

The morphology and reproductive structures of Mediterranean species of the genus *Nemastoma* J. Agardh, nom. cons. (Nemastomataceae, Nemastomatales): *Nemastoma dichotomum* and *N. dumontioides*

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Abstract

We investigated a collection of *Nemastoma* J. Agardh, nom. cons. (Nemastomatales, Rhodophyta), including the Mediterranean type material of species described by J. Agardh, Kützing and Ercegovic, and recent specimens from the Mediterranean Sea, Adriatic Sea and northeastern Atlantic Ocean. Based on their habit and vegetative and reproductive structures, we confirm that: (i) all the Mediterranean specimens studied belong to the genus *Nemastoma*; (ii) two species of *Nemastoma* co-exist in the Mediterranean Sea: (a) *N. dichotomum*, which is characterized by great plasticity of habit, a cartilaginous thallus in its basal and middle parts, and presumed reproduction exclusively by apomixis; and (b) *N. dumontioides*, which is characterized by a regularly pseudodichotomous, saccate-membranous thallus, and which reproduces both sexually and probably apomictically; and (iii) the Atlantic specimens attributed to *N. dichotomum* belong to other genera, and so, *N. dichotomum* is probably endemic to the Mediterranean Sea. Thus, three species described by Kützing have been reduced to varieties of *Nemastoma dichotomum*: (a) var. *biasolettianum* (Kützing) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov., with ligulate upper parts, (b) var. *caulescens* (Kützing) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov., with wedge-shaped upper parts, and (c) var. *incrassatum* (Kützing) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov., with spatulate upper parts. Finally, *Nemastoma constrictum* and *N. constrictum* var. *longitrichogynum* are placed in synonymy with *N. dichotomum* var. *caulescens*.

Keywords: Mediterranean Sea; morphology; *Nemastoma*; Nemastomataceae; reproductive structures.

Introduction

The genus *Nemastoma* J. Agardh nom. cons. (Nemastomataceae, Nemastomatales) includes less than 10 spe-

cies distributed in the tropical, subtropical and warm temperate waters of both hemispheres (G.T. Kraft pers. comm.). In the Mediterranean Sea, it is currently represented by *N. dichotomum* J. Agardh and three other species of uncertain taxonomic position, *N. dumontioides* J. Agardh, *N. constrictum* Ercegovic and *N. inconspicuum* Reinsch (Furnari et al. 1999, Athanasiadis 2002, Guiry and Nic Dhonncha 2003).

Agardh (1842: 89–90; as *N. dichotoma*) first described *Nemastoma dichotomum*, the type species of the genus (Silva 1980), from Trieste (Italy, Adriatic Sea) and Nice (France, western Mediterranean Sea). Of the original material, only that from the Adriatic Sea remains in the Herbarium of the Botanical Museum, Lund, Sweden (LD). In the absence of a holotype indicated by Agardh, one specimen (LD 22089) was selected by P. Robins (27 November 1984) as a lectotype, the other specimens being labeled as syntypes (P. Robins, msc.) (see Athanasiadis 1988, 1996). Kützing (1843: 390, pl. 74, figs iv 1–2) described *Gymnophlaea dichotoma* Kützing from Trieste, a taxon later considered conspecific with *N. dichotomum* by Kützing himself (1849), Agardh (1851), Berthold (1884) and Kylin (1932), and distinguished some particular morphological variations of *N. dichotomum* at a species or sub-species level: *G. incrassata* Kützing (1843: 390), *G. biasoletiana* Kützing (1843: 390–391), *G. dichotoma* var. *tenuis* Kützing (1866: 21, pl. 58) and *G. caulescens* Kützing (1866: 22, pl. 61). He also described a further species as *Ginnania irregularis* Kützing (1845: 299), later reduced to synonymy with *N. dichotomum* by G.B. De Toni (1905: 1662). The generic name *Ginnania*, which was proposed by Montagne (1839–1841), is a taxonomic synonym of *Scinaia* Bivona-Bernardi. It was based on *G. furcellata* (Turner) Montagne (basonym: *Ulva furcellata* Turner 1801), a synonym of *Scinaia furcellata* (Turner) J. Agardh. Because of the wide morphological diversity of all these taxa, Athanasiadis (1988) considered that, until a critical comparative study of the types or new collections from the type localities had been carried out, the relationship of *N. dichotomum* to these taxa described by Kützing would remain uncertain.

Some years after Kützing made his observations, Agardh (1851, 1876) considered *G. biasoletiana* and *G. incrassata* as synonyms of *N. dichotomum* and described a new Mediterranean species of *Nemastoma*, *N. dumontioides* J. Agardh (1851: 164), on the basis of a single putatively sterile specimen collected at Marseille (France). He characterized it by its saccate-membranous, *Halymenia*-like thallus which closely adhered to paper, in contrast to *N. dichotomum* which possesses a partly cartilaginous and less adherent thallus. According to Athan-

asiadis (1988), an examination of the holotype confirmed that the vegetative structure of the species is similar to *N. dichotomum*, although, in the absence of reproductive organs, its identity remains obscure. *Nemastoma dumontioides* is not mentioned in AlgaeBase (Guiry and Nic Dhonncha 2003).

In 1875, Reinsch described another species from the Adriatic Sea, *Nemastoma inconspicuum* Reinsch (1875: 55: pl. xli, figs 1A–D; as *N. inconspicua*). According to the original description, the species grew epiphytically on *Hypnea musciformis* (Wulfen in Jacquin) J.V. Lamouroux, was 2–3 mm high and produced zonately divided tetrasporangia. This species has never been found subsequently. G.B. De Toni (1905: 1668) placed it in *taxa excludenda*, and Athanasiadis (2002) considered it to be of uncertain taxonomic position, mentioning that the occurrence of zonately divided tetrasporangia is the only reference of such structures in the genus.

Ercegovic described *Nemastoma constrictum* Ercegovic (1949: 40–44, figs 19–20; as *N. constricta*) from the Kornati Islands (Croatia, Adriatic Sea), which differs from *N. dichotomum* mainly by the presence of intercalary constrictions, situated at 0.5 or 1.0 cm below the bifurcations of the thallus, and by the 2-celled carpogonial branches inserted subapically on cortical fascicles. In *N. dichotomum*, no constrictions are reported and the carpogonial branch is usually 3-celled and situated laterally on rhizoidal adventitious filaments (Berthold 1884, Athanasiadis 1988). Ercegovic also described a variety of *N. constrictum* from the Adriatic Sea, *N. constrictum* var. *longitrichogynum* Ercegovic (1949: 44–47, figs 21–22; as *N. constricta* var. *longitrichogyna*), which differs from *N. constrictum* mainly by the presence of longer trichogynes (up to 1000 µm in length). This latter variety has not been found again since its description, whereas *N. constrictum* is recorded from a later Adriatic collection by Ercegovic (1963).

Kylin (1956: 248) considered the absence of gland cells as a distinctive generic feature of the genus *Nemastoma*, although he acknowledged the occurrence of terminal gland cells in *N. dumontioides* (Kylin 1932). However, gland cells in *Nemastoma dichotomum* had been described and illustrated by Agardh (1879: 109, pl. iv, fig. 5) and their occurrence has been confirmed in all the confirmed species of *Nemastoma* (Reinsch 1875, Feldmann 1942, Ercegovic 1949, Ardré 1980, Athanasiadis 1988, Masuda and Guiry 1994, 1995).

