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An assessment of the taxonomic status of the Mediterranean endemic genus *Acrodiscus* Zanardini (Halymeniales, Rhodophyta)

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Abstract. *Acrodiscus* Zanardini is a poorly known monotypic endemic Mediterranean genus based on *A. vidovichii* (Menegh.) Zanardini. Rarely reported, its reproductive structures have remained undocumented, leaving its exact taxonomic position uncertain. Solely on the basis of its vegetative structure, Zanardini provisionally placed it in the family Cryptonemiaceae of the order Cryptonemiales (currently the Halymeniaceae of the Halymeniales), although he was uncertain as to whether the new genus actually belonged to that family or should instead be included in the Gigartinaceae of the Gigartinales (where Meneghini had originally placed it). In the present study we have extensively sampled *A. vidovichii* and documented its vegetative and tetrasporangial features. As well, we provide molecular-sequence data (COI-5P, *rbc*L, LSU) that indicate its phylogenetic affinities. We confirm *Acrodiscus* as a member of the Halymeniaceae and its status as an independent genus. Searches of several institutional herbaria have allowed us to locate and lectotypify Meneghini's *Chondrus? vidovichii* by the discovery of his original material now held at the Herbarium Horti Pisani (Pisa, Italy).

Keywords. *Acrodiscus*, DNA barcoding, Mediterranean endemic, lectotype, morphology, phylogeny.

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Introduction

Acrodiscus Zanardini is a poorly known and persistently enigmatic genus endemic to the Mediterranean Sea. Based on *Chondrus? vidovichii* Menegh. (Savi 1841), it was later proposed as the type and only

species of the genus *Acrodiscus* by Zanardini (1868) based on vegetative structure and position of the tetrasporangial sori. Throughout its long history it has only rarely been collected, is known only from asexual material, and has remained of uncertain family affinity. Zanardini (1868) provisionally placed the genus in the Cryptonemiaceae (currently the Halymeniaceae), but he questioned whether the new genus was correctly placed in that family or whether it should instead be included in the Gigartinaceae. In the absence of female reproductive structures, it has not been possible to relate *Acrodiscus* to existing members of the Halymeniaceae owing to the importance of carpogonial and auxiliary-cell "ampullae" in the taxonomy of that family (Chiang 1970; Gargiulo *et al.* 2013; Saunders & Kraft 1996). Halymeniacean vegetative structures are on the contrary quite similar to those of other families within the Gigartinales. Presumably based on the similarity of morphology and presence of tetrasporangial sori, Schmitz (1889) proposed that *A. vidovichii* should be transferred to the genus *Polyopes*. Guiry & Guiry (2016) have suggested that *Acrodiscus* is congeneric with *Cryptonemia*, although it lacks the differentiated stipes and especially the refractive medullary cells typical of that genus (Kraft & Saunders 2014: 165).

The aim of the present study is to ascertain the taxonomic position of *A. vidovichii* through several lines of evidence that include DNA-sequence data, a thorough description of both vegetative and tetrasporangial features, and the full characterization of the type material on which our lectotypification is based.

Material and Methods

Specimens from several localities (Table 1) were used for anatomical and DNA studies.

Fresh material was pressed as herbarium specimens, with fragments preserved in 4% formalin for permanent wet-preservation or dried in silica gel for molecular studies. Vouchers are housed in the Herbarium of the University of Barcelona, Spain (BCN-Phyc), the Muséum national d'Histoire naturelle in Paris, France (PC), or in the *Herbarium Messanaensis* of the University of Messina, Italy (MS). Additional material (dried or formalin preserved) was examined in the herbaria of Spanish universities and institutions involved in the project "*Flora Phycologica Iberica*" (Manghisi *et al.* 2010a). For the lectotypification, the *Herbarium Centrale Italicum* of Florence (FI), the herbarium of the Civic Natural History Museum of Venice (MCVE), the *Herbarium Patavinum* of the University of Padua (PAD), the *Herbarium Horti Botanici Pisani* of the University of Pisa (PI), the herbarium of the University of Leiden (L), the herbarium of the Natural History Museum in London (BM), and the cryptogamic herbarium of the Muséum national d'Histoire naturelle in Paris (PC) were searched for type material (Table 2; Appendix 1). Herbarium designations are given as directed by the Index Herbariorum (Thiers continuously updated).

For morpho-anatomical observations, thalli were hand-sectioned with single-edged razor blades, stained with 1% aniline blue, and observed using a Nikon Optiphot-2 equipped with a Nikon Coolpix 4500 camera (Leica Microsystems, Italy).

Sequence data generated for COI-5P, *rbc*L and LSU genes were submitted to BOLD (http://www.barcodinglife.org) and to GenBank (Clark *et al.* 2016). Accession numbers, together with collection information, are given in Table 1.

DNA extraction was performed as outlined in Manghisi *et al.* (2010b). The barcode region COI-5P was PCR amplified as detailed in Saunders & McDevitt (2012), the nuclear LSU rRNA gene was PCR amplified as detailed in Harper & Saunders (2001), and the plastid *rbc*L gene was amplified with various primer combinations as specified by Freshwater & Rueness (1994) and Wang *et al*. (2000).

Sequences were generated using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (PE Applied Biosystems [ABI], Foster City, CA, USA) and analyzed using an ABI Prism 3130XL genetic analyzer at

Table 1. List of specimens of *Acrodiscus vidovichii* examined in the present paper (a = COI5P; b = LSU; c = *rbc*L). [continued on next two pages]

Voucher identification	Collection locality	Collection date	Collector(s)	Notes	BOLD Process ID / GenBank accession no.
LLG0393 PC0152091	Vis, Croatia	20 Jun. 2007	*J.M. Utge, L. Le Gall*	–	KJ594944[a] – KJ594947[b] – KJ594952[c]
LLG0394 PC0152089	Vis, Croatia	20 Jun. 2007	*J.M. Utge, L. Le Gall*	–	ACROV001-16[a,c]
LLG2193 PC0152957	La Gabinière, Port Cros, France	19 Oct. 2008	*L. Le Gall, Y. Turpin*	–	REDEU456-11[a]
LLG2223 PC0152936	Montremian, Port Cros, France	20 Oct. 2008	*L. Le Gall, F. Rousseau*	–	REDEU466-11[a]
LLG2246 PC0152915	Montremian, Port Cros, France	20 Oct. 2008	*L. Le Gall, F. Rousseau*	Tetra-sporic	REDEU488-11[a,c]
LLG2549 PC0162311	Montremian, Port Cros, France	7 Jun. 2009	*L. Le Gall, J.M. Utge*	–	REDEU539-11[a]
LLG2560 PC0162425	Montremian, Port Cros, France	7 Jun. 2009	*L. Le Gall, J.M. Utge*	–	REDEU544-11[a]
LLG2717 PC0162264	La Gabinière, Port Cros, France	8 Jun. 2009	*L. Le Gall, J.M. Utge*	–	REDEU575-11[a]
LLG2733 PC0162395	La Gabinière, Port Cros, France	8 Jun. 2009	*L. Le Gall, J.M. Utge*	–	REDEU580-11[a]
LLG2801 PC0162435	La Gabinière, Port Cros, France	9 Jun. 2009	*L. Le Gall, J.M. Utge*	–	REDEU590-11[a]
LLG2846 PC0162436	La Gabinière, Port Cros, France	9 Jun. 2009	*L. Le Gall, J.M. Utge*	–	REDEU604-11[a]
LLG4197 PC0145757	Corsica, France	3 Oct. 2011	*L. Le Gall*	–	ACROV004-16[a]
LLG4209 PC0145758	Corsica, France	3 Oct. 2011	*L. Le Gall*	–	–

Voucher identification	Collection locality	Collection date	Collector(s)	Notes	BOLD Process ID / GenBank accession no.
ACR002	Scilla, Reggio Calabria, Calabria, Italy	29 Jun. 2005	*A. Manghisi*	–	ACROV003-16[a,b,c]
LLG3679 PC0145759	Capo dell'Armi, Reggio Calabria, Calabria, Italy	13 Apr. 2011	*L. Le Gall, J.M. Utge*	–	–
LLG3839 PC0145760	Scala dei Turchi, Agrigento, Sicily, Italy	17 Apr. 2011	*L. Le Gall, A. Manghisi, M. Morabito*	–	–
LLG3890 PC0145761	S. Maria La Scala, Acireale, Sicily, Italy	18 Apr. 2011	*L. Le Gall, J.M. Utge*	–	ACROV002-16[a]
LLG3902 PC0145762	S. Maria La Scala, Acireale, Sicily, Italy	18 Apr. 2011	*L. Le Gall, J.M. Utge*	–	–
LLG3993 PC0145763	S. Maria La Scala, Acireale, Sicily, Italy	21 Apr. 2011	*L. Le Gall, J.M. Utge*	–	–
LLG4102 PC0144391	Kalithea, Rhodos I., Greece	7 Sep. 2011	*L. Le Gall*	–	ACROV005-16[a]
LLG4103 PC0144392	Kalithea, Rhodos I., Greece	7 Sep. 2011	*L. Le Gall*	–	–
BCN-Phyc 5900	Illa dels Porros, Menorca, Spain	22 Jun. 2003	*A. Manghisi, L. Lavelli, J. Rull, N. Salvador*	–	–
BCN-Phyc 5901	Faro, Cap Caballeria, Menorca, Spain	23 Jun. 2003	*A. Manghisi, L. Lavelli, J. Rull, N. Salvador*	–	–
BCN-Phyc 5902	es Cavall, Mallorca, Spain	2 Jun. 2004	*A. Manghisi,J. Rull, N. Salvador*	–	–
BCN-Phyc 5903	el Laberint, cala Bona, Mallorca, Spain	3 Jun. 2004	*J. Rull, N. Salvador*	Tetra-sporic	–
BCN-Phyc 5905	cala Pi, Mallorca, Spain	4 Jun. 2004	*A. Manghisi, J. Rull, N. Salvador*	–	–

