Mediterranean Marine Science

Vol 16, No 2 (2015)

First record of Dichotomaria obtusata (Ellis & Solander) Lamarck (Nemaliales, Rhodophyta) in the Mediterranean Sea

R. HOFFMAN, S.-L. LIU, Y. LIPKIN, M. STERNBERG

doi: 10.12681/mms.1107

To cite this article:

HOFFMAN, R., LIU, S.-L., LIPKIN, Y., & STERNBERG, M. (2015). First record of Dichotomaria obtusata (Ellis & Solander) Lamarck (Nemaliales, Rhodophyta) in the Mediterranean Sea. *Mediterranean Marine Science*, *16*(2), 325–330. https://doi.org/10.12681/mms.1107

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.1107

First record of the calcified genus *Dichotomaria* Lamarck (Nemaliales, Rhodophyta) in the Mediterranean Sea

R. HOFFMAN[1], S.-L. LIU[2], Y. LIPKIN[1] and M. STERNBERG[1]

[1] Department of Molecular Biology and Ecology of Plants, Tel-Aviv University, Tel-Aviv 69978, Israel
[2] Department of Life Science, Tunghai University, Taichung 40704, Taiwan

Corresponding author: razyho@hotmail.com

Handling Editor: Athanasios Athanasiadis

Received: 22 October 2014; Accepted: 11 March 2015; Published on line: 23 April 2015

Abstract

Global climate change is causing the Mediterranean coastal area of Israel to gradually acquire tropical characteristics. Rising sea surface temperatures in the eastern Mediterranean basin have facilitated the introduction, settlement and establishment of hundreds of alien species (Zenetos et al. 2012). The vast majority of these exotic species are of Indo-Pacific origin. We report the occurrence of the genus *Dichotomaria* in the eastern Mediterranean on the basis of specimens identified as *Dichotomaria* cf. *obtusata* (J. Ellis & Solander) Lamarck. Tetrasporophytes with sporangial initials were identified morphologically and confirmed molecularly using plastid *rbc*L sequences. We also discuss possible paths of introduction of this and other alien species into the Levantine Sea.

Keywords: Alien species, invasive species, Levantine Sea, Mediterranean Sea, *Dichotomaria* cf. *obtusata*, *rbc*L, Israel.

Introduction

Over twenty alien species of seaweeds have been reported thus far from the Israeli Mediterranean (IM) coast. Amongst those recorded during the first decade of the twenty-first century, *Codium arabicum* Kützing, *Codium parvulum* (Bory ex Audouin) P.C. Silva, *Galaxaura rugosa* (J. Ellis et Solander) J.V. Lamouroux and *Stypopodium schimperi* (Kützing) M. Verlaque et Boudouresque showed a prominent occurrence along the Mediterranean shores of Israel. Their presence was obvious from enormous drift events that cast ashore thousands of tons of algal biomass indicating large subtidal blooms of these invasive seaweeds (Hoffman *et al.*, 2011; Hoffman, 2013). The increasing presence of invasive non-native species threatens the local marine biota and has the potential for disrupting ecosystem function (Hoffman, 2013).

Galaxauraceae is a red algal family of calcified seaweeds that has a primarily tropical and subtropical distribution, with few members in temperate seas. The family comprises four genera: *Actinotrichia* Decaisne, *Dichotomaria* Lamarck, *Galaxaura* J.V. Lamouroux and *Tricleocarpa* J.M. Huisman & M.A. Borowitzka, with over 50 species currently accepted (Guiry & Guiry, 2014). *Dichotomaria* was reestablished as distinct from *Galaxaura* by Huisman *et al.* (2004) on the basis of morphological and molecular evidence, and this segregation was further supported by new morphological characters (Wang *et al.*, 2005). Within Galaxauraceae, *Dichotomaria* is characterized by having ± isomorphic gametophytic and tetrasporophytic phases, a pericarp enclosing the cystocarp, a multinucleate fusion cell formed by gonimoblast cells, and cruciately divided tetrasporangia produced on stalk cells (Wang *et al.*, 2005; Kurihara & Huisman, 2006; Wiriyadamrikul *et al.*, 2014).

During an algal survey that took place in late June 2014 on an islet made of Kurkar (a rocky substratum of calcite-cemented sandstone) at Sdot-Yam, near the old port of Caesarea (built by Herod the Great about 13-25 B.C.), three specimens of *Dichotomaria* sp. were found and collected from a natural intertidal habitat.