Current knowledge of the life history and reproductive structures of the Mediterranean *Nemastoma* is limited. Kützing (1843: pl. 74, fig. iv–2) illustrated carposporophyte-like structures ('Kapselfrucht') in *Gymnophlaea dichotoma* Kützing, but the first detailed description and illustration of the female reproductive structures and development of the carposporophyte were made from Gulf of Naples specimens by Berthold (1884; as *G. dichotoma* Kützing). Berthold described carpogonial branches, auxiliary cells and the development of gonimoblasts on connecting filaments at a point close to the union of the connecting filament with the auxiliary cell (Berthold 1884, Oltmanns 1904, Kylin 1932). In the Adriatic Sea and in the Mediterranean Sea up to the present day, the only specimens (including the lectotype LD

22089) that have been reported as fertile, lack connecting filaments and have gonimoblasts that develop directly on the auxiliary cells (Athanasiadis 1988; as *Nemastoma dichotomum*) as a result of presumed apomixis. Male reproductive structures have never been described, although the syntypes LD 22088 and LD 22090 have been labeled as male gametophytes by Robins (msc. in Agardh Herbarium, LD). A filamentous stage reproducing by monospores has been observed in cultures of carpospores from plants of unknown origin (Cortel-Breeman and van den Hoek 1970, van den Hoek et al. 1972). Reinsch (1875: pl. xli, figs 2A, 2B; as *Nemastoma dichotoma*) described 'monospores' in *N. dichotomum*, but it appears on closer inspection of his figures that this author has confused gland cells with monosporangia.

The present paper aims at clarifying and describing in detail the morphology and the reproductive processes of the taxa of *Nemastoma* that occur in the Mediterranean Sea. We have studied an extensive collection of fresh and herbarium specimens from the Mediterranean Sea and elsewhere, including the type material of the species described by J. Agardh, Kützing and Ercegovic. This has allowed us to examine the vegetative and reproductive features of each species, enabling us to evaluate the range of variation and to select those characteristics that are most useful for species delineation.

Materials and methods

The following type materials and other collections were obtained on loan from LD and the Rijksherbarium, Leiden, The Netherlands (L):

- *Ginnania irregularis* Kützing: L 0487962, holotype, Trieste, Italy;
- *Gymnophlaea biasoletiana* Kützing: L 0487960, holotype, Trieste, Italy;
- *G. caulescens* Kützing: L 0487961, syntype, Pirano, Trieste, Italy;
- *G. dichotoma* var. *tenuis* Kützing: L 0487959, syntype, Trieste, Italy;
- *G. incrassata* Kützing: L 0487958, Trieste, Italy, October 1889, specimen collected by Kützing; the holotype is lacking in the Rijksherbarium, Leiden;
- *Nemastoma dichotomum* J. Agardh: LD 22089, lectotype, Trieste, Italy; syntypes: LD 22087, LD 22088, LD 22090, LD 22091, LD 22092, Trieste, Italy;
- *Nemastoma dumontioides* J. Agardh: LD 22078, holotype, Marseille, France.

Unfortunately, we failed to locate the holotypes of *Gymnophlaea dichotoma* Kützing and *Nemastoma inconspicuum* Reinsch. For the latter, the original drawing of the illustration published by Reinsch (1875) is the only document preserved in the Herbarium of the Naturhistorisches Museum, Vienna, Austria.

We also failed to locate the holotype of *Nemastoma constrictum* Ercegovic, although we did manage to obtain one specimen collected by Ercegovic in October 1938 at the type locality (Kornati Islands, Croatia, mid-Adriatic Sea) that is deposited in the J. Feldmann Herbarium, Muséum National d'Histoire Naturelle, France.

Loans also included specimens deposited in the Herbaria of the Universities of Trieste, Italy (TSB-2639, Trieste, Italy; TSB-2646, Elaphonesos Island, Pariki, Greece; TSB-2642, Istria, Croatia) and Catania, Italy (CAT-870, Augusta, Italy), and the Centre d'Océanologie de Marseille, France (H-3527, Monaco; H-2354, Corsica, France; H-2355, Corsica, France). A photograph of the specimen studied by Berthold (1884) (no. 001502, Berthold Herbarium, Stazione Zoologica di Napoli, Italy) was kindly sent by Professor Maria Cristina Buia.

Atlantic specimens from the north of Spain referred to *Nemastoma dichotomum* and deposited in the Rijksherbarium, Leiden (L 0487967–L 0487969, Punta Abeleira, Pontevedra, Spain; L 0487968, Santa Clara, San Sebastián, Spain, leg. H. Stegenga, M.V. Wissen, I. Mol, A. Mulder and A.V. Beem) were also studied.

Specimens of Mediterranean *Nemastoma* were collected by SCUBA diving at different localities and preserved in 4% formalin/seawater or dried as herbarium specimens, supplemented by some specimens kindly given to the authors by Dr E. Ballesteros. Liquid-preserved specimens and exsiccata have been deposited in the Phycological Herbarium of the University of Girona, section algae (HGI-A 2512, Corsica, France, 26.viii.1992; HGI-A 4783, Corsica, France, 9.x.2001; HGI-A 5200, HGI-A 5295, and HGI-A 5296, Ibiza, Spain, 26.v.2001; HGI-A 5315, Pollença, Spain; HGI-A 5688, HGI-A 5689, HGI-A 5690, HGI-A 5691, HGI-A 5692 and HGI-A 5693, Columbretes Islands, Spain). A summary of the material examined is provided in Table 1.

Finally, we also examined the following specimens of *Nemastoma canariense* (Kützinger) J. Agardh deposited in the Rijksherbarium, Leiden:

- L 0487955, leg. F.T. Kützinger, Canary Islands;
- L 0487956, leg. F.T. Kützinger, Canary Islands (?);
- L 0487963, Las Palmas, Canary Islands, 1.ii.1896, leg. A. Vickers;
- L 0487964 and L 0487965, Graciosa Island, Canary Islands, 28.iii.1983, leg. W.F. Prud'homme van Reine;
- L 0487966, Montaña Clara Island, Canary Islands, 31.iii.1983, leg. W.F. Prud'homme van Reine.

Herbarium and liquid-preserved specimens were studied by hand-sectioning with a razor blade and by squash preparations previously treated with acidified aniline blue (1–5%), a stain particularly effective for the examination of the reproductive structures of Nemastomataceae. Material was examined under a light microscope and features were drawn with a *camera lucida*. All morphological data are based on herbarium specimens. Habit photographs were taken with a Pentax Programa camera (objective Macro Sigma F28 of 50 mm) and the light photomicrographs with a Nikon F-601M camera.

Results

The morphology and the vegetative and reproductive structures of the Mediterranean specimens show that all of them belong to the genus *Nemastoma* J. Agardh. All are gametophytic and possess: (i) a multiaxial structure and complete lack of secondary pit-connections; (ii) a lax

filamentous medulla; (iii) a cortex of subdichotomous fascicles of cells that progressively decrease in size outwards; (iv) rhizoidal adventitious filaments produced by scattered inner cortical or medullary cells; and (v) terminal gland cells borne in the mid cortex. Fertile specimens have: (vi) a non-procarpic or a procarp-like (i.e., carpogonial branch and auxiliary cell borne on the same rhizoidal filament but carposporophytes develop on the auxiliary cell without any connection or fusion between auxiliary cell and neighboring carpogonia or other cells of the carpogonial branch) female structure; (vii) a simple 3-celled, occasionally 2- or 4-celled, carpogonial branch; (viii) rounded auxiliary cells intercalary in adventitious rhizoidal filaments and differentiating independently of fertilization; and (ix) connecting filaments (when present), multicellular, branched and able to effect successive diploidizations; (x) gonimoblast development laterally and more or less outwardly directed (Table 2). Based on habit and vegetative and reproductive characters, it was confirmed that at least two species of *Nemastoma* co-exist in the Mediterranean Sea: *N. dichotomum*, which is characterized by a high morphological plasticity, basal and middle parts cartilaginous and the plants presumably reproducing exclusively by apomixis, and *N. dumontioides*, which is characterized by a uniformly pseudodichotomous, saccate-membranous thallus and both sexual and presumably apomictic reproduction.