Voucher identification	Collection locality	Collection date	Collector(s)	Notes	BOLD Process ID / GenBank accession no.
BCN-Phyc 5906	cala Figuera (Norte), Mallorca, Spain	5 Jun. 2004	*A. Manghisi, J. Rull, N. Salvador*	–	–
BCN-Phyc 5907	P.ta Colibre, I. Columbret Gran, Illes Columbrets, Castelló de la Plana, Spain	9 Jul. 2004	*N. Salvador, J. Rull*	–	–
BCN-Phyc 5908	Freu de Terra, Hospitalet de l'Infant, Tarragona, Spain	7 Jul. 2004	*A. Manghisi, N. Salvador, J. Rull*	–	–
MS35027-1	Catania, Sicily, Italy	1 Jan. 1980	*M. Cormaci*	–	–
MS35027-2	Capo S. Gregorio, Lecce, Italy	1 Jul. 1980	*S. Santisi*	–	–
MS35027-3	Bagni della Regina, Sorrento, Gulf of di Naples, Italy	1 Sep. 1980	–	–	–
MS35027-4					–
MS35027-5					–
MS35027-6					–
MS35027-7					–
MS35027-8					–
MS35027-9					–
MS35027-10	S. Maria la Scala Catania, Sicily, Italy	5 Apr. 1983	–	–	–
MS35027-11					–
MS35027-12					–
MS35027-13					–
MS35027-14					–
MS35027-371F	Punta Gallo Palermo, Sicily, Italy	28 May 1985	–	–	–

the CEMAR, University of New Brunswick, Canada, or an ABI Prism 3730XL at the Genoscope (www.genoscope.fr) in Evry, France. Forward and reverse sequence reads were assembled into contigs with the software ChromasPro (v. 1.7.6.1, Technelysium Pty Ltd) and edited. Multiple sequence alignments were constructed in SeaView v. 4.3.3 (Gouy *et al.* 2010) and included both data from GenBank and sequences generated for the present study. Newly generated COI-5P sequences were subjected to distance analysis in PAUP* (Swofford 2002).

For *rbc*L analyses, an initial alignment of 728 sequences and 1258 nucleotide positions was subjected to neighbor-joining (NJ) distance analysis under a K2 nucleotide substitution model in PAUP* to identify species groups. The resulting tree was used to prepare a second alignment for subsequent phylogenetic analyses with 36 sequences representative of most genera of the order Halymeniales, including the generitypes, by the exclusion of duplicate or similar sequences (poor quality sequences, i.e., those missing more than 30% of data, were also removed), and 1211 nucleotide positions.

Similarly, an initial alignment was built with 122 LSU sequences and 2921 nucleotide positions, from which, after NJ analyses performed as above, a fourth alignment was built with 33 LSU sequences representative of most genera of Halymeniales, including the generitypes, and 2640 nucleotide positions, excluding those ambiguously aligned.

A fifth alignment consisted of the concatenation of LSU and *rbc*L sequences for the same species and, when possible, for the same specimen, these including 21 sequences and 3820 nucleotide positions. GenBank accession numbers of sequences used for the final alignments are listed in Table 3.

All phylogenetic analyses were performed in MrBayes v. 3.1.2 or 3.2.1 (parallel version; Altekar *et al.* 2004; Ronquist & Huelsenbeck 2003), RAxML (online version, http://embnet.vital-it.ch/raxml-bb/; Stamatakis et al. 2008), and PhyML 3.0 (online version, http://atgc.lirmm.fr/phyml; Guindon & Gascuel 2003).

Maximum Likelihood analyses were performed with bootstrap resampling to estimate robustness of the internal nodes (Felsenstein 1985), based on 1000 replicates in PhyML, with a GTR+G+I substitution model (with all parameters estimated during the search), starting from ten random BIONJ trees (Gascuel 1997) with subtree pruning and regrafting (SPR) as branch-swapping algorithm. Furthermore, Maximum Likelihood (ML) analyses were also performed with bootstrap resampling based on 100 replicates in RAxML, with a GTR+G+I substitution model with three partitions for *rbc*L, corresponding to each codon position, and with four partitions for the concatenate analyses corresponding to LSU and each codon position of *rbc*L.

For Bayesian inference both the *rbc*L and the concatenate data sets were also partitioned as above. The covarion-like model (Huelsenbeck 2002) was combined with the GTR+G model of sequence evolution linking or unlinking parameters among partitions (shape, statefreq, revmat, switchrates, Tratio) and setting the prior for the site specific rates as "variable". Different analytical strategies were tested in order to reach convergence. Each analysis consisted of two parallel runs, each run using four chains, one cold and three incrementally heated.

A single run consisted of 5 million generations that were sampled every 1000th tree. After completion of the two runs, likelihood values were plotted against the number of generations to evaluate when MCMC chains reached stability, in order to set an appropriate burn-in value for each analysis. Only trees saved during the stationary phase were used to reconstruct a majority-rule consensus tree and calculate the distribution of posterior probabilities.

In all phylogenetic analyses, unrooted trees were constructed, the root being subsequently designated based on previous knowledge (Withall & Saunders 2006).

Table 2. Historical herbarium specimens examined for lectotypification of *Chondrus vidovichii* Menegh. BM = Natural History Museum, London, UK; FI = *Herbarium Universitatis Florentinae*, Florence, Italy; L = *Nationaal Herbarium Nederland*, Leiden, the Netherlands; MCVE = Civic Natural History Museum, Venice, Italy; PAD = *Herbarium Patavinum*, Padua, Italy; PC = Muséum national d'Histoire naturelle, Paris, France; PI = *Herbarium Horti Botanici Pisani*, Pisa, Italy. [continued on next page]

Herbarium	Voucher identification	Material type	Collection	Taxon name on label	Locality on label	Date	Collector
BM	000619430		Herb. Griffiths	*Cryptonemia dichotoma* J. Agardh	Nizza	1841	*J. Agardh*
BM	000563717		Herb. Hookerianum ex Herb. J.G. Agardh	*Cryptonemia dichotoma* J.Ag.	Nizza	–	–
BM	000563718		Herb. W.H. Harvey ex Herb. J.G. Agardh	*Cryptonemia dichotoma* J.Ag.	Nizza	–	–
BM	000569934		Herb. E.M. Holmes ex Herb. Weber van Bosse	*Acrodiscus vidovichii*	Dalmazien	–	*Vidovich*
FI	4796-1	4 sub sheets, 1 envelope	Herb. Meneghini		–	–	–
L	0833934		Herb. Suringar	*Cryptopleura vidovichii*	*Dalmazia!*	–	–
L	0833935		Herb. Kützing	*Cryptonemia vidovichii* * *Chondrus vidovichii* Menegh. 1841 *Cryptonemia dichotoma* J.Ag. 1842	*Dalmazia*	–	*Meneghini*
L	0833936	sub sheet 1 sub sheet 2		*Acrodiscus vidovichii* *Acrodiscus vidovichii*	Dalmazia Dalmazien	–	*Vidovich* *Vidovich, com. Zanardini*

Herbarium	Voucher identification	Material type	Collection	Taxon name on label	Locality on label	Date	Collector
MCVE	1 folder	sheet 1	Herb. Zanardini		Gulf of Naples	–	*Pedicino*
		sheet 2	Herb. Zanardini		Dalmatia		
		envelope 1	Herb. Zanardini				
		envelope 2	Herb. Zanardini				
		envelope 3	Herb. Zanardini	*Chondrus vidovichii* Menegh.			
		envelope 4	Herb. Zanardini	*Chryptonemia vidovichii* Z.			
		drawings					
PAD	A00389	1 envelope	Herb. Forti ex Herb. Ardissone Ex Herb. Mgh!	*Chondrus vidovichii* Mgh.	Dalmazia	–	*Vidovich*
PC	0047928		Herb. Montagne	*Cryptonemia vidovichii* Zan. (*Chondrus ? vidovichii* Menegh.) (*Cryptonemia dichotoma* J.Ag.)	Dalmazia	–	*Meneghini*
PC	0474569		Herb. Zanardini	*Acrodiscus vidovichii* Zan.	–	–	–
PC	0523534			*Cryptonemia dichotoma* J.Ag.	Nizza	1840	*J. Agardh*
PI	Folder 742. *4. Euhymenia dichotoma Kg.*	1 envelope 1 sheet 2 sheets	Ex H. Mgh.	*Chondrus vidovichii* Menegh. *Cryptonemia dichotoma* J.Ag.	*Dalm.*	–	*Vidovich*

Table 3. List of sequences from GenBank used in phylogenetic analyses.