Materials and Methods

Study area

Following the finding of the species at Sdot-Yam, algal surveys were carried out along the entire IM coastline, during the summer of 2014. These surveys were conducted in the intertidal zone as well as the shallow subtidal, to a depth of 8 m. Specimens were collected from natural habitats as well as from algal drifts, placed in plastic bags, and labelled with location and collection data. Herbarium specimens were stored as dried or preserved in 70% alcohol.

Dichotomaria specimens were observed exclusively at two sites (Fig. 1). Specimens were found growing attached

Fig. 1: Distribution (black dots) of *Dichotomaria* cf. *obtusata*. along the Israeli Mediterranean coastline.

to vertical walls located inside a pothole at 1m depth, on a small islet made of Kurkar at Sdot-Yam (32.4912°N, 34.8866°E). Specimens were also collected at the city of Haifa (32.8324°N, 34.9725°E) ca. 40 km north of the first site. Most specimens collected at Haifa were found scattered in the summer algal drift, however, one was found growing attached to a flat limestone foundation at about 4 m depth.

Morphological studies

The following specimens were examined: **Sdot-Yam**: sterile thalli, 27.vi.2014-(III), coll. *R. Hoffman* (TAU unnumbered; Hoffman s.n.); sterile thalli, 27.vi.2014-(IV), coll. *R. Hoffman* (TAU unnumbered; Hoffman s.n.); **Haifa**: thallus with sporangial initials, 20.vii.2014-(III), coll. *R. Hoffman* (TAU unnumbered; Hoffman s.n.).

Tissues were decalcified in 0.1M HCl solution, and cellular morphology was examined using a Zeiss Axioplan 2 imaging (Carl Zeiss, Jena, Germany). Digital images were acquired with an Olympus DP71 microscope digital camera and an Olympus SP 820UZ digital camera (Olympus Corp. Tokyo, Japan). We compared the anatomical and cellular features of the Mediterranean specimens of *Dichotomaria* to those published in the literature (Littler & Littler, 2000; Wang *et al.*, 2005; Huisman & Kurihara, 2006; Kurihara & Huisman, 2006; Wiriyadamrikul *et al.*, 2014), and also to *Dichotomaria* specimens stored at the national seaweed herbaria of Israel.

The algaebase.org site (Guiry & Guiry, 2014) and the national seaweed herbaria of Israel (TAU and Hebrew University in Jerusalem) were checked to confirm the assumption that this is the first record of the genus *Dichotomaria* in the Mediterranean.

Molecular and phylogenetic analyses

Molecular examination and sequencing of specimen TAU Sdot-Yam, Hoffman s.n., 27.vi.2014-(I) took place in Taiwan. Total DNA was extracted using the ZR Plant/Seed DNA kit (Zymo Research, CA, USA) following the manufacturer's instructions. Gene amplification and sequencing followed the protocols described in Wang *et al.* (2005). Gene-specific primers are listed in Lin *et al.* (2001) as follows: F7+R753, F492+R1150, and F993+RrbcS for *rbc*L. Sequencing was done using an ABI3730 DNA Sequencer (Applied Biosystems, Foster, CA, USA) at the Mission Biotechnology Company (Taipei, Taiwan). The newly generated sequence was deposited at GenBank under the accession KM659128. Additional *rbc*L sequences were obtained from GenBank with the accession numbers listed in Wang *et al.* (2005), Kurihara *et al.* (2005), Kurihara & Huisman (2006), Liu *et al.* (2013), and Wiriyadamrikul *et al.* (2014). Three genera of Galaxauraceae, including *Actinotrichia*, *Galaxaura*, and *Tricleocarpa*, were used as outgroups. Prior to the phylogenetic analysis, the nucleotide sequences were aligned using the software MUSCLE with default settings (Edgar, 2004). Phylogenetic analysis was conducted using the software MEGA v.6 (Tamura *et al.*, 2013) with a maximum likelihood method. The best-fit nucleotide substitution model of sequence evolution was selected using MEGA v. 6 as the Tamura 3-parameter model (T93) + G + I due to the lowest BIC (Bayesian Information Criterion) score. The parameters were as follows: assumed nucleotide frequencies A = 0.324, T = 0.310, C = 0.157, G = 0.209; nucleotide substitution matrix with A-T = 0.041, A-C = 0.021, A-G = 0.081, T-A = 0.043, T-C = 0.177, T-G = 0.028, C-A = 0.043, C-T = 0.349, C-G = 0.028, G-A = 0.126, G-T = 0.041, G-C = 0.021; proportion of sites assumed to be invariable = 0.56; and rates for variable sites assumed to follow a gamma distribution with the shape parameter = 0.88. Statistical support for each node of the phylogenetic tree was computed using 1000 replicates of bootstrapping analyses (Felsenstein, 1985) with the maximum likelihood method in MEGA v.6.