In comparison with the collection of *Nemastoma* from the western Mediterranean Sea, the specimens from the Adriatic Sea have a high morphological diversity. Further investigations are required to determine the degree of genetic isolation between these variable Adriatic phenotypes, but the lack of significant differences in vegetative and reproductive structures and the fact that most of the specimens are from the same general locality (Trieste region, northern Adriatic Sea) have led us to attribute them all provisionally to *N. dichotomum* and to propose that the three most distinctive forms described as separated species by Kützinger be reduced to varieties of *Nemastoma dichotomum*:

- *N. dichotomum* var. *biasolettianum* (Kützinger) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov.;
- *N. dichotomum* var. *caulescens* (Kützinger) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov.;
- *N. dichotomum* var. *incrassatum* (Kützinger) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov.

The loss of the holotype means that the identity of *Gymnophlaea dichotoma* Kützinger remains uncertain. The illustrations of its habit (Kützinger 1843: pl. 74, fig. iv 1, Kützinger 1866: 21, pl. 58g) show an alga with a regular pseudodichotomous saccate thallus similar to those of *N. dumontioides*, which seems to indicate that the latter species also occurs in the Adriatic Sea (see also Ercegovic 1963: fig.17a) although it has not been collected there recently.

Also, the identity of the minute *Nemastoma inconspicuum* Reinsch cannot be resolved without a study of the holotype. The illustrations of its vegetative structure and

Table 1 The collection of *Nemastoma* specimens studied, grouped by species and varieties.

Specimen	Locality	Date	Labelled as	Leg.	Type of reproduction
<i>Nemastoma dichotomum</i> LD 22089, lectotype of <i>N. dichotomum</i> , ♀	Trieste (IT)	—	<i>Halymenia elongata</i> ?	J. Agardh	Apomixis
LD 22087, syntype of <i>N. dichotomum</i> , ♀	Trieste (IT)	—	<i>Gymnophlaea incrassata</i> ? <i>Nemastoma dichotoma</i> <i>Gymnophlaea furcellata</i> ?	J. Agardh	Apomixis
LD 22090, syntype of <i>N. dichotomum</i> , ♀ ♂	Trieste (IT)	—	<i>Gymnophlaea dichotoma</i> tab. 58 <i>Gymnophlaea furcellata</i> ? <i>Gymnophlaea dichotoma</i> ?	J. Agardh	Apomixis
L 0487970, ♀	Miramare, Trieste (IT)	ix.1875	<i>Nemastoma dichotoma</i>	F. Hauck	Apomixis
L 0487971, ♀	Miramare, Trieste (IT)	—	<i>Nemastoma dichotoma</i>	F. Hauck	Apomixis
L 0487959, ♀, syntype of <i>Gymnophlaea dichotoma</i> var. <i>tenuis</i>	Trieste (IT)	—	<i>Nemastoma dichotoma</i> <i>Gymnophlaea dichotoma</i> var. <i>tenuis</i>	F. T. Kützing	Apomixis
L 0487962, holotype of <i>Ginnania irregularis</i>	Trieste (IT)	—	<i>Ginnania irregularis</i> <i>Gymnophlaea irregularis</i> <i>Nemastoma dichotoma</i>	F. T. Kützing	
TSB 2634, ♀	Miramare, Trieste (IT)	—	<i>Nemastoma dichotoma</i>	F. Hauck	Apomixis
TSB 2635, ♀	Venice (IT)	vii.1960	<i>Nemastoma dichotoma</i>	S. Pignatti	Apomixis
TSB 2639, ♀	Duino, Trieste (IT)	14.xii.1966	<i>Nemastoma dichotoma</i>	G. Giaccone	Apomixis
TSB 2643, ♀	St. Croce, Trieste (IT)	13.vii.1966	<i>Nemastoma dichotoma</i>	S. Pignatti	Apomixis
<i>N. dichotomum</i> var. <i>biasolettianum</i>					
L 0487960, holotype of <i>G. biasolettianum</i> , ♀	Trieste (IT)	—	<i>Gymnophlaea biasolettianum</i> <i>Nemastoma dichotoma</i>	F. T. Kützing	Apomixis
LD 22092, syntype of <i>N. dichotomum</i> , ♀	Trieste (IT)	—	<i>Gymnophlaea biasolettianum</i> tab. 59	J. Agardh	Apomixis
TSB 2646, ♀	Elaphonesos I., Pariki (GR)	18.viii.1967	<i>Nemastoma dichotoma</i> f. <i>ligulata</i>	G. Giaccone	Apomixis
TSB 2651, ♀	Rovigno, Istria (HR)	x.1891	<i>Nemastoma dichotoma</i>	Lucas	Apomixis
<i>N. dichotomum</i> var. <i>caulescens</i>					
L 0487961, syntype of <i>G. caulescens</i> , ♀	Pirano (IT)	—	<i>Gymnophlaea caulescens</i> <i>Nemastoma dichotoma</i>	F. T. Kützing	Apomixis
LD 22088, syntype of <i>N. dichotomum</i> , ♀ ♂	Trieste (IT)	—	<i>Gymnophlaea caulescens</i> tab. 61 <i>Gymnophlaea dichotoma</i>	J. Agardh	Apomixis
Unlabelled specimen Herb. J. Feldmann, ♀	Kornati Islands (HR)	x.1938	<i>Nemastoma constricta</i>	A. Ercegovic	Apomixis
<i>N. dichotomum</i> var. <i>incrassatum</i>					
L 0487958	Trieste (IT)	x.1889	<i>Gymnophlaea incrassata</i> <i>Nemastoma dichotoma</i>	F. T. Kützing	
LD 22091, syntype of <i>N. dichotomum</i> , ♀ ♂	Trieste (IT)	—	<i>Gymnophlaea incrassata</i> tab. 59	J. Agardh	Apomixis
TSB 2642	Canale di Leme, Rovigno, Istria (HR)	11.iii.1903	<i>Nemastoma dichotoma</i>	N. Wille	
<i>Nemastoma dumontioides</i>					
LD 22078, holotype, ♂	Marseilles (FR)	1837	<i>Nemastoma dumontioides</i>	J. Agardh	
no 001502, ♀, Berthold Herbarium, Stazione Zoologica di Napoli, Italy	Naples (IT)	vi.1879	<i>Nemastoma dichotoma</i>	G. Berthold	Sexual
CAT- 870, ♀	Augusta, Sicily (IT)	28.vii.1975	<i>Nemastoma dichotomum</i>	M. Cormaci	Sexual
H 2354, ♀	Cavallu island, Lavezzi Islands, Corsica (FR)	21.x.1988	<i>Nemastoma dichotomum</i>	M. Verlaque	Sexual
H 2355 ♂	Corsica (FR)	21.x.1988	<i>Nemastoma dichotomum</i>	M. Verlaque	
H 3527, ♀	Larvotto, Monaco (FR)	3.iv.1997	<i>Nemastoma dichotomum</i>	M. Verlaque	Apomixis
HGI-A 2512, ♀	Galéria, Corsica (FR)	26.viii.1992	<i>Nemastoma dichotomum</i>	C. Rodríguez-Prieto	Sexual
HGI-A 4783 ♂	Corsica (FR)	9.x.2001	<i>Nemastoma dichotomum</i>	E. Ballesteros	
HGI-A 5200	Ibiza (ES)	26.v.2001	<i>Nemastoma dichotomum</i>	E. Ballesteros	
HGI-A 5295	Ibiza (ES)	26.v.2001	<i>Nemastoma dichotomum</i>	E. Ballesteros	
HGI-A 5296, ♀	Ibiza (ES)	26.v.2001	<i>Nemastoma dichotomum</i>	E. Ballesteros	Apomixis
HGI-A 5315	Pollenca (ES)		<i>Nemastoma dichotoma</i>	E. Ballesteros	
HGI-A 5688	Columbretes I. (ES)	10.v.2002	<i>Nemastoma dichotomum</i>	C. Rodríguez-Prieto	
HGI-A 5689	Columbretes I. (ES)	10.v.2002	<i>Nemastoma dichotomum</i>	C. Rodríguez-Prieto	
HGI-A 5690	Columbretes I. (ES)	10.v.2002	<i>Nemastoma dichotomum</i>	C. Rodríguez-Prieto	
HGI-A 5691	Columbretes I. (ES)	10.v.2002	<i>Nemastoma dichotomum</i>	C. Rodríguez-Prieto	
HGI-A 5692, ♀	Columbretes I. (ES)	17.ix.2002	<i>Nemastoma dichotomum</i>	C. Rodríguez-Prieto and N. Sánchez	Apomixis
HGI-A 5693, ♀	Columbretes I. (ES)	17.ix.2002	<i>Nemastoma dichotomum</i>	C. Rodríguez-Prieto and N. Sánchez	Apomixis

The number of the herbarium specimen, the locality, the date of collection, the name or names labelling the specimen, the collector (Leg.) and the type of reproduction are indicated.

zonate tetrasporangia (Reinsch 1875: 55: pl. xli, figs 1A–D) suggest a possible tetrasporophyte of *Nemastoma*-taceae, but give no further relevant information.