Species	GenBank accession numbers LSU	*rbc*L
Aeodes nitidissima J.Agardh	GWS1525 [1]	–
Aeodes nitidissima J.Agardh	–	KJ739733
Amalthea freemaniae D'Archino & W.A.Nelson	–	KJ606651
Carpopeltis phyllophora (J.D.Hooker & Harvey) F.Schmitz	–	FN908164
Codiophyllum natalense J.E.Gray	–	FN908160
Corynomorpha prismatica (J.Agardh) J.Agardh	KJ594950	KJ594955
Cryptonemia lomation (Bertoloni) J.Agardh	KJ594948	KJ594953
Cryptonemia luxurians (C.Agardh) J.Agardh	–	AB061374
Cryptonemia rotunda (Okamura) Kawaguchi	–	AB061375
Cryptonemia undulata Sonder	AF419133	–
Dermocorynus dichotomus (J.Agardh) Gargiulo, M.Morabito & Manghisi	KJ511247	–
Dermocorynus horridus (Kützing) Gargiulo, M.Morabito & Manghisi	KJ511244	–
Dermocorynus montagnei P.Crouan & H.Crouan	–	AY435171
Epiphloea bullosa (Harvey) De Toni	DQ343693	–
Epiphloea bullosa (Harvey) De Toni	–	FN908149
Felicinia marginata (Roussel) Manghisi, Le Gall, Ribera, Gargiulo & M.Morabito	KJ594949	KJ594954
Galene profundae D'Archino & Zuccarello	–	KJ606645
Gelinaria ulvoidea Sonder	GQ471910	–
Gelinaria ulvoidea Sonder	–	FN908154
Glaphyrosiphon intestinalis (Harvey) Leister & W.A.Nelson	–	GU252166
Glaphyrosiphon aucklandicus (Montagne) W.A.Nelson, S.Y.Kim & S.M.Boo	–	KJ739734
Grateloupia filicina (J.V.Lamouroux) C.Agardh	KJ511243	JX070629
Grateloupia ovata Womersley & J.A.Lewis	GQ471911	–
Grateloupia proteus Kützing	KJ511245	–
Halymenia abyssicola E.Y.Dawson	–	GU598119
Halymenia actinophysa M.Howe	–	GU598118
Halymenia dilatata Zanardini	–	AB038604
Halymenia floresii (Clemente) C.Agardh	KJ594951	KJ594956
Halymenia maculata J.Agardh	GQ471913	–
Halymenia maculata J.Agardh	–	AB061397
Halymenia plana Zanardini	GQ471914	–
Halymenia pseudofloresii Collins & M.Howe	GQ471915	–
Halymeniales sp.	GQ471916	–
Isabbottia ovalifolia (Kylin) Balakrishnan	EF033616	KM360033
Kintokiocolax aggregato-ceranthus Tak.Tanaka & Y.Nozawa	–	KF475733
Mariaramirezia osornoensis M.S.Calderon, G.H.Boo, A.Mansilla & S.M.Boo	–	AF488827
Norrissia setchellii (Kylin) Balakrishnan	DQ343694	–
Pachymenia carnosa (J.Agardh) J.Agardh	DQ343695	–
Pachymenia carnosa (J.Agardh) J.Agardh	–	AF385640
Pachymenia cf. *orbicularis* (Zanardini) Setchell & N.L.Gardner	DQ343696	–
Pachymenia lusoria (Greville) J.Agardh	GQ471917	–
Pachymenia orbicularis (Zanardini) Setchell & N.L.Gardner	GQ471918	–
Pachymeniopsis gargiuli S.Y.Kim, A.Manghisi, M.Moribato & S.M.Boo	KJ511246	–
Phyllymenia belangeri (Bory de Saint-Vincent) Setchell & N.L.Gardner	AY772035	–
Polyopes constrictus (Turner) J.Agardh	DQ343697	–
Polyopes constrictus (Turner) J.Agardh	–	AB084535
Polyopes lancifolius (Harvey) Kawaguchi & Wang	KF543072	–
Polyopes tasmanicus (Womersley & J.A.Lewis) Kawaguchi & J.A.Lewis	GQ471919	–
Prionitis lanceolata (Harvey) Harvey	–	AY772037
Prionitis sternbergii (C.Agardh) J.Agardh	EF033617	–
Spongophloea tissotii (Weber van Bosse) Huisman, De Clerck, *et al.*	–	FN908162
Thamnoclonium dichotomum (J.Agardh) J.Agardh	–	FN908152
Thamnoclonium latifrons Endlicher & Diesing	–	FN908158
Tsengia comosa (Harvey) Womersley & Kraft	DQ343702	–
Tsengia laingii (Kylin) Womersley & Kraft	DQ343703	–
Tsengia lanceolata (J.Agardh) Saunders & Kraft	DQ343701	–
Tsengia lanceolata (J.Agardh) Saunders & Kraft	–	AY294386
Yonagunia tenuifolia Kawaguchi & Masuda	–	AB116248
Zymurgia chondriopsidea (J.Agardh) J.A.Lewis & Kraft	DQ343698	KM360035

[1] LSU sequence unpublished, courtesy of Dr. Gary W. Saunders, University of New Brunswick, Fredericton, NB, Canada.

Results

Phylum Rhodophyta Wettst. (Wettstein 1901)
Subphylum Eurhodophytina G.W.Saunders & Hommers. (Saunders & Hommersand 2004)
Class Florideophyceae Cronquist (Cronquist 1960)
Subclass Rhodymeniophycidae G.W.Saunders & Hommers. (Saunders & Hommersand 2004)
Order Halymeniales G.W.Saunders & Kraft (Saunders & Kraft 1996)
Family Halymeniaceae Bory (Bory de Saint-Vincent 1828)

Genus ***Acrodiscus*** Zanardini (Zanardini 1868)

Acrodiscus vidovichii (Menegh.) Zanardini
Figs 1–7; Tables 1–2

Memorie del Reale Istituto Veneto di Scienze, Lettere ed Arti 14: 201 (1868). – *Chondrus? vidovichii* Menegh. in Savi, *Sezione di Botanica, e Fisiologia Vegetabile*, *Adunanza del dì 16 Settembre 1841*. *In: Atti della terza Riunione degli Scienziati Italiani tenuta in Firenze*: 427 (1841). – *Cryptonemia vidovichii* (Meneghini) Zanardini, *Saggio di classificazione naturale delle Ficee*: 42 (1843). – *Euhymenia dichotoma* (J.Agardh) Kütz. var. *vidovichii* Menegh. ex Kütz., *Tabulae phycologicae; oder, Abbildungen der Tange. Vol. 17*: 22, tab. 72 (1867). – Lectotype (designated here): Dalmatia [Šibenik, Croatia], Jul. 1841, *Vidovich* folder 742.4, n.11, fragments at the top and at the bottom left (PI!) (Fig. 7).
Cryptonemia dichotoma J.Agardh, *Algae maris Mediterranei et Adriatici*: 100 (1842). – *Cryptonemia* (section *Acrodiscus*) *dichotoma* J.Agardh, *Species genera et ordines algarum*: 225 (1851). – *Euhymenia dichotoma* (J.Agardh) Kütz., *Species algarum*: 742 (1849). – Type: France, Nice, near St. Hospice, 1841, *J. Agardh* s.n. (LD, BM!, BM000619430, PC!, PC0523534).
Acrodiscus vidovichii Zanardini f. *cochlearis* Erceg., *Acta Adriatica* IV: 76 (1949). syn. nov. – Type: Adriatic, Croatia.

Description

Thalli up to 10 cm in length, the blade segments typically 1–3 cm long by (4–)7(–10) mm broad by 200–250 µm thick (Fig. 1), compressed to flattened, thin, membranous-fleshy, dark red in color, erect from a very short cuneate stipe with a discoid holdfast, the blades subdichotomously branched, linear and slightly channeled, the apices broadly rounded (Figs 1C–D, 2A, 3A). Constrictions occasional between forks (Fig. 1D) and especially at sites of branching (Fig. 1B, D); proliferous blades frequent at constrictions (Fig. 1B) or from lower stipes (Fig. 1B–D).