Results

Morphological results

Specimens have a slightly calcified, segmented thallus rising from a discoid holdfast, up to 1.5 cm in diameter, forming stiff, terete, erect bushy clusters, up to 7 cm high, pale pink to cream or red in color (Fig. 2a).

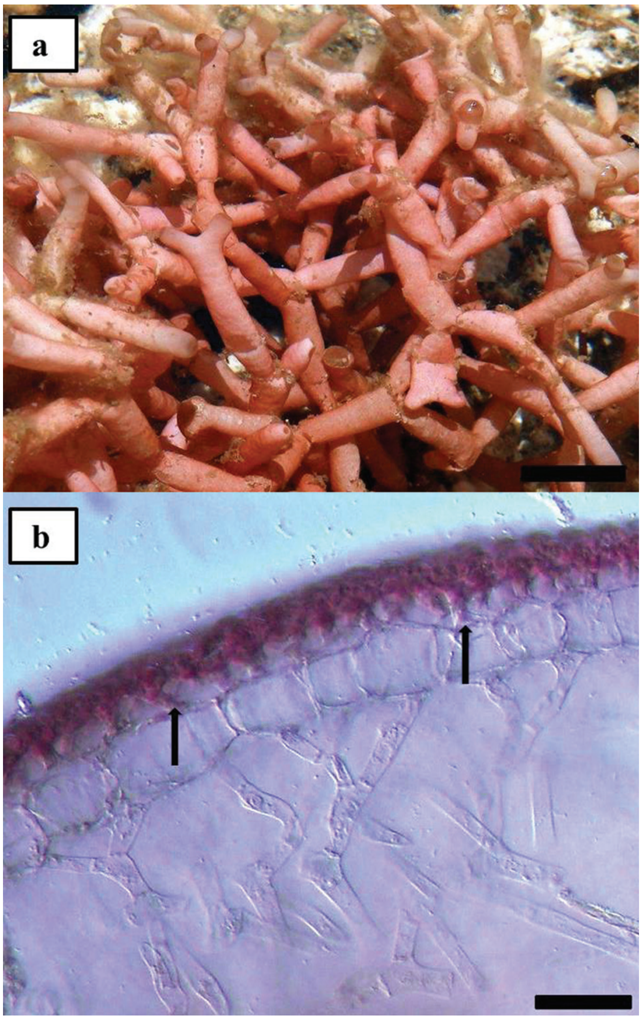

Fig. 2: Tetrasporangial thallus of specimen TAU Sdot Yam, Hoffman s.n., 27.vi.2014-(III), (a) habit (scale bar = 8 mm) and (b) Cross-section showing the tristromatic cortex with distinct stalk-cells (arrows) in the middle portion (scale bar = 50 µm).

Branching is dichotomous. Segments are slightly calcified at joints, covered with scattered microscopic hairs, fragile, brittle and obtuse at apices, 2–3 mm in diameter and 4 to 10 mm long. The medulla is mucilage-filled and largely cell-free, however, dichotomous filaments (12-18 µm in diameter) cross the medullary space.

The cortex is three cells thick throughout the segmented thallus (Fig.2b). Cells in the outermost (epidermal) layer are tightly arranged, triangular in transverse section and polygonal in surface view (Fig. 3) with 4-8 sides (15–25 µm long by 20–35 µm wide). Cells of the middle cortical layer are elongated and stalk-like, 8-14 µm in diameter (Fig. 2b). Cells of the innermost layer are broadly oval in transverse section and polygonal in surface view (50–70 µm long by 45–110 µm wide).

Specimen 20.vii.2014-(III) (TAU, Hoffman s.n.) is provided with terminal sporangial initials, on stalk cells cut off from the outermost cortical cells. Immature tetrasporangia are spherical to ovoid in shape, and 20–23µm wide by 28-36 µm long.