Finally, *Nemastoma constrictum* Ercegovic and *N. constrictum* var. *longitrichogynum* Ercegovic do not differ in any significant way from each other or from *N. dichotomum* var. *caulescens*, and are here relegated to synonymy with the latter.

***Nemastoma dichotomum* J. Agardh** (Figures 1–23)

Protologue and illustrations: Agardh (1842), *Algae maris Mediterranei et Adriatici, observationes in diagnosis*

specierum et dispositionem generum: 91 (as *Nemastoma dichotoma*); Agardh (1879: pl. iv, fig. 5).

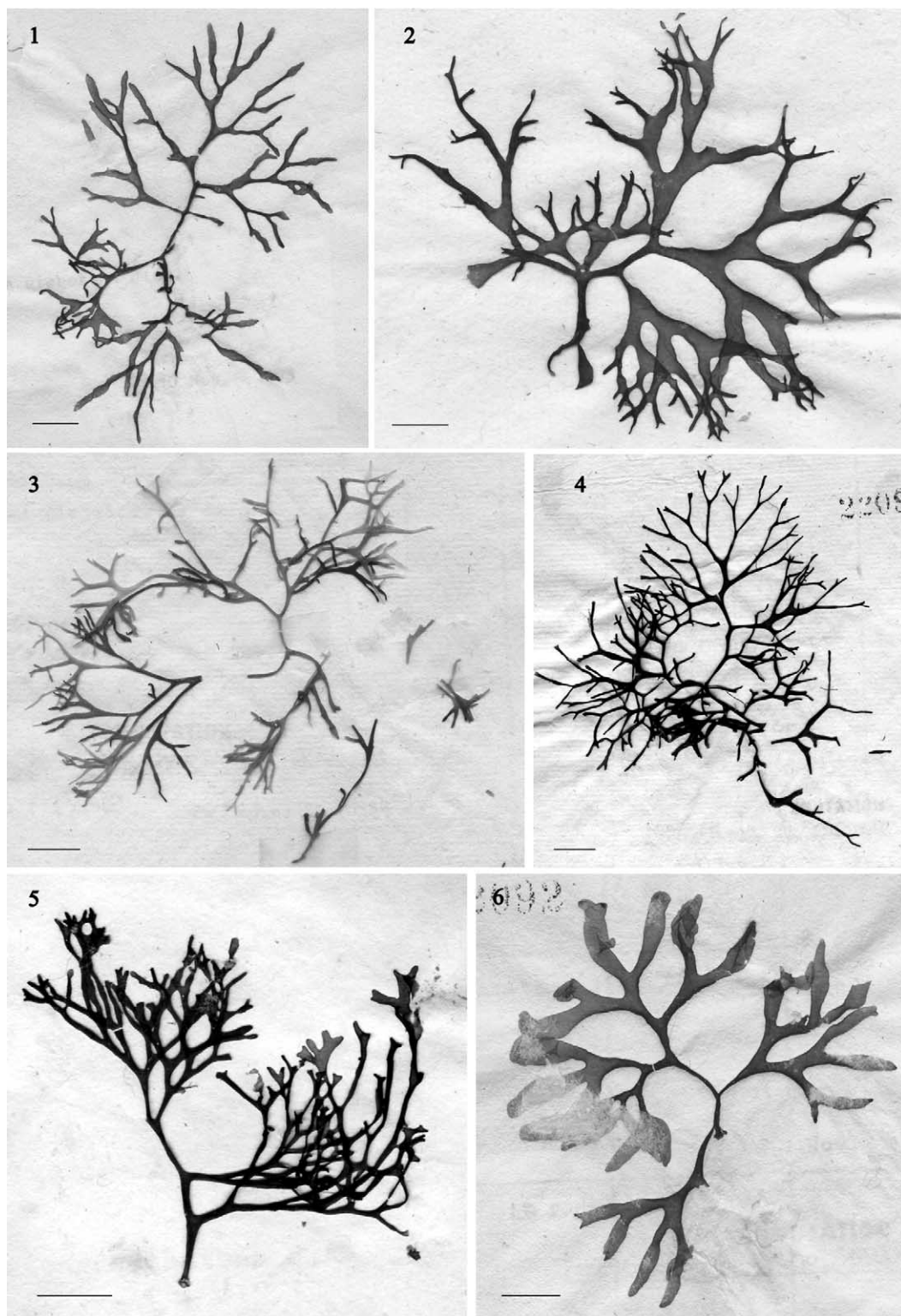
Lectotype: LD 22089, ♀, Trieste, Italy, leg. J. Agardh (Figure 1).

Syntypes: LD 22087, ♀, LD 22088, ♀ ♂, LD 22090, ♀ ♂, LD 22091, ♀ ♂, LD 22092, ♀, Trieste, Italy, Adriatic Sea, leg. J. Agardh (Figures 2–6).

Heterotypic synonyms: *Gymnophlaea dichotoma* var. *tenuis* Kützing (1866: 21, pl. 58k); holotype: L 0487959, ♀, Trieste, Italy, leg. F.T. Kützing (Figure 7). *Ginnania irre-*

Table 2 A comparison between the taxonomic characteristics and the distribution of the types and authentic (i.e., collected by the author) specimens of *Nemastoma*.