Fronds multiaxial (Fig. 2A), the cortex anticlinal (Fig. 2A–B), the medulla densely filamentous (Fig. 2D); cortical filaments 5- or 6-layered, pseudo-dichotomously branched, the cells subspherical to ellipsoid (Fig. 2B), progressively smaller outwardly, 5–9 µm in diameter proximally, 2–3 µm in diameter at surface (Fig. 2B) and evenly spaced or slightly paired (Fig. 2C). Subsurface cortical cells secondarily pit-connected; short longitudinal multicellular 'bridges' sometimes linking adjacent cortical cells or growing into the medulla from the transition zone between cortex and medulla; stellate ("ganglionic" *sensu* Womersley & Lewis 1994) and refractive medullary cells or filaments absent. Medulla occupying half of the blade sections, composed of interwoven longitudinal or, less commonly, oblique and transverse filaments ca 5 µm in diameter (Fig. 2D).

Female and male reproductive structures not observed. Tetrasporangia developing in eliptical nemathecia located subapically (Fig. 3A). Tetrasporangial filaments usually three-celled, the terminal cell a cruciate/decussate-cruciate tetrad (30–)33(–35) µm long by 12–15 µm wide (Fig. 3C–D); subapical cells ellipsoid, bearing two simple or once-dichomous sterile filaments that surround the sporangia (Fig. 3C); basal

cells deltoid, sterile nemathecial paraphyses four-celled (Fig. 3C), the proximal cells narrowly falcate, the terminal cells smaller in diameter and subspherical (Fig. 3C).

Distribution and habitat

Acrodiscus is uncommon but widely distributed across the Mediterranean Sea (Fig. 4; see also Guiry & Guiry 2016; Manghisi *et al*. 2010a); it is a sciaphilous species, found throughout the year on rocky substrata from depths of 0–50 m. Tetrasporophytes were collected in spring and autumn (Table 1).

Taxonomic history

In the year following Meneghini's (Savi 1841) proposal of *Chondrus? vidovichii* from Dalmatia [collected by Vidovich, *fide* Zanardini (1868)], J. Agardh (1842) described *Cryptonemia dichotoma* from collections near Nice. Zanardini (1843) soon after regarded *Chondrus? vidovichii* and *Cryptonemia dichotoma* as conspecific and a true member of *Cryptonemia*, creating *Cryptonemia vidovichii* based on Meneghini's prior naming. Apparently disregarding Zanardini's (1843) proposals, Kützing (1849) transferred *C. dichotoma* to his own new genus *Euhymenia*, which is now regarded as synonymous with *Kallymenia*

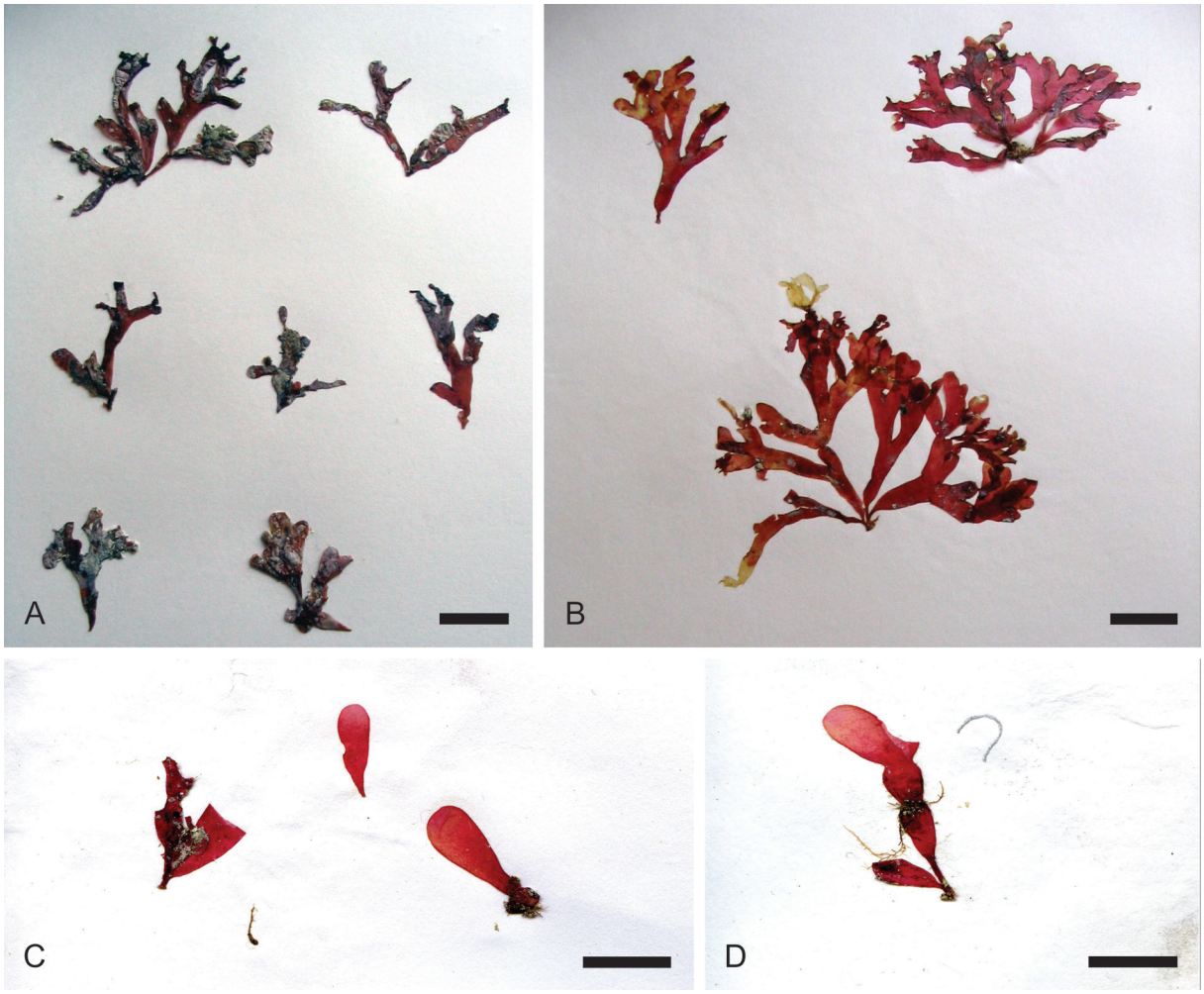

Fig. 1. A–B. Herbarium sheets MS35027-1 and MS35027-14, respectively, both from Sicily. **C–D**. Herbarium sheets PC0152091 (LLG0393) and PC0152089 (LLG0394), respectively, both from Vis Island, Croatia. Scale bars: A–B = 2 cm; C–D = 1 cm.

(Guiry & Guiry 2016). Next, J. Agardh (1851), ignoring Kützing's *Euhymenia*, put *Cryptonemia vidovichii* into his newly proposed section *Acrodiscus* of *Cryptonemia*, along with *Phyllophora crenulata* J.Agardh and *C. denticulata* J.Agardh, on the base of their morphology and the presence of subapical tetrasorangial sori. Kützing (1867) then made Meneghini's species a variety of J. Agardh's *Cryptonemia dichotoma*, thus not acknowledging the synonymy of the two as had been advocated by Zanardini (1843). Soon afterwards, Zanardini (1868) removed *Cryptonemia vidovichii* (which he still regarded as synonymous with *C. dichotoma*) from the genus *Cryptonemia* and placed it into a newly created one, *Acrodiscus*, named after the section made by J. Agardh. De Toni (1905) transferred J. Agardh's *C. crenulata* and *C. denticulata* to *Acrodiscus* (as *A. crenulatus* (J.Agardh) De Toni and *A. denticulatus* (J.Agardh) De Toni), although both are currently regarded as genuine species of *Cryptonemia* (Guiry & Guiry 2016).