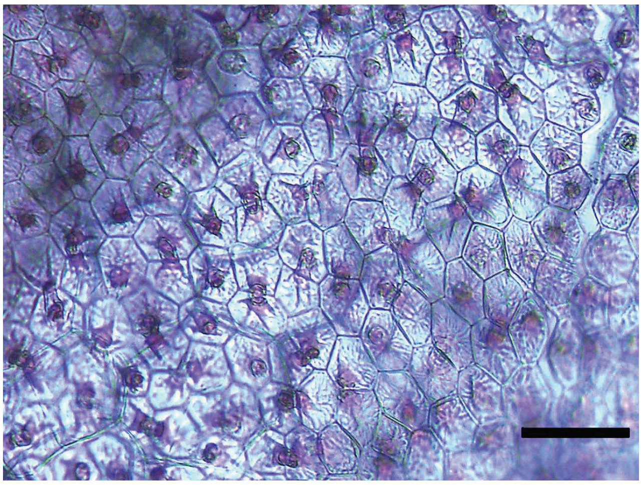

Fig. 3: Surface view of the polygonal epidermal cells of the outermost layer (scale bar = 40 µm).

Molecular results

A total of 1,363 nucleotides were used in our phylogenetic analysis after trimming the alignment to account for missing data at the 5' end of some of the aligned sequences. Maximum-likelihood (ML) analysis, revealed five lineages in the genus *Dichotomaria* with moderate to strong statistical support (Fig. 4). The Mediterranean specimen was grouped with members of the *D. obtusata* assemblage, being more closely related to the specimen from Phang Nga (Thailand), and differing by only 0.3% divergence in the *rbc*L sequence (Table 1). As the molecular affiliation of *D. obtusata* from its type locality (Bahamas) remains unknown, and a second Atlantic taxon is recognized as *D. obtusata* var. *major*, we concluded that the Mediterranean material should be identified as *D. cf. obtusata*, representing an undescribed entity from the Indo-Pacific Ocean.

Discussion

The results of our morphological observations and molecular analyses indicated that the specimens from the IM belong to the *Dichotomaria obtusata* assemblage. In this genus, there are two different types of thallus: (1) the flattened form and (2) the terete form. Our specimens clearly belong to the second group. To the best of our knowledge, there are only four taxa of *Dichotomaria* with terete thallus: *D. hommersandii* S.L. Liu & S.M. Lin, *D. papillata* (Kjellman) A. Kurihara & Masuda, *D. obtusata*, and *D. obtusata* var. *major* Taylor (Tanaka, 1936; Liu *et al.*, 2013; Wiriyadamrikul *et al.*, 2014). *Dichotomaria papillata* can be excluded from further consideration since its branches lack nodal constriction (Tanaka, 1936). In contrast, both *D. hommersandii* and *D. obtusata* possess the latter character, but differ by the width of branches. Branch width in *D. obtusata* (1.5-3.0 mm) and *D. obtusata* var. *major* (2.5-3.0 mm) is greater than in *D. hommersandii* (0.5-1.5 mm) (Liu *et al.*, 2013; Wiriyadamrikul *et al.*, 2014). The branch width of our