		<i>Nemastoma dichotomum</i>					<i>N. dichotomum</i> var. <i>bisolettianum</i>		<i>N. dichotomum</i> var. <i>caulescens</i>			<i>N. dichotomum</i> var. <i>incrassatum</i>		<i>Nemastoma dumontioides</i>	
		LD 22089	LD 22087	LD 22090	L 0487959	L 0487962	L 0487960	LD 22092	L 0487961	LD 22088	Feldmann	L 0487958	LD 22091	LD 22078	
HABIT															
Height (cm)		5	6–7	5	2–3	7–8	5–6	5	5	5–6	7	3–4	5–6	4–5	
Thallus	Base	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	
	Middle	Cylindrical-compressed	Cylindrical-compressed	Cylindrical-compressed	Cylindrical-compressed	Cylindrical-compressed	Cylindrical-compressed	Cylindrical-compressed	Cylindrical	Cylindrical	Cylindrical-compressed	Cylindrical	Cylindrical	Compressed	
	Upper part	Compressed	Compressed	Cylindrical-compressed	Cylindrical-compressed	Cylindrical-compressed	Compressed	Compressed	Compressed	Cylindrical-compressed	Compressed	Compressed	Compressed	Compressed	
Texture	Base	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	Cartilaginous	
	Middle	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	Cartilaginous-membranous	
	Upper part	Membranous	Membranous	Cartilaginous-membranous	Cartilaginous-membranous	Membranous	Membranous	Membranous	Membranous	Cartilaginous-membranous	Membranous	Membranous	Membranous	Membranous	
Base caespitose		?	No	?	No	No	No	Yes	?	No	Yes	No	?		
Segments	Maximal width (mm)	2	4–5	1.5	1	1–2	3	3	3	1	3	1.5	1.5	5	
Length of segments to the apex		Increasing	Decreasing	Decreasing	Decreasing	Decreasing	Increasing	Increasing	Decreasing	Decreasing	Decreasing	Decreasing	Decreasing	Decreasing	
Type of apex		Ligulate	Wedge-shaped	Digitate	Digitate	Digitate	Ligulate	Ligulate	Wedge-shaped	Wedge-shaped	Wedge-shaped	Spathulate	Spathulate	Digitate	
VEGETATIVE STRUCTURES															
Outer cortical cells	L. × diam. (μm)	4–8 × 4–6	4–10 × 4–6	2–15 × 6–8	4–10 × 6–8	4–10 × 6–8	4–8 × 6–8	4–8 × 8	4–8 × 6–8	4–15 × 4–6	4–18 × 2–6	4 × 6–8	4–8 × 6–8	4–6 × 2–6	
	Max. length (μm)	200	360	200	320	280	375	440	220	210	400	160	175	350	
	Diameter (μm)	6–8	6–10	8–12	6–10	6–10	6–10	8–12	6–10	6–10	4–8	6–10	5–10	6–10	
Rhizoidal cells	Max. length (μm)	60	60	70	60	50	70	60	60	60	50	60	60	70	
	L. × diam. (μm)	10–24 × 8–20	10–22 × 8–18	18–36 × 16–30	18–22 × 16–18	20–26 × 12–20	17–25 × 12–16	18–24 × 18	24–28 × 16–22	16–26 × 16–18	10–24 × 8–20	10–18 × 10–16	12–26 × 8–16	12–23 × 12–18	
Gland cells		No	No	No	No	No	No	No	No	Yes	No	No	No		
Presence of hyaline hairs		No	No	No	No	No	No	No	No	No	Yes	No	No		
REPRODUCTIVE STRUCTURES															
Carpogonial branch	On rhizoidal filaments	Yes	Yes	Yes	Yes	–	Yes	Yes	Yes	Yes	Yes	–	Yes	–	
	No. of cells	2–4	3	(2-) 3	3	–	3	3	(2-) 3	3	3	–	3	–	
	Supporting cell diam. (μm)	10–15 × 8–10	5–12	10–13	?	–	10–12	10–12	10–12	10–12	12–14 × 12	–	10–14	–	
	Hypogynous cell diam. (μm)	8–10	5–10	6–8	?	–	6–8	8–10	6–8	5–10	8–10	–	6–8	–	
	Carpogonium length (μm)	5–40	5–25	10–40	?	–	5–10	5–220	5–10	5–10	20	–	5–25	–	
	Auxiliary cells	Diameter (μm)	7–13	10–12	?	10–12	–	10–12	8–10	10–12	18	12–15	–	10–12	–
	Connecting filaments	Absent	Absent	Absent	Absent	–	Absent	Absent	Absent	Absent	Absent	Absent	–	Absent	–
Gonimoblast	Presence	Yes	Yes	Yes	Yes	–	Yes	Yes	Yes	Yes	Yes	–	Yes	–	
	On auxiliary cell	Yes	Yes	Yes	Yes	–	?	Yes	?	Yes	?	–	Yes	–	
	Carpogonia diam. (μm)	12–15	10–24	10–18	10–24	–	12–24	17–22	12–24	12–20	10–22	–	10–22	–	
	Spermatangia	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent	Present	Absent	Absent	Present	Present	
LOCALITY		Trieste (I)	Trieste (I)	Trieste (I)	Trieste (I)	Trieste (I)	Trieste (I)	Trieste (I)	Trieste (I)	Trieste (I)	Kornati I. (CR)	Trieste (I)	Trieste (I)	Marseille (F)	



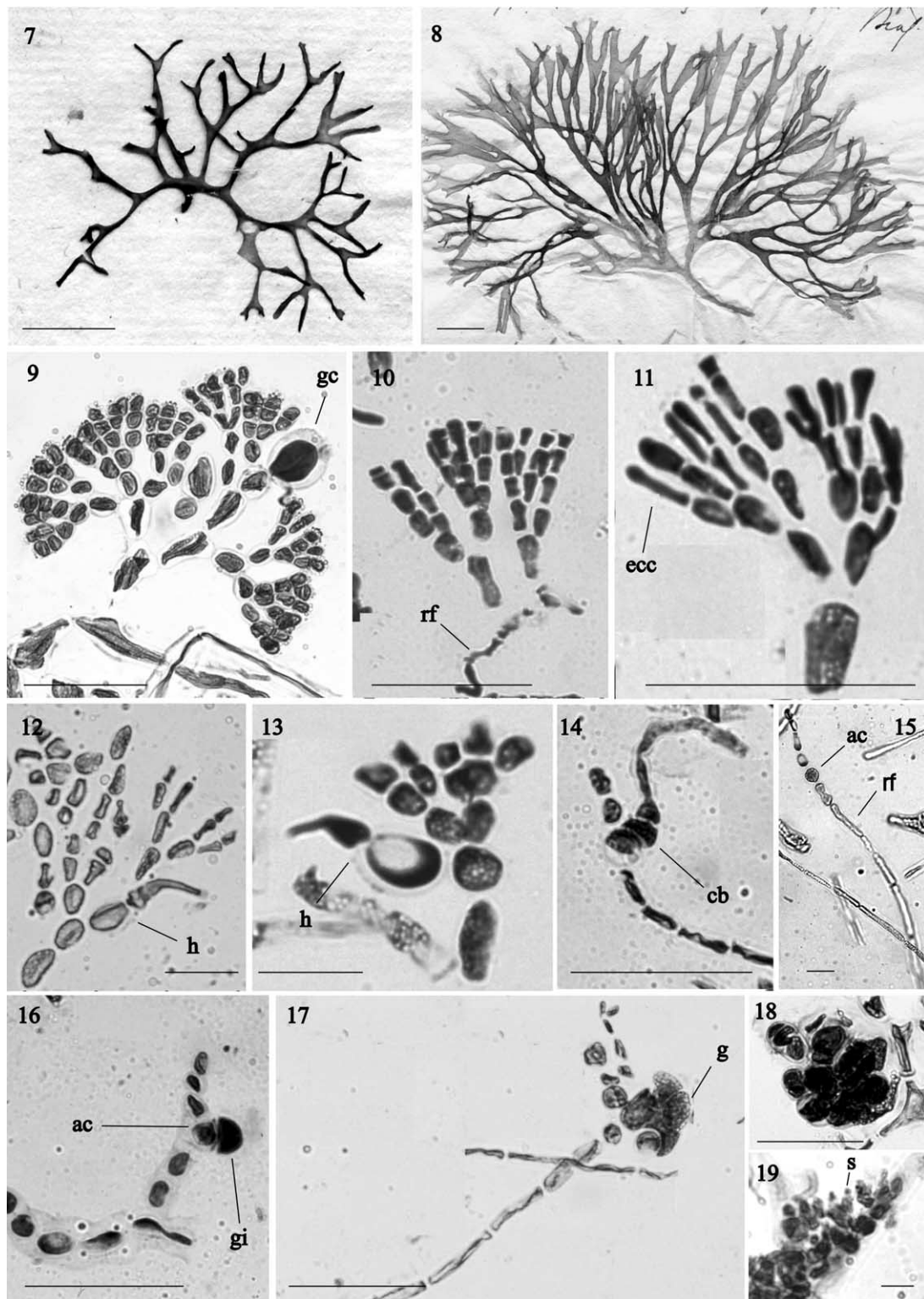
Figures 1–6 *Nemastoma dichotomum* J. Agardh, the type collection in the Herbarium J. Agardh (LD).