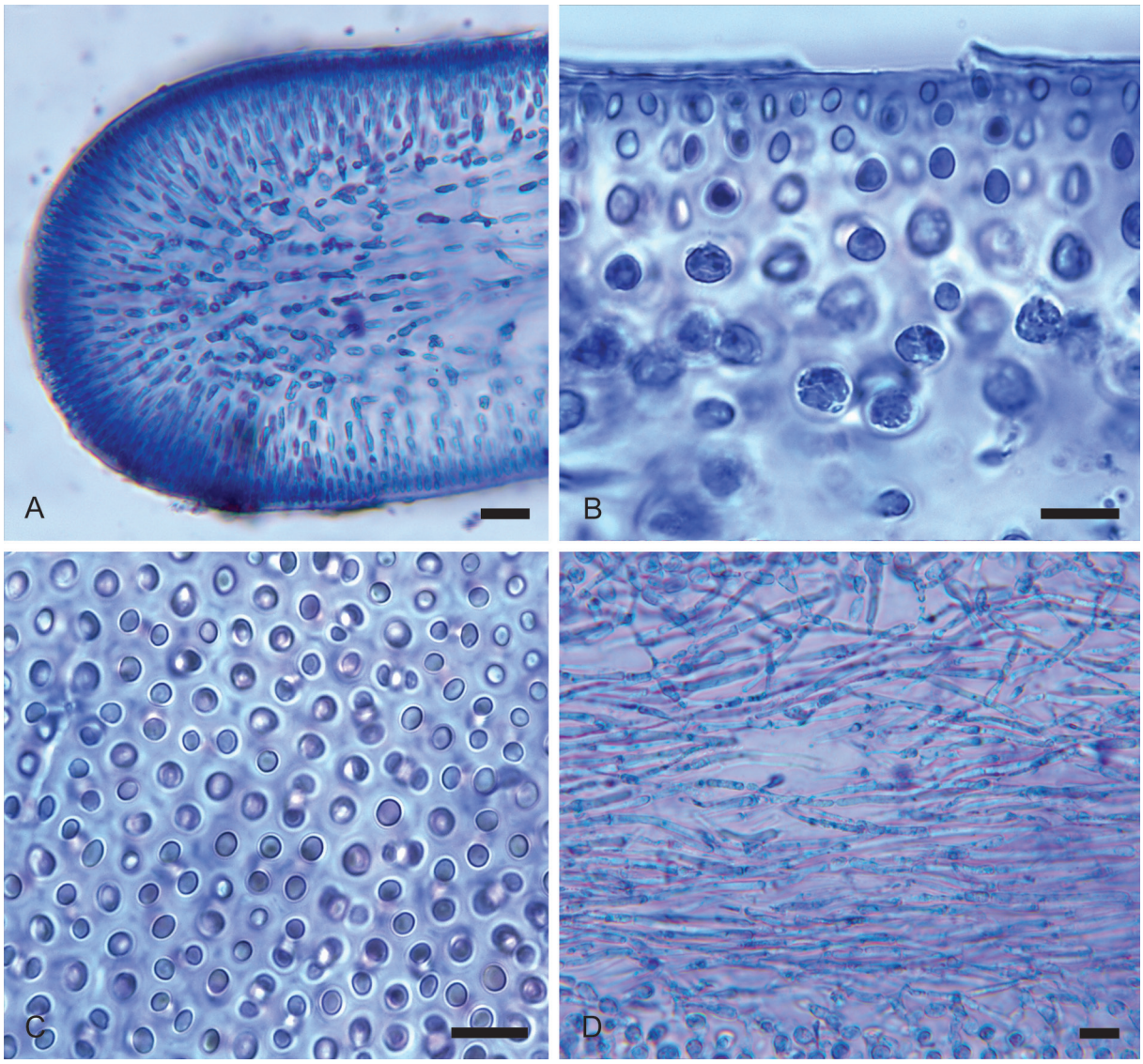

Fig. 2. Analine-blue stained vegetative features. **A**. A transapical longitudinal section indicative of its multiaxial structure. **B**. The anticlinal orientation of pseudo-dichotomous cortical filaments. **C**. Mostly paired cortical cells in surface view. **D**. Longitudinally aligned, peripherally inverwoven medullary filaments. Scale bars: A, D = 20 μm; B–C = 10 μm.

In 1949, Ercegović proposed *Acrodiscus vidovichii* f. *cochlearis*, arguing that the specimens from Dalmatia (Croatia) had "spoon-shaped", rather than the flattened fronds described by several other workers (Ardissone 1883; Hauck 1885; Preda 1908; Zanardini 1868). Ercegović overlooked, however, the fact that populations from throughout the Mediterranean had been described as both compressed-flat or plane (Ardissone 1883; Hauck 1885; Kylin 1956; Savi 1841; Preda 1908; Zanardini 1868) and with bent/curved sub-grooved margins (Agardh 1842; Aleem 1993; De Toni 1905; Feldmann 1939). In our experience, freshly collected specimens normally have bent/curved margins but flatten once pressed on herbarium sheets; younger and thinner specimens, on the other hand, can be planar throughout and usually adhere to paper, whereas older, more coriaceous specimens may remain canaliculate and non-adherent. We therefore find little reason to recognize a separate forma *cochlearis*.

Lectotypification

Chondrus? vidovichii Menegh. was validly published in what is commonly reported as Menghini's *Algologia Dalmatica* (Guiry & Guiry 2016) in *Atti della terza Riunione degli Scienziati italiani tenuta in*

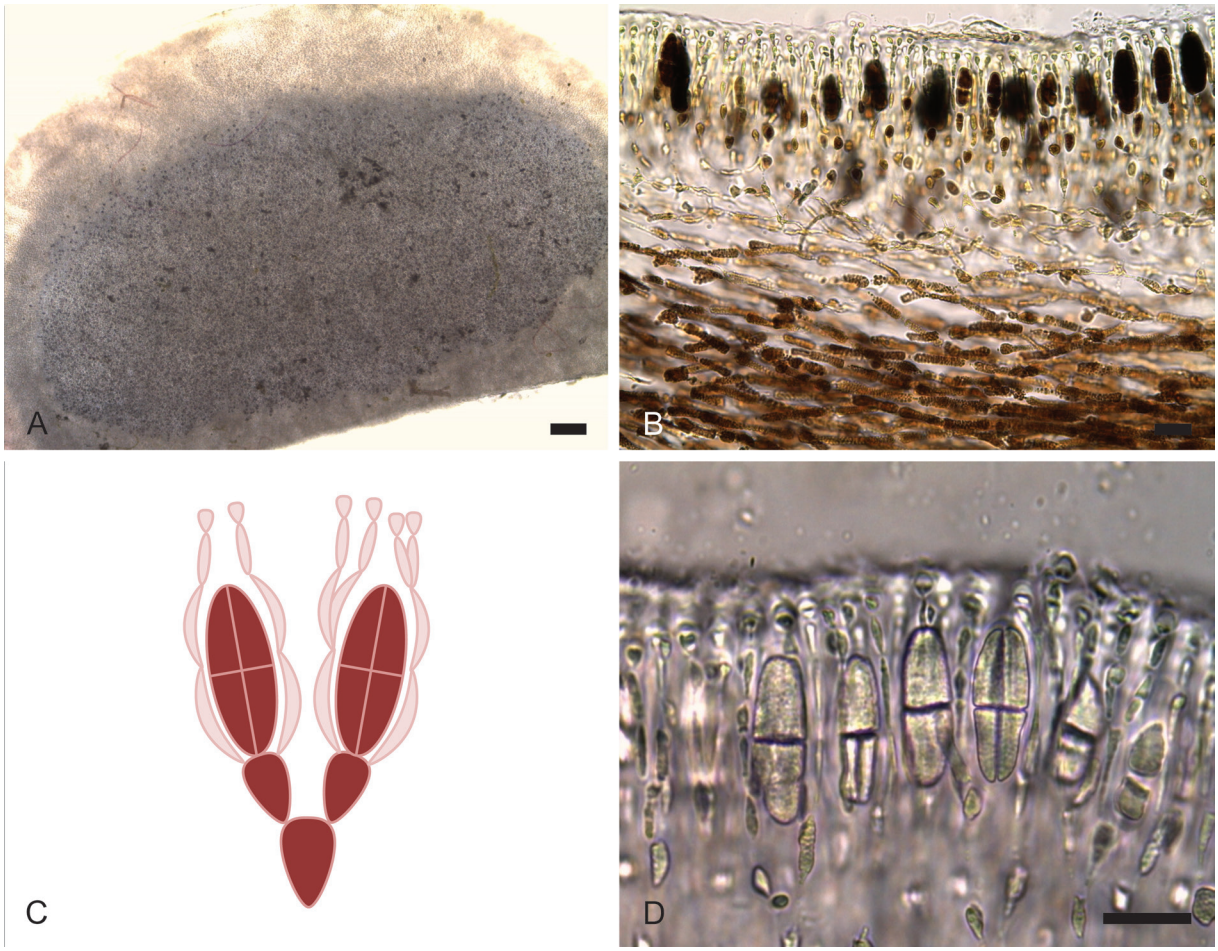

Fig. 3. A. Elliptical outline of a subapical nemathecial sorus. **B**. Lugol-stained l.s. of a tetrasporangial nemathecium. **C**. Schematic drawing of terminal tetrasporangia and jacketing nemathecial filaments. **D**. Horizontally aligned cruciate and decussate-cruciate tetrasporangia within the palisade of nemathecial filaments. Scale bars: A = 200 μm; B, D = 20 μm.

Firenze nel Settembre 1841 (Savi 1841). Indeed, the latter is a congress acta in the form of a book with various sections. During the meeting of the Botany and Plant Physiology group, Meneghini showed his manuscript to the assembly. The secretary of the group, Pietro Savi, recorded the meeting events in the acta, and transcribed part of Meneghini's manuscript, reportedly titled *Algologia Dalmatica*, including its novelties. With specific reference to *Chondrus? vidovichii*, Savi copied the Latin diagnosis and noted the lack of reproductive structures, and made reference to an illustration that does not appear in the acta. No holotype is designated, nor is there an iconotype that might serve as one.

In the Library of Natural and Environmental Sciences of the University of Pisa, Italy, we found numerous manuscript documents belonging to Meneghini, among them the original complete manuscript of the so-called *Algologia Dalmatica,* actually *Alghe Dalmate, enumerate ed illustrate dal professor Giuseppe Meneghini* (Fig. 5 A–B), along with plates including his illustration of *Chondrus? vidovichii* (Fig 6). Unfortunately, the manuscript was never published.