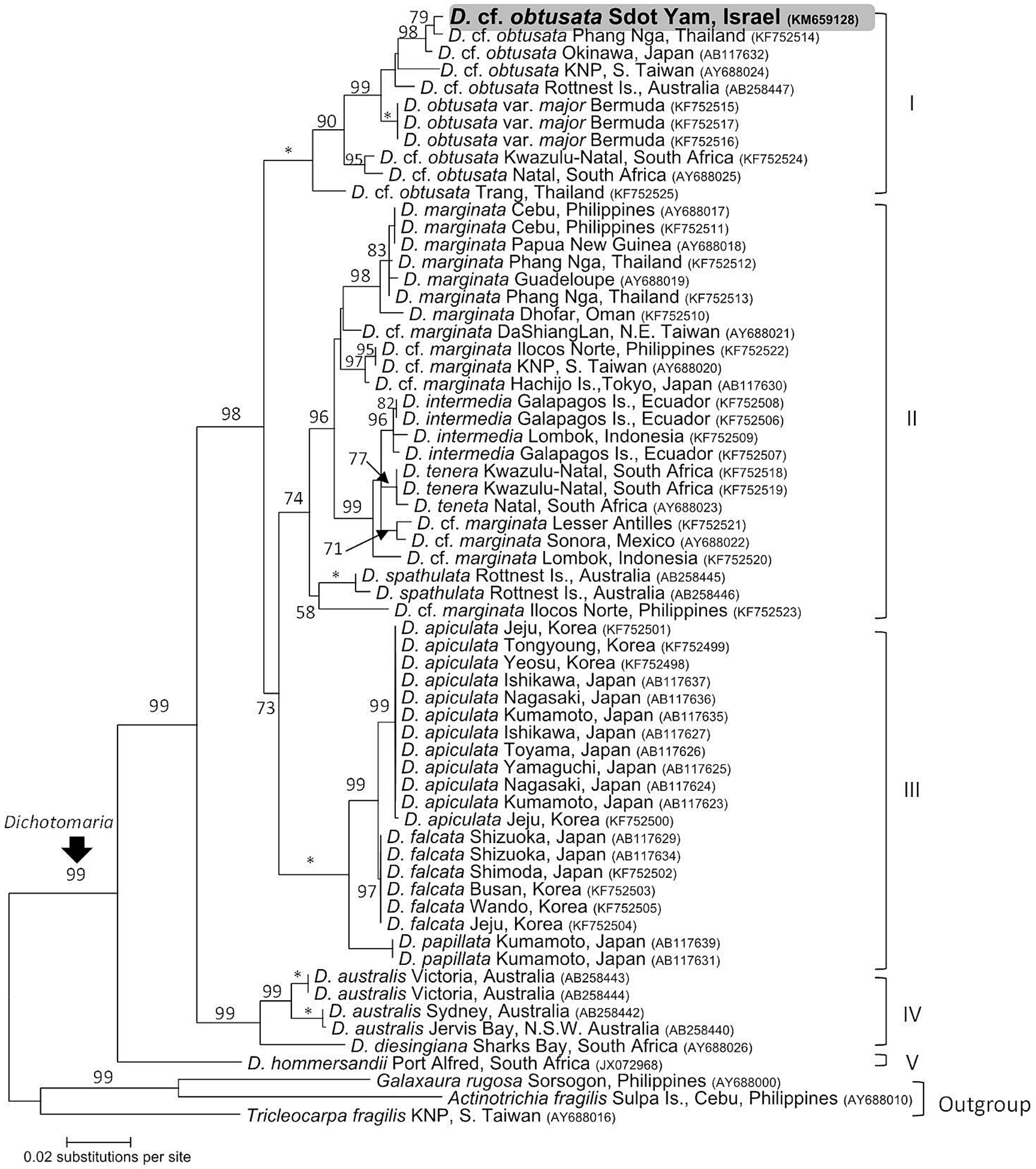

Fig. 4: The phylogram of the genus *Dichotomaria* inferred from the maximum likelihood method. Species from three different genera of Galaxauraceae (i.e., *Actinotrichia*, *Galaxaura* and *Tricleocarpa*) were used as outgroup. Statistical support from 1000 replicates of bootstrapping analyses is indicated on branches (> 50%). Asterisk indicates 100% statistical support from the bootstrapping analyses.

specimens agrees well with the descriptions of *D. obtusata* and *D. obtusata* var. *major*. Moreover, the particular arrangement of cortical cells in our material (Fig. 2b) supports its identification with the tetrasporophyte phase of these species (Huisman & Kurihara, 2006).

At the molecular level, Wiriyadamrikul *et al.* (2014, fig. 4) observed high genetic divergence in *rbc*L and *cox*1 sequences, between widely separated strains of *D. obtusata,* and recognized at least 3 groups: one from Australia, Japan, Taiwan, and Thailand, a second one from South Africa, and a third one from Bermuda (var. *major*). Wiriyadamrikul *et al.* concluded that these groups may represent distinct species, but in the absence of data from the type locality (Bahamas) they refrained from taxonomic implications. Our molecular analyses of the Mediterranean material showed that it is affiliated with the first group of Wiriyadamrikul *et al.* and in particular with the specimen from Phang Nga (Thailand). Accordingly, we tentatively identified our materials as *D.* cf. *obtusata*, pending genetic information of populations from the type locality (Bahamas).

The present report of the tropical genus *Dichotomaria* from IM increases the number of Galaxauraceae in the Mediterranean Sea to three. The other two members are *Tricleocarpa fragilis* (Linnaeus) Huisman & R.A.Townsend, considered to be native (Guiry & Guiry, 2014), and the tropical *Galaxaura rugosa* (J.Ellis & Solander) J.V. Lamouroux, which was also observed recently on the Israeli

Table 1. Uncorrected pairwise P-distance (%) between species in the "D. obtusata" assemblage based on rbcL sequences.