(1) Lectotype (LD 22089, ♀), Trieste (IT). (2) Syntype (LD 22087, ♀), Trieste (IT). (3) Syntype (LD 22088, ♀ ♂), Trieste (IT) (= *N. dichotomum* var. *caulescens* (Kützing) Rodríguez-Prieto, Verlaque et Vergés). (4) Syntype (LD 22090, ♀ ♂), Trieste (IT). (5) Syntype (LD 22091, ♀ ♂), Adriatic Sea (IT) (= *N. dichotomum* var. *incrassatum* (Kützing) Rodríguez-Prieto, Verlaque et Vergés). (6) Syntype (LD 22092, ♀), Trieste (IT) (= *N. dichotomum* var. *biasolettianum* (Kützing) Rodríguez-Prieto, Verlaque et Vergés). Scale bars for Figures 1–6=1 cm.

gularis Kützing (1845: 299), Kützing (1866): 24, pl. 69a–c); holotype: L 0487962, ♀, Trieste, Italy, leg. F.T. Kützing (Figure 8).

Specimens examined: see Table 1.

Habit: gametophytes to 7–8 cm high, arising from a discoid holdfast, occasionally caespitose; rosy, deeply red to red-brown or violet in colour; fronds cylindrical and cartilaginous basally, usually softer above; central parts cartilaginous, either cylindrical or compressed in dry specimens; plants irregularly pseudodichotomous, the



Figures 7–19 (7) *Nemastoma dichotomum* J. Agardh [*Gymnophlaea dichotoma* var. *tenuis* Kützing, holotype (L 0487959, ♀), Trieste (IT)]. (8) *Nemastoma dichotomum* J. Agardh [*Ginnania irregularis* Kützing, holotype (L 0487962), Trieste (IT)]. (9–19) Vegetative and reproductive structures of *Nemastoma dichotomum* J. Agardh. (9) Cortical fascicle showing a gland cell (gc) (TSB 2639). (10) Cross section of cortex showing the beginning of a rhizoidal filament (rf), (*Nemastoma constrictum* Ercegovic, lectotype, PC). (11) Cross section of cortex showing some elongated outer cortical cells (ecc) (*Nemastoma constrictum* Ercegovic, lectotype, PC). (12–13). ‘Glandular-like’ hairs (h) (*Nemastoma constrictum* Ercegovic, lectotype, PC). (14) Three-celled carpogonial branch (cb) supported by a divided supporting cell and with a well-developed trichogyne (TSB 2639). (15) Auxiliary cell (ac) on a rhizoidal filament (rf) (*Nemastoma constrictum* Ercegovic, lectotype, PC). (16–17) Auxiliary cells (ac) giving rise to a gonimoblast initial (gi) (Figure 16) and young gonimoblast (g) (Figure 17) (TSB 2639). (18) Gonimoblasts in cross section, with carpospores developing *in situ* (TSB 2639). (19) Spermatangia (s) (LD 22088). Scale bars for Figures 7–8=1 cm; for Figures 9–18=50 μm ; for Figure 19=10 μm .

branches, 1–5 mm broad, increasing slightly in breadth at the bifurcations, often fastigiata; lengths of the segments uniform except in upper parts; upper segments variable in length, digitate, ligulate, wedge-shaped or spatulate depending on the variety (see below); margins smooth, sometimes proliferous (Figures 1–8).

Vegetative structure: multiaxial, fronds composed of a broad, laxly-filamentous medulla of unpigmented elongated cells to 440 μm long and 5–12 μm in diameter, and a compact cortex subdichotomously divided to the sixth order, the outer cortical cells ovoid, club-shaped, slightly elongated or, occasionally very elongated, (4–) 8 (–15) μm long and (4–) 6–8 μm in diameter (Figures 9–11); secondary pit-connections absent; rhizoidal filaments adventitious, simple or subdichotomously branched, developed on the inner cortical cells or sometimes on the medullary filaments and transversing the thallus; cells of rhizoidal filaments shorter (up to 70 μm in length) and with denser content than medullary cells; terminal gland cells present within the sub-surface layers of the cortex, subspherical to ovoid, 10–28 (–38) μm long and 8–22 (–30) μm in diameter; ‘glandular-like’ hairs occasionally present at the place of a cortical dichotomy (Figures 12–13).

Reproduction: plants monoecious; female reproductive structures non-procarpic or procarp-like (carpogonial branch and auxiliary cell borne on the same rhizoidal filament); female gametophytes monocarpogonial; carpogonial branches 3-celled (occasionally 2- or 4-celled), borne laterally on a modified intercalary vegetative cell of rhizoidal filaments, the supporting cell rounded or ovoid, (5–) 12–14 μm long and (5–) 8–12 μm in diameter, occasionally divided into two hemispherical cells; hypogynous cells 5–10 μm in diameter; trichogynes, simple or occasionally bifurcate, usually 5–40 μm in length, occasionally up to 220 μm (Figure 14); auxiliary cells intercalary on rhizoidal filaments, enlarged and dark-staining prior to presumed diploidization, 8–18 μm in diameter (Figure 15); occasionally, two auxiliary cells borne on the same rhizoidal filament; nutritive cells absent; connecting filaments not observed; gonimoblast initial arising from auxiliary cell, presumably by apomixis (Figures 16–17); gonimoblast development lateral or outward; carposporophytes scattered over the thallus, covered by a thin hyaline membrane and apparently formed by two gonimolobes; all gonimoblast cells becoming carposporangia, 10–24 μm in diameter, occasionally germinating *in situ* (Figure 18); male gametophytes apparently uncommon; spermatangia rounded, 1–3 μm in diameter and cut off from the terminal cortical cells, single or in groups of 2–3 (Figure 19); tetrasporophytes unknown.

Distribution: Adriatic, Aegean and Ionian Seas. Atlantic specimens from the north of Spain and deposited as *Nemastoma dichotomum* in the Rijksherbarium, Leiden (L) are misidentifications and do not belong to the genus *Nemastoma*, as having a filamentous medulla with some stellate cells.

***N. dichotomum* var. *biasolettianum* (Kützing)**

Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov. (Figures 6, 20)

Basionym: *Gymnophlaea biasolettiana* Kützing (1843), *Phycologia generalis (oder Anatomie, Physiologie und Systemkunde der Tange)*: 390–391.

Illustrations: Kützing (1866: 21, pl. 59d–e).

Holotype: L 0487960, ♀, Trieste, Italy, leg. F.T. Kützing (Figure 20).

Specimens examined: see Table 1. The specimen LD 22092, ♀ (syntype of *N. dichotomum* labeled ‘*G. biasolettiana*, Trieste, Italy’) is probably from the same collection that included the holotype of *G. biasolettiana* (Figure 6).

Habit: gametophytes to 6 cm high, basal parts cylindrical, cartilaginous; median parts cylindrical or compressed; interdichotomies increasing in length distally, <3 mm wide except at the bifurcations, where they can slightly increase in breadth; differs from var. *dichotomum* in its upper parts ligulate, compressed and membranous in dry specimens.

Distribution: Adriatic, Aegean and Ionian Seas.

