://doi.org/10.1016/j.ecss.2017.03.031

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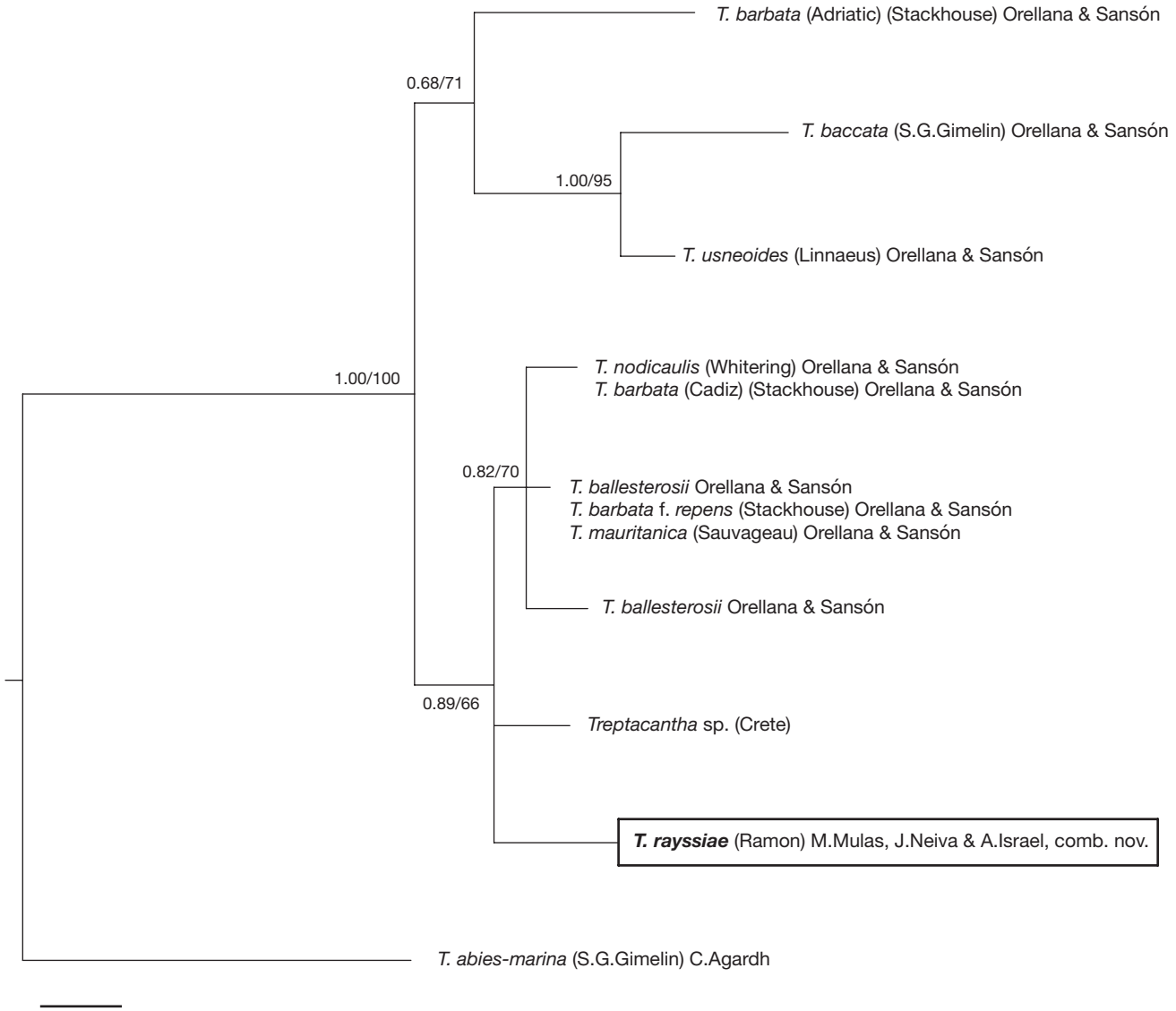
APPENDICES

Appendix 1. — Bayesian 50% majority-rule consensus COI tree of *Treptacantha* Kützing (sensu Orellana *et al*. (2019) synonym of *Cystoseira* C.Agardh clade VI of Draisma *et al*. (2010), unique sequences only), showing the phylogenetic position of *T. rayssiae* (Ramon) M.Mulas, J.Neiva & Á.Israel, comb. nov. Bayesian posterior probabilities (>0.50) and maximum likelihood bootstrap support values, respectively. The outgroup used is *T. abies-marina* (S.G.Gmelin) C.Agardh, the most divergent of the genus (Bruno de Sousa *et al*. 2019). Scale bar: 0.006 BIPP (Bayesian Inference Posterior Probability).



Appendix 2. — Sequences extracted from GenBank (Bruno de Sousa *et al.* 2019; Silberfeld *et al.* 2010†; McDevit & Saunders 2009‡). The dash between the COI GenBank accession numbers indicates the first and the last sequence in the included range.

Species	COI GenBank accession nº	# sequences
Bifurcaria bifurcata	EU681394†	1
Cystoseira geminata (synonym of *Stephanocystis geminata*)	FJ409138‡	1
Cystoseira compressa	MF768036-44	8
Cystoseira humilis	MF768045-47	3
Cystoseira foeniculacea	MF768048-49	2
Carpodesmia amentacea	MF768050-52	3
Carpodesmia tamariscifolia	EU681401†, MF768053-63	12
Carpodesmia mediterranea	MF768064-65	2
Treptacantha abies-marina	MF768066-71	6
Treptacantha ballesterosii	MF768072	1
Treptacantha mauritanica	MF768073	1
Treptacantha barbata f. *repens* (synonym of *C. barbata* f. *aurantia in* Bruno de Sousa *et al.* 2019)	MF768074	1
Treptacantha barbata	KY682970, MF768075	2
Treptacantha nodicaulis	EU681400†, MF768076-77	3
Treptacantha baccata	EU681399†, MF768078-79	3
Treptacantha usneoides	MF768080-83	4
Treptacantha baccata	EU681399†, MF768078-79	3
Treptacantha usneoides	MF768080-83	4