Interestingly, in other documents there is evidence that: a) Meneghini received material from Dalmatia (Croatia) collected either by Vidovich in Sebenico (Šibenik), by Sandri in Zara (Zadar), or by Stalio in Spalato (Split); b) Menegnini received material from Vidovich in July 1841 (including a *Chondrus?*); and c) Meneghini dedicated to Vidovich all the new species collected by him. Consequently, it can be inferred that the type material should have been collected by Vidovich in Sebenico in July 1841.

Finally, we found that the *Herbarium Horti Botanici Pisani* (PI) holds a number of Meneghini specimens. In a folder labelled "*742. 4. Euhymenia dichotoma Kg.*" is an envelope and three sheets numbered "11". The envelope contains ten specimens of *A. vidovichii*, three of them on numbered sheets (224, 778, 783). Two of the three sheets (upper and lower left) each have a fragment of the specimen drawn in the abovementioned plate (Fig. 7). Consequently, we designate as type material of *Chondrus? vidovichii*

Fig. 4. Recorded sites of *Acrodiscus vidovichii* (Menegh.) Zanardini along the shores of the Mediterranean Sea.

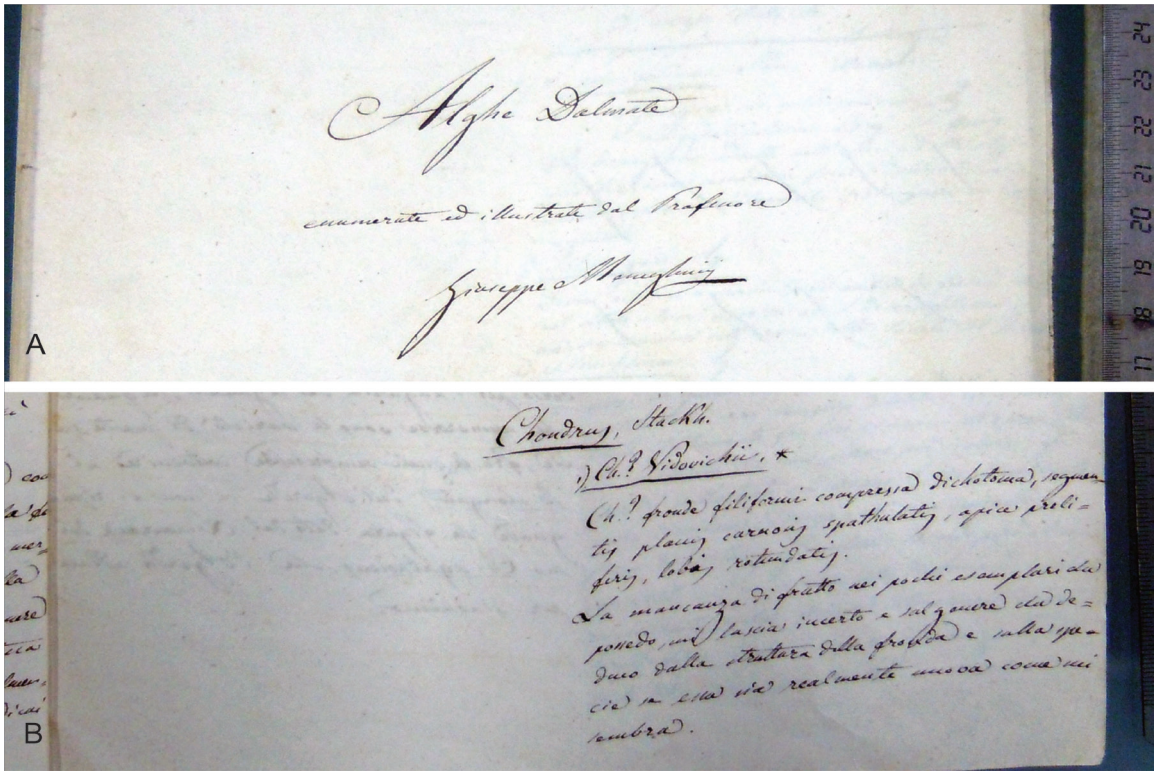

Fig. 5. A. Detail of front page of Meneghini's 1841 manuscript "*Alghe Dalmate, Enumerate ed Illustrate dal Professor Giuseppe Meneghini*". **B**. Detail of protologue page of *Chondrus? vidovichii* (Menegh.) Zanardini.

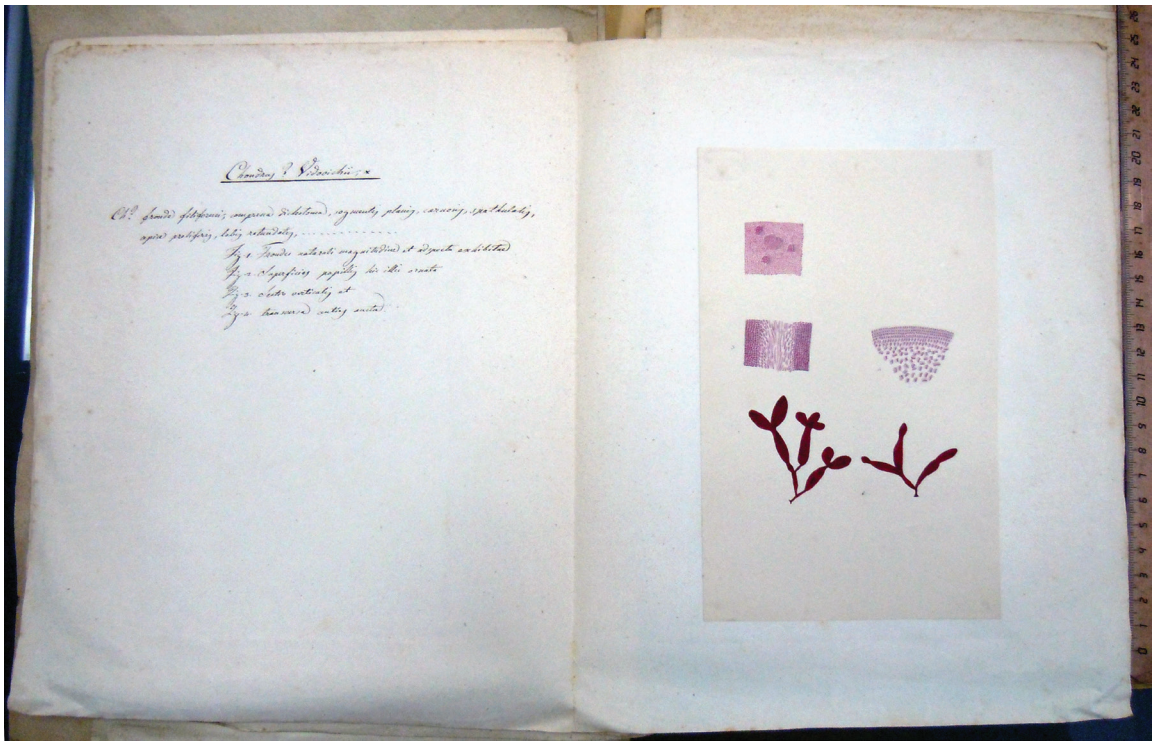

Fig. 6. Plate and caption of *Chondrus? vidovichii* (Menegh.) Zanardini from Meneghini's manuscript "*Alghe Dalmate, Enumerate ed Illustrate dal Professor Giuseppe Meneghini*".