***N. dichotomum* var. *caulescens* (Kützing) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov.**

(Figures 3, 21–22)

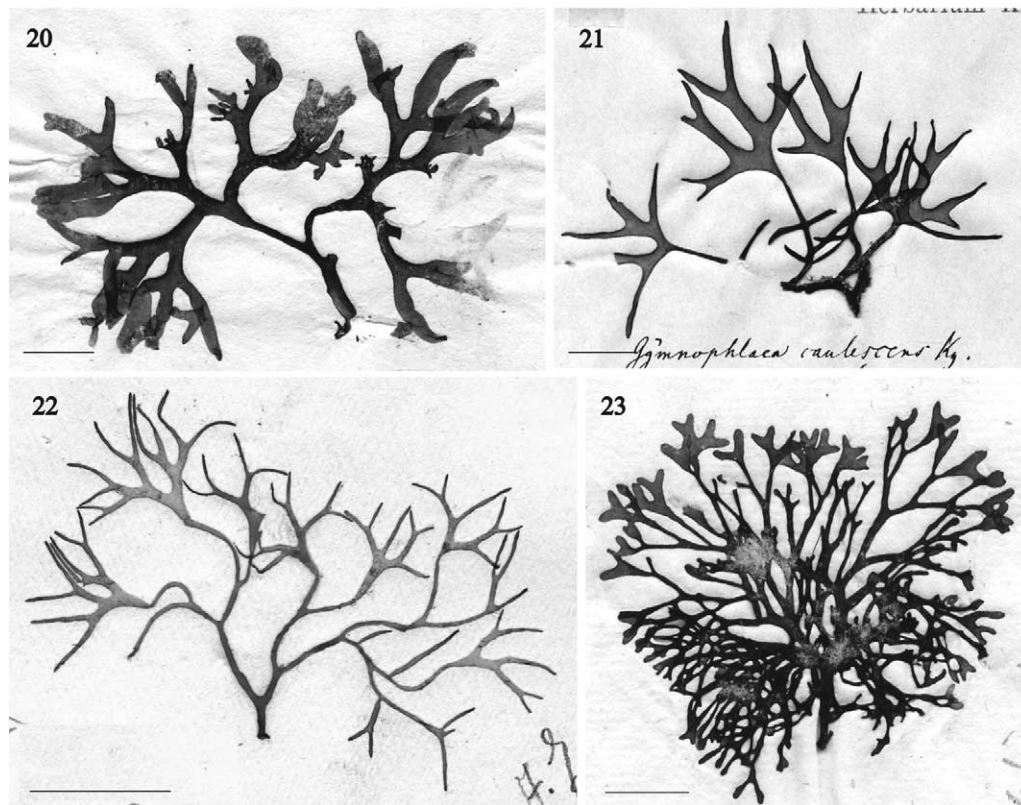
Basionym: *Gymnophlaea caulescens* Kützing (1866), *Tabulae phycologicae, oder Abbildungen der Tange*: 22, pl. 61a–c.

Syntype: L 0487961 (two specimens), ♀, Pirano, Trieste, Italy, leg. F.T. Kützing (Figure 21).

Heterotypic synonyms: *Nemastoma constrictum* Ercegovic (1949: 40–44, figs 20–21; as *N. constricta*); type locality: Kornati Islands, middle Adriatic Sea; holotype presumed to have been destroyed; lectotype designated herein: unnumbered specimen collected by Ercegovic himself, in October 1938, at the type locality (one month after the collection of the holotype), ♀, labeled “*Nemastoma constrictum*, leg. Ercegovic, October 1838, Kornati Islands, Croatia” and deposited in the J. Feldmann Herbarium, Muséum National d’Histoire Naturelle (PC) (Figure 22). *N. constrictum* var. *longitrichogynum* Ercegovic (1949): 44–47, figs 22–23; as *N. constricta* var. *longitrichogyna*); type locality: middle Adriatic Sea; holotype presumed to have been destroyed.

Other specimens examined: see Table 1. The specimen LD 22088, ♀ ♂ (syntype of *N. dichotomum* labeled ‘*G. caulescens*, Trieste, Italy’) belongs to this variety (Figure 3).

Habit: gametophytes to 7 cm high, basal and medium parts cylindrical or slightly compressed, cartilaginous in texture; interdichotomies <3 mm wide throughout



Figures 20–23 *Nemastoma dichotomum*.

(20) *Nemastoma dichotomum* var. *biasolettianum* (Kützing) Rodríguez-Prieto, Verlaque et Vergès [*Gymnophlaea biasoletiana* Kützing, holotype (L 0487960, ♀), Trieste (IT)]. (21) *Nemastoma dichotomum* var. *caulescens* (Kützing) Rodríguez-Prieto, Verlaque et Vergès [*Gymnophlaea caulescens* Kützing, syntype (L 0487961, ♀), Trieste (IT)]. (22) *Nemastoma dichotomum* var. *caulescens* [*Nemastoma constrictum* Ercegovic, lectotype, Kornati islands (CR); published by permission of the Muséum National d'Histoire Naturelle, Herbarium Cryptogamique (PC)]. (23) *Nemastoma dichotomum* var. *incrassatum* (Kützing) Rodríguez-Prieto, Verlaque et Vergès [*Gymnophlaea incrassata* Kützing, lectotype (L 0487958), Trieste (IT)]. Scale bars for Figures 20–23=1 cm.

except for the bifurcations, which are up to 5 mm wide; differs from var. *dichotomum* in its upper parts wedge-shaped, compressed and membranous in dry specimens.

The lectotype of *Nemastoma constrictum* showed no differences from the syntype of *Gymnophlaea caulescens* Kützing (Table 2). As suggested by Athanasiadis (1988), the structures regarded by Ercegovic as being 2-celled carpogonial branches with long trichogynes are hairs ('glandular-like' hairs) (Figures 12–13). *Nemastoma constrictum* var. *longitrichogynum* Ercegovic probably corresponds to *N. dichotomum* var. *caulescens*, as it differs only by its exceptionally long 'glandular-like' hairs (up to 1000 µm long).

Distribution: Adriatic Sea.

***N. dichotomum* var. *incrassatum* (Kützing)**

Rodríguez-Prieto, Verlaque et Vergès comb. nov. et stat. nov. (Figures 5, 23)

Basionym: *Gymnophlaea incrassata* Kützing (1843), *Phycologia generalis (oder Anatomie, Physiologie und Systemkunde der Tange)*: 390.

Illustrations: Kützing (1866: 21, pl. 59).

Holotype: not located in the Rijksherbarium, Leiden; type locality: Adriatic Sea.

Lectotype designated herein: L 0487958, sterile, labeled "*Gymnophlaea incrassata*, leg. Kützing, x.1889, Trieste, Italy" (Figure 23).

Specimens examined: see Table 1. The specimen LD 22091, ♀ ♂ (syntype of *N. dichotomum* labeled *G. incrassata*, Adriatic Sea, belongs to this variety (Figure 5).

Habit: gametophytes to 6 cm high; basal and medium part of the thallus terete, filiform and cartilaginous; segments decreasing in length towards the upper parts and less than 1.5 mm wide except in the bifurcations where they can slightly increase in breadth (1.5–2.0 mm); differs from var. *dichotomum* in its upper parts spathulate and membranous in dry specimens.

Distribution: Adriatic Sea.

***Nemastoma dumontioides* J. Agardh** (Figures 24–44)

Protologue: Agardh (1851), *Species genera et ordines algarum, seu descriptiones succinctae specierum, generum et ordinum, quibus constituitur. Vol. 2: Florideae. Part 1*: 164.

Illustrations: Kylin (1932: pl. i, fig. 1, holotype); Berthold (1884: pl. iv, fig. 3, pl. vi, figs 9–15, as *Gymnophlaea dichotoma* Kützing); Oltmanns (1904: fig. 326–1, as *Gymnophlaea dichotoma*).

Holotype: LD 22078, ♂, Marseille, France, leg. J. Agardh (Figure 24). The holotype is a male gametophyte, although spermatangia are not described in the protologue.

Specimens examined: see Table 1. An examination of the photograph of the specimen studied by Berthold (1884): specimen 001502, labeled “*Gymnophlaea dichotoma* Kützing, Gulf of Naples”, Berthold Herbarium, courtesy of the Stazione Zoologica di Napoli, Italy (Figure 25), showed that the alga is *N. dumontioides*, although the author failed to describe or illustrate gland cells.

Habit: gametophytes to 8 cm high, arising from a discoid holdfast; fronds often caespitose, rosy, deeply red to red-brown in colour; thallus membranous throughout in dry specimens, cylindrical or compressed and gelatinous in fresh specimens (Figures 26–27), very regularly pseudodichotomously branched; branches often fastigiated and corymbiform in outline, 4–5 mm broad, increasing slightly in breadth at the bifurcations; length of the interdichotomies uniform proximally, shorter distally; terminal segments digitate or occasionally ligulate; axes smooth but sometimes proliferous (Figures 24–27).