Localities	GenBank Accession No.	Ocean/Sea	1	2	3	4	5	6	7	8	9	10	11
1. Sdot-Yam, Israel	KM659128	Mediterranean Sea	-										
2. Phang Nga, Thailand	KF752514	Indian Ocean	0.3	-									
3. Okinawa, Japan	AB117632	Pacific Ocean	0.4	0.6	-								
4. KNP, S. Taiwan	AY688024	Pacific Ocean	1.8	2.0	1.8	-							
5. Rottnest Island. Australia	AB258447	Pacific Ocean	1.7	1.6	1.3	1.6	-						
6. Bermuda	KF752515	Atlantic Ocean	2.0	1.8	1.5	1.8	1.3	-					
7. Bermuda	KF752516	Atlantic Ocean	2.0	1.8	1.5	1.8	1.3	0.0	-				
8. Bermuda	KF752517	Atlantic Ocean	2.0	1.8	1.5	1.8	1.3	0.0	0.0	-			
9. Kwazulu-Natal, South Africa	KF752524	Indian Ocean	3.0	3.1	2.8	2.7	2.3	2.0	2.0	2.0	-		
10. Natal, South Africa	AY688025	Indian Ocean	2.8	2.6	2.5	2.6	2.3	1.9	1.9	1.9	0.6	-	
11. Trang, Thailand	KF752525	Indian Ocean	3.5	3.5	3.3	3.3	2.9	2.9	2.9	2.9	2.2	2.0	-

coast (Hoffmann *et al.*, 2008; Hoffman & Dubinsky, 2010). The survival of D. *cf. obtusata* and the proliferation of G. rugosa could be explained by global warming in the Levant Sea, where sea surface temperatures increased dramatically in the past few years (Hoffman, 2013).

Dichotomaria obtusata is well represented in the algal herbarium of TAU, where, most collections originate from the Red Sea (Gulf of Aqaba and Sinai Peninsula) and a few from Taiwan. The common occurrence of the species in the Red Sea supports the hypothesis that our Mediterranean material is a Lessepsian immigrant that simply crossed the Suez Canal. Yet, we may also speculate that the vector of introduction was shipping. This assumption implicates bulk carriers as the vector that introduced *D.* cf. *obtusata* to the Mediterranean. These ships which transport coal from Australia, South Africa and Colombia to the power station located near Sdot-Yam, anchor about 3 km south west of the islet. As *D. obtusata* is commonly recorded on the shores that surround South Africa, Australia and Colombia (Guiry & Guiry, 2014), it is quite possible that one of these ships carried this rhodophyte either in its ballast water or as filamentous holdfasts attached to its hull. Assuming a first introduction to Sdot-Yam, the occurrence at Haifa may indicate a northward spreading, probably with the local north-flowing marine current. Across the globe, the northern boundaries of *D. obtusata* are in southern Japan for the western Pacific, North Carolina (USA) for the western Atlantic and the Madeira Archipelago for the eastern Atlantic (Guiry & Guiry, 2014). Madeira is located ca. 6,500 km west of the collecting site at Haifa and at the same latitude (32°50').

Zenetos *et al.* (2012) reported the highest number of alien species in the eastern Mediterranean basin, with a total of 775 species as compared to 308 species in the western basin. Seaweeds including sea-grasses were the only group that contributed with a higher number of non-native species in the western Mediterranean than the eastern Mediterranean, with 93 and 70 species respectively. This fact does not necessarily demonstrate the actual situation since algal surveys from the Levant Sea and the Egyptian coast are comparatively few.

Acknowledgements

The main author is a post-doctoral VATAT-supported fellow at the Steinhardt Museum of Natural History and National Research Center, and at the Department of Molecular Biology and Ecology of Plants at Tel Aviv University. This research was supported by an Israeli Taxonomy Initiative grant. The second author was financially supported by a grant from the Ministry of Science and Technology, Taiwan (MOST103-2611-M-029-001). We thank Prof. Michael Wynne for his useful advice and Miss Tsai-Yin Hsieh for her help with DNA extractions. Finally, the authors wish to thank the associate editor, A. Athanasiadis and four anonymous reviewers for their helpful and constructive comments that enabled us to significantly improve this paper.