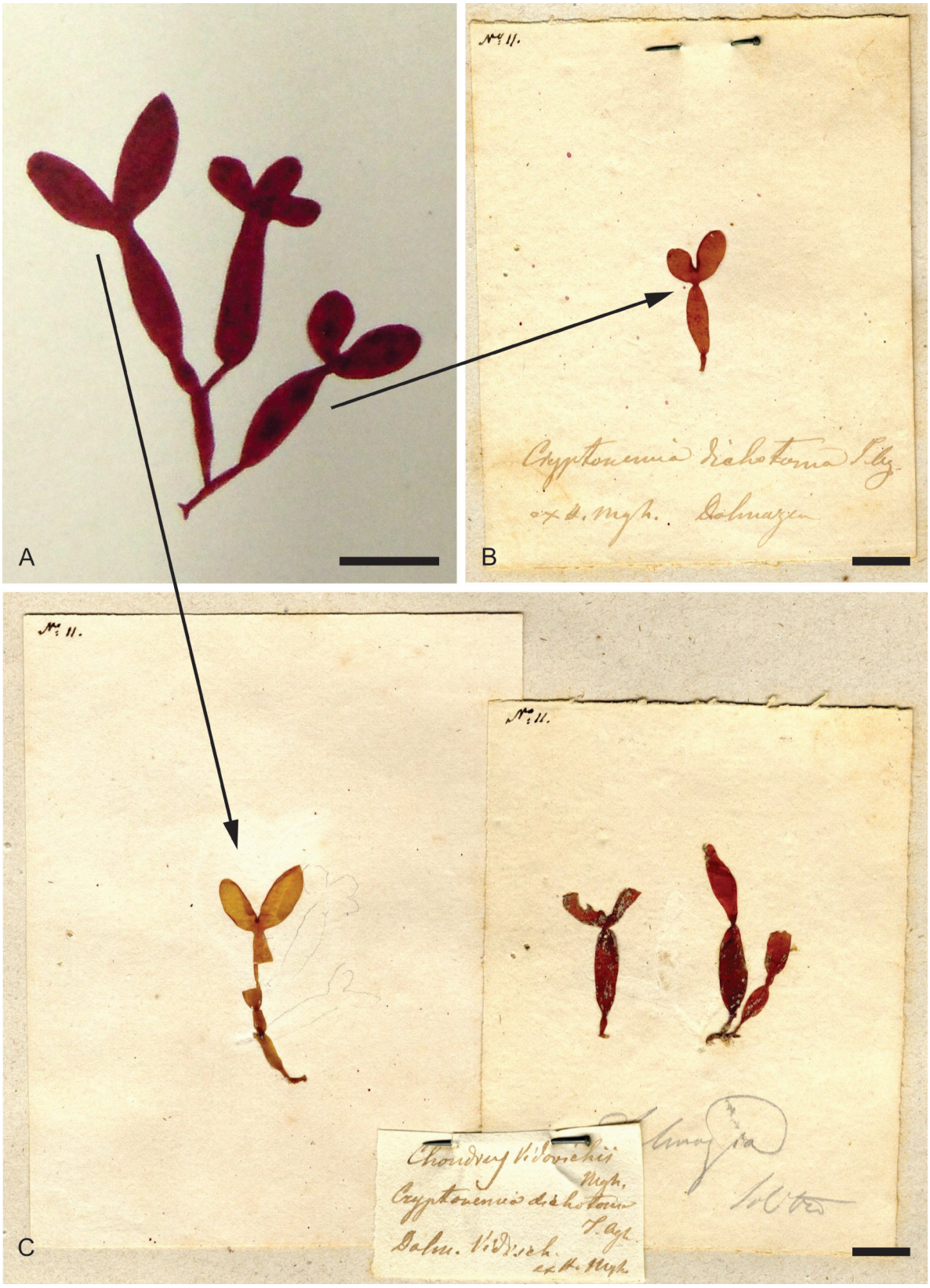

Fig. 7. Two fragments (**B–C**, arrows) of the specimen (**A**) of *Chondrus? vidovichii* (Menegh.) Zanardini illustrated in Meneghini's protologue manuscript. The sheets are currently filed in folder *742.4*, labelled "*Euhymenia dichotoma Kg*." at the *Herbarium Orti Botanici Pisani* (PI). Scale bars = 1 cm.

Meneghini the two fragments on two of the "11" sheets that were portions of the single specimen he illustrated in the unpublished figure accompanying his manuscript.

Phylogenetic analyses

The DNA barcode region was generated for 15 samples from different Mediterranean localities, including the type area; the sequences are now lodged in BOLD and Genbank (Table 1). Divergence among generated sequences ranged from 0–3 bp (0–0.53%), which is a typical level of within-species variation.

Phylogenetic analyses inferred from both *rbc*L and LSU markers (Fig. 8 and trees not shown) resolved three strongly supported supergeneric lineages within the Halymeniales: a) one of *A. nitidissima* J.Agardh and species of *Pachymenia* J.Agardh; b) a second comprised of *Polyopes* J.Agardh and *Glaphyrosiphon intestinalis* (Harv.) Leister & W.A.Nelson; and c) a third consisting of *Grateloupia* C.Agardh, *Yonagunia* Kawag. & Masuda, *Pachymeniopsis* Yamada ex Kawab., *Prionitis* J.Agardh, *Phyllymenia* J.Agardh, *Mariaramirezia* M.S.Calderon, G.H.Boo, A.Mansilla & S.M.Boo, *Kintokiocolax* Tak.Tanaka & Nozawa and *Dermocorynus* P.Crouan & H.Crouan. The relationships among the remaining halymeniacean genera included in our analyses were poorly or not resolved. *Cryptonemia* J.Agardh was polyphyletic and *Thamnoclonium* Kützing was paraphyletic in *rbc*L analyses, and the genus *Halymenia* C.Agardh was polyphyletic in both *rbc*L and LSU trees.

The exact alliance of *Acrodiscus* was uncertain, as it varied depending on the phylogenetic reconstruction methods and the marker. In *rbc*L analyses, *A. vidovichii* was included in an unsupported lineage encompassing *Felicinia marginata* (Roussel) Manghisi, L.Le Gall, Ribera, Gargiulo & Morabito and *Corynomorpha prismatica* (J.Agardh) J.Agardh. This assemblage in turn grouped with particular, especially type, species of *Halymenia*, *Cryptonemia*, *Carpopeltis* F.Schmitz, *Codiophyllum* J.E.Gray, *Spongophloea* Huisman, De Clerck, Prud'homme & Borow., *Thamnoclonium* Kütz., *Epiphloea* J.Agardh and *Gelinaria* Sond. In LSU trees, *Acrodiscus* grouped with *Corynomorpha* and species of *Pachymeniopsis*, *Dermocorynus*, *Grateloupia* and *Prionitis*. In concatenate LSU-*rbc*L analyses (Fig. 8), it was sister to *Corynomorpha* with variable degrees of support, both genera being included in a deeper lineage encompassing *Felicinia* Manghisi, L.Le Gall, Ribera, Gargiulo & Morabito, *Halymenia*, *Cryptonemia*, *Gelinaria*, *Epiphloea* and *Isabbottia* M.S.Balakr.

Discussion

Our molecular analyses have highlighted the well-known fact that some genera within the Halymeniaceae are not monophyletic assemblages, with various tree topologies for the family as a whole depending both on taxon sampling and phylogenetic signals of the markers (Fig 8). None of our analyses conclusively resolved the family position of *Acrodiscus* Zanardini*,* as its alliances changed depending on the reconstruction method.

The three supergeneric lineages within the Halymeniaceae emerging from our phylogenies have already been found in previous works (Manghisi *et al.* 2014; Mineur *et al.* 2010; Nelson *et al.* 2014), but morphological/anatomical characteristics unifying the members of the three lineages can still not be precisely specified. The relationships among the several remaining genera represented in our analyses were poorly or not at all resolved, the polyphyly of some of them, such as *Halymenia*, *Thamnoclonium* and *Cryptonemia,* being clearly indicated. Studies such as those recently initiated for the complex that includes *Grateloupia*, *Pachymeniopsis*, *Yonagunia* and related genera (Calderon *et al.* 2014; Gargiulo *et al.* 2013) are needed before the generic relationships for the whole of the family Halymeniaceae can be satisfactorily resolved.

The anatomy of *Acrodiscus* is typically halymeniaceous, although in the absence of molecular data this evidence would not be definitive for family placement. Gametangial and gonimoblast structures