Vegetative structure: similar to that of *N. dichotomum*; unpigmented elongated medullary cells, to 350 μm long and 6 (–10) μm in diameter; cortex compact, subdichotomously divided to the sixth order (Figures 28–30); outer cortical cells ovoid, club-shaped or, occasionally, slightly elongated (2-) 6–8 \times 4–6 μm (Figure 29); rhizoidal filaments adventitious, to 70 μm in length, transversing the thallus (Figure 30); terminal subsurface gland cells always present (Figure 30), subspherical, 12–23 \times 12–18 μm ; vegetative ‘glandular-like’ hairs hyaline, up to 2000 μm long, observed in only one specimen (H 3527, Monaco) (Figures 31–32).

Reproduction: plants dioecious; female gametophytes monocarpogonial and non-procarpic (Figures 33–36) or occasionally procarpic-like (carpogonial branch and auxiliary cell borne on the same rhizoidal filament) (Figures 37–38); carpogonial branches 3-celled (occasionally 2-celled) borne laterally (terminally in one specimen: CAT 870, Sicily) on a modified vegetative cell of a rhizoidal filament (Figures 33–34) or, very rarely, lateral on cortical fascicles (Figure 39); supporting cell rounded or ovoid, 10–14 long and 10–12 μm in diameter, usually divided into two hemispherical cells; hypogynous cells 8–10 μm in diameter; trichogynes simple or occasionally bifurcate, to 240 μm in length; auxiliary cells rounded, intercalary in rhizoidal filaments, enlarged and dark-staining prior to presumed diploidization, 14–17 μm in diameter (Figures 35–38); two auxiliary cells occasionally borne on the same rhizoidal filament (Figure 36); nutritive cells absent; presumably following fertilization, a connecting filament

is initiated by the basal cell of the carpogonial branch and issue head on from it (Figure 40) growing parallel to the frond surface; connecting filaments septate, branched, 1–2 μm in diameter, connecting laterally with auxiliary cells and able to effect successive diploidizations (Figure 41); gonimoblast initial arising from the connecting filament adjacent to its point of fusion to the auxiliary cell (Figure 42); carposporophytes scattered over the thallus and covered by a thin hyaline membrane, all gonimoblast cells becoming carposporangia, 12–23 μm in diameter; in some individuals, connecting filament absent and gonimoblast initial arising from auxiliary cell presumably by apomixis; gonimoblast development outward; male gametophytes apparently not very frequent (Table 1); spermatangia rounded, to 1–2 μm in diameter, cut off from the terminal cortical cells, singly or in groups of 2–3 (Figures 43–44); tetrasporophyte unknown.

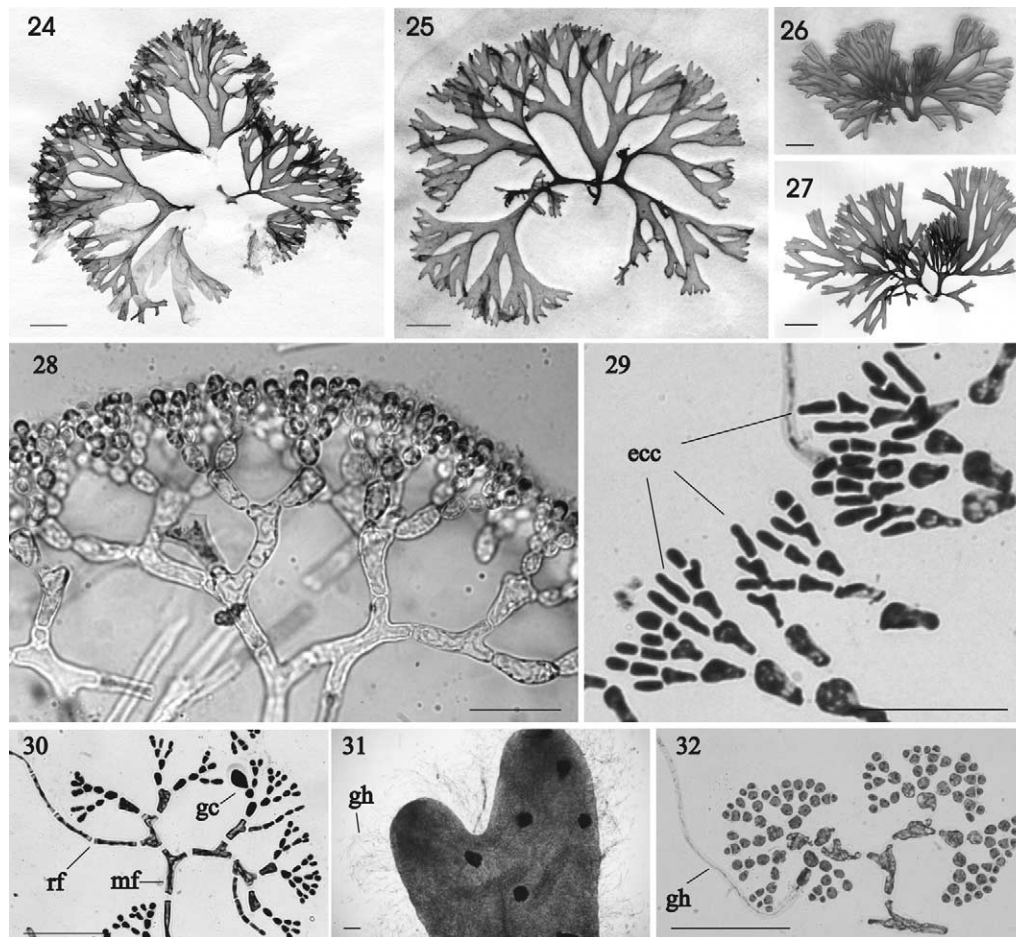
Distribution: western Mediterranean Sea, Adriatic Sea (?).

Discussion

The red alga family Nemastomataceae, placed now in the resurrected order Nemastomatales Kylin emend. G.W. Saunders et Kraft (2002), currently includes the following four genera: *Adelophycus* Kraft in Womersley (1994), *Itonoa* Masuda and Guiry (1995), *Nemastoma* J. Agardh (1842) and *Predaea* G. De Toni (1936). The vegetative and reproductive structures of type materials and recent collections of Mediterranean specimens confirm that the diagnostic features of the genus *Nemastoma* J. Agardh (Masuda and Guiry 1994, 1995) are: (i) terminal subsurface gland cells; (ii) supporting cells and auxiliary cells intercalary on adventitious filaments; (iii) carpogonial branches lacking sterile laterals; (iv) no nutritive cells on hypogynous or supporting cells; (v) no nutritive auxiliary cells; and, (vi) connecting filaments (when present), multicellular, branched and able to effect successive diploidizations; (vii) gonimoblast initial arising from the connecting filament adjacent to its point of fusion with the auxiliary cell; (viii) gonimoblast development lateral or outward; and (ix) tetrasporophyte unknown.

According to Saunders and Kraft (2002), the Nemastomatales have 3–5-celled carpogonial branches, but 2-celled carpogonial branches have occasionally been observed in *N. dumontioides* and are the normal features of several *Predaea* species (Kraft 1984, Verlaque 1990).

Moreover, our findings indicate that the Nemastomataceae generally include non-procarpic genera, where the zygote is successively transferred to several auxiliary cells, and that connecting filaments in *Nemastoma* can be issued from the basal cell of the carpogonial branch (rather than the carpogonium itself). The latter feature appears to be the only one presently segregating *Nemastoma* from *Adelophycus*. On the other hand, the procarpic-like condition (occasionally seen in both apomictic and sexual plants of *Nemastoma*) can be fortuitous (connec-



Figures 24–32 *Nemastoma dumontioides* J. Agardh.