References

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acid Research,* 32, 1792-1797.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution,* 39, 783-791.

Guiry, M.D., Guiry, G.M., 2014. *AlgaeBase*. http://www.algaebase.org (Accessed 1 September 2014).

Hoffman, R., Israel, A., Lipkin, Y., Dubinsky, Z., Iluz D., 2008. First record of two seaweeds from the Israeli Mediterranean: *Galaxaura rugosa* (J. Ellis and Solander) J.V. Lamouroux (Rhodophyta) and *Codium adhaerens* C. Agardh (Chlorophyta). *Israel Journal of Plant Sciences,* 56, 123-126.

Hoffman, R., Dubinsky, Z., 2010. Invasive and Alien Rhodophyta in the Mediterranean and along the Israeli shores. p. 47-60. In: *Red Algae in Genome Age. Cellular origin, life in extreme habitats and astrobiology*. Seckbach, J., Chapman, D.J. (Eds). Springer Publishers, Netherlands, 498 pp.

Hoffman, R., Shemesh, E., Ramot, M., Dubinsky, Z., Pinchasov-Grinblat, Y. *et al.*, 2011. First record of *Codium arabicum* Küt (Bryopsidales, Chlorophyta) in the Mediterranean Sea. *Botanica Marina,* 54, 487–495.

Hoffman, R., 2013. Alien benthic algae and seagrasses in the Mediterranean Sea and their connection to global warming. p. 159-181. In: *The Mediterranean Sea: Its History and Present Challenges*. Goffredo, S., Dubinsky, Z. (Eds). Springer Publishers, Netherlands, 678 pp.

Huisman, J.M., Harper, J.T., Saunders, G.W., 2004. Phylogenetic study of the Nemaliales (Rhodophyta) based on large-subunit ribosomal DNA sequences supports segregation of the Scinaiaceae fam. nov. and resurrection of Dichotomaria Lamarck. *Phycological Research,* 52 (3), 224-234.

Huisman, J.M., Kurihara, A., 2006. Dichotomaria. p. 16-21. In: *Algae of Australia Nemaliales*. Huisman, J.M. (Ed). Australian Biological Resources Study, Canberra, 164 pp.

Kurihara, A., Arai, S., Shimada, S., Masuda, M., 2005. The conspecificity of *Galaxaura apiculata* and *G. hystrix* (Nemaliales, Rhodophyta) inferred from comparative morphology and *rbc*L and ITS1 sequences. *European Journal of Phycology,* 40, 39-52.

Kurihara, A., Huisman, J.M., 2006. The *Dichotomaria marginata* assemblage in Australia. p. 120-136. In: *Algae of Australia: Nemaliales*. Huisman, J.M. (Ed). Canberra: Australian Biological Resources Study, 164 pp.

Lin, S.M., Fredericq, S., Hommersand, M., 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and *rbc*L sequences, including the Phycodryoideae, subfam. nov. *Journal of Phycology,* 37, 881-899.

Littler, D.S., Littler, M.M., 2000. *Caribbean reef plants. An identification guide to the reef plants of the Caribbean, Bahamas, Florida and Gulf of Mexico*. Offshore Graphics, Washington, 542 pp.

Liu, S.L., Lin, S.M., Wang, W.L., 2013. Molecular phylogeny of the genus *Dichotomaria* (Galaxauraceae, Rhodophyta) from the Indo-Pacific region, including a new species *D. hommersandii* from South Africa. *European Journal of Phycology,* 48, 221-234.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30, 2725-2729.

Tanaka, T., 1936. The genus *Galaxaura* from Japan. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido Imperial University,* 1 (2), 141-173.

Wang, W.L., Liu, S.L., Lin, S.M., 2005. Systematics of the calcified genera of the Galaxauraceae (Nemaliales, Rhodophyta) with an emphasis on Taiwan species. *Journal of Phycology,* 41, 685-703.

Wiriyadamrikul, J., Wynne, M.J., Boo, S.M., 2014. Phylogenetic relationships of *Dichotomaria* (Nemalionales, Rhodophyta) with the proposal of *Dichotomaria intermedia* (R.C.Y. Chou) comb. nov. *Botanica Marina,* 57 (1), 27-40.

Zenetos, A., Gofas, S., Morri, C., Rosso, A., Violanti, D. *et al*., 2012. Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD), Part 2, Introduction trends and pathways. *Mediterranean Marine Science,* 13, 328-352.