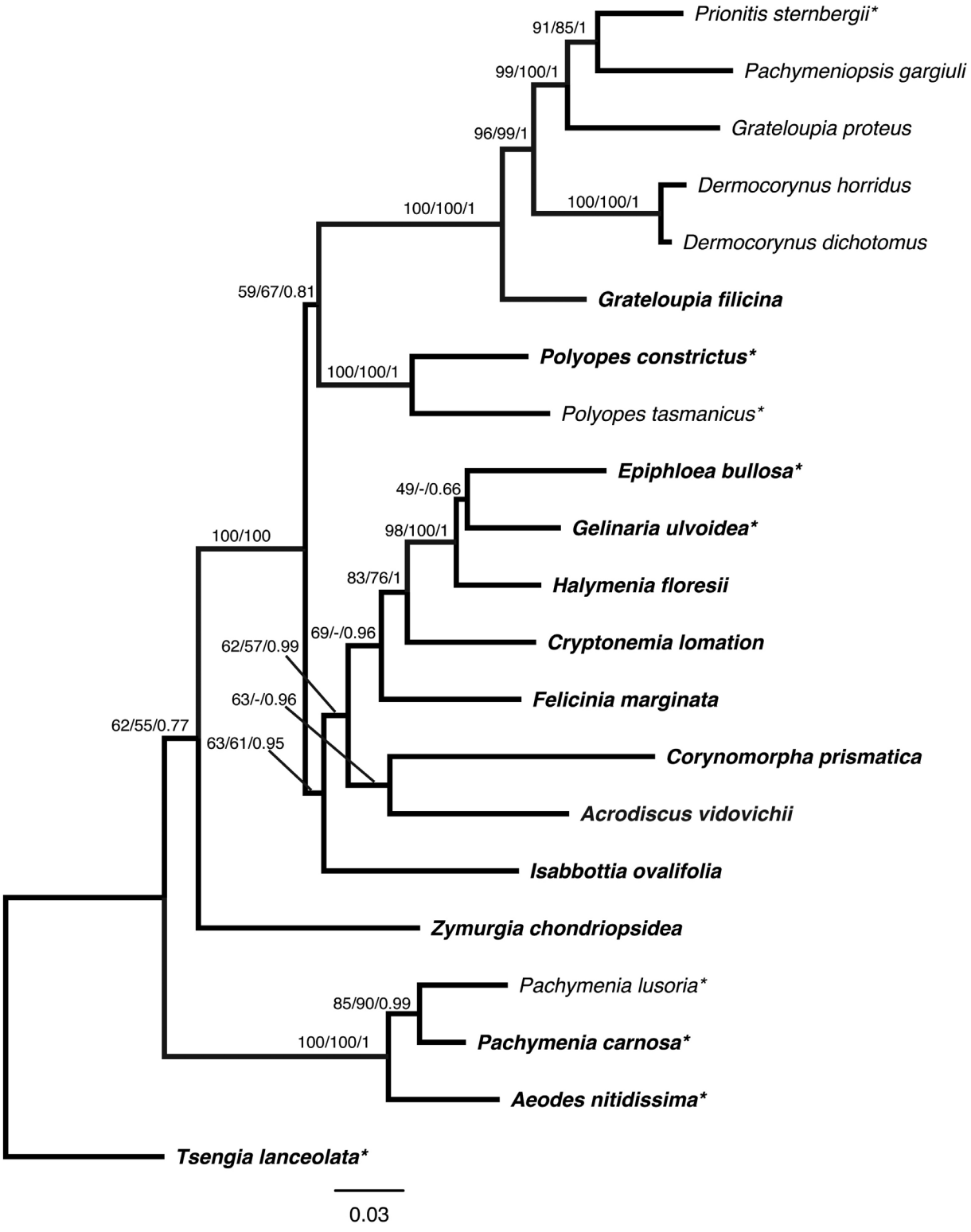

Fig. 8. ML phylogram inferred from the combined LSU-*rbc*L data by PhyML. Supports at nodes indicate bootstrap values (from PhyML and RaxML) and posterior probabilities inferred from Bayesian analysis. Generitype species are indicated in bold; concatenate sequences obtained from different samples for LSU and *rbc*L are indicated by "*".

remain unknown despite extensive herbarium collections and our examination of some 44 specimens, although the discovery of nemathecial tetrasporangia is a strong indication that gametophytes exist in some form yet to be discovered. Should they prove to be heteromorphic or cryptic stages, this would be a first for a large family in which the members otherwise uniform in displaying isomorphic alternations of generations.

Although Schmitz (1889) provisionally included *Acrodiscus* in the genus *Polyopes*, the molecular data show that the two genera are not closely related. The grouping of *Acrodiscus* with the tropical genus *Corynomorpha* is not robust in our phylogenies, nor do habit and morphologies suggest a natural alliance. Nevertheless, this unlikely relationship should be further tested, as should a possible association with *Felicinia* and sistership with the *Cryptonemia*/*Halymenia* clade. For the moment we conclude on morphological and anatomical, as well and particularly on molecular, grounds that *Acrodiscus* is unquestionably an independent genus of the Halymeniaceae. The two poorly known species *Cryptonemia crenulata* and *C. denticulata*, both described by J. Agardh (1851) as having subapical sori of tetrasporangia similar to those of *Acrodiscus*, should be investigated as possible additional members of this presently monotypic genus.

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Appendix

Description of the historical herbarium material studied for the lectotypification of *Chrondrus ? vidovichii* Menegh.

Koster (1969) suggested that the Meneghini collection might be in Florence (FI) or in Kützing's collections, which are in Leiden (L) or London (BM). Therefore, the Herbarium Universitatis Florentinae was searched for the type of Meneghini, and we found that sheet FI 4796-1 holds five sub-sheets, each with a specimens of *Acrodiscus vidovichii*, without locality, date or collector. Four of them are from Herbarium Meneghini; the fifth one is mounted on a slide and accompanied by a manuscript annotation "*Chondrus vidovichii col frutto, un pezzo del quale si staccò dalla cima e lo comprendo. – Sentirò con piacere che a quest'alga ha levato per sempre il punto interrogativo*". ["*Chondrus vidovichii* with fruit, a fragment of which detached from the tip and I include it. – I will hear with pleasure that you have removed the question mark from this alga for ever".]

The manuscript annotation has been compared to Meneghini manuscript documents, and the writing is different. The annotation is from someone sending a fertile specimen to Meneghini and hoping he will eliminate the question mark from *Chondrus ? vidovichii*. The fact that the specimen is fertile is actually an argument to posit that it cannot belong to Meneghini's original material at the time he described the species. For the remaining specimens there is no clear evidence of their being paratypes.

From the Nationaal Herbarium Nederland (L) we received on loan three herbarium sheets of *A. vidovichii*. L 0833934 belonged to Herbarium Suringar and has a single specimen with the manuscript label "*Cryptopleura vidovichii* Dalmazia!", but without mention of either a date or a collector. L 0833935 belonged to Herbarium Kützing and has two specimens, with the manuscript label "*Cryptonemia vidovichii*, * *Chondrus vidovichii*, Menegh. 1841 *Cryptonemia dichotoma*, J. Ag. 1842", the collector/sender is Meneghini and the locality is Dalmatia; nevertheless, there is no mention of a date. The two specimens were drawn by Kützing in his *Tabulae Phycologicae* vol. 17 (Kützing 1867: tav. 72, fig. d–e). Kützing in his *Species Algarum* (1849: 742) wrote "*specim. dedit amic.* Menghini", maybe in reference to these specimens. L 0833936 consists of two sheets. The first one holds a specimen and fragment in a small envelope, with the manuscript label "*Acrodiscus vidovichii* Dalmazia lg. Vidovich", but without a date. The second sheet holds a single specimen, with the manuscript label "*Acrodiscus vidovichii* lg. Vidovich, com. Zanardini Dalmazien", and without date, but has another annotation in the upper part referring to Zanardini's *Iconographia Phycologica Adriatica* tv. LXIX, fig. 3. In fact, the specimen corresponds to the one drawn in that figure.

In the Herbarium of the Natural History Museum of London (BM) we found the herbarium sheet BM000619430, with the manuscript label "*Cryptonemia dichotoma* J. Agardh, 1841 Nizza J. Agardh" (ex Herbarium Griffiths), which is original material of *Cryptonemia dichotoma* J. Agardh 1842. Also BM000563717 and BM000563718 are both from Nizza and ex Herbarium J.G. Agardh, but without a date. Another interesting sheet is BM000569934, with the manuscript label "*Acrodiscus vidovichii*, Dalmazien, lg. Vidovich" (ex Herb. Weber van Bosse), because the specimen was collected at the type locality (topotype) by Vidovich, the same collector that sent material to Meneghini.

Unable to find suitable material for the lectotypification in the herbaria suggested by Koster (1969), we extended our search to other herbaria. In the Muséum national d'Histoire naturelle de Paris (PC) there are three interesting specimens/sheets. The first one, PC0047928 (MA2052), is labelled "*Cryptonemia vidovichii* Zan. (*Chondrus ? vidovichii* Menegh., *Cryptonemia dichotoma* J. Ag.) Meneghini – Dalmazia"; thus, it belonged to Meneghini and was collected in Dalmatia, but there is no reference to the date of collection. The second one, PC0523534 (AR24258), is labelled "*Cryptonemia dichotoma* J Ag. , Nizza –

J. Agardh 1840"; it is original material of *Cryptonemia dichotoma* J.Agardh. The third one, PC0474569, is labelled *Acrodiscus vidovichii*, Zanard. and belonged to Herbarium Zanardini.

We also searched in the Natural History Museum of Venice (MCVE), in the Herbarium Zanardini, where we found two sheets, four envelopes and two hand-drawn tables. The first sheet has two specimens of *A. vidovichii* from the Gulf of Naples, collected by Pedicino, while the second sheet has a specimen in two pieces/fragments from Dalmatia, with no collector cited. The first envelope on the left holds various small specimens. The second envelope, above in the middle, has a hand-written notation *(con fruttificazione)* and an unclear name, maybe the collector. It holds three small fertile specimens. The third envelope, below in the middle, is marked "*Chondrus vidovichii* Menegh." and holds a single specimen. The fourth envelope, on the right, is marked "*Chryptonemia vidovichii, Z.*" and holds four small specimens. The two hand-drawn tables correspond to table VIII published in Zanardini (Zanardini 1868) or LXIX in (Zanardini 1871). Note that the drawn specimen n. 4 corresponds to the one in two fragments from Dalmatia.

Subsequently, following Meneghini's biography we also searched in Padua, where he taught until 1848, and then in Pisa, where he resided and taught after 1849.

The *Herbarium Patavinum* (PAD) of Padua, Italy, holds a small specimen of *A. vidovichii* in an envelope (PAD A00389). The envelope recites "*Chondrus vidovichii Mgh! Dalmatia leg. Vidovich ex Herb Mgh! Beccari*". However, lacking a date, it is not a good candidate for type material.

All the above-mentioned herbarium material is listed in Table 2.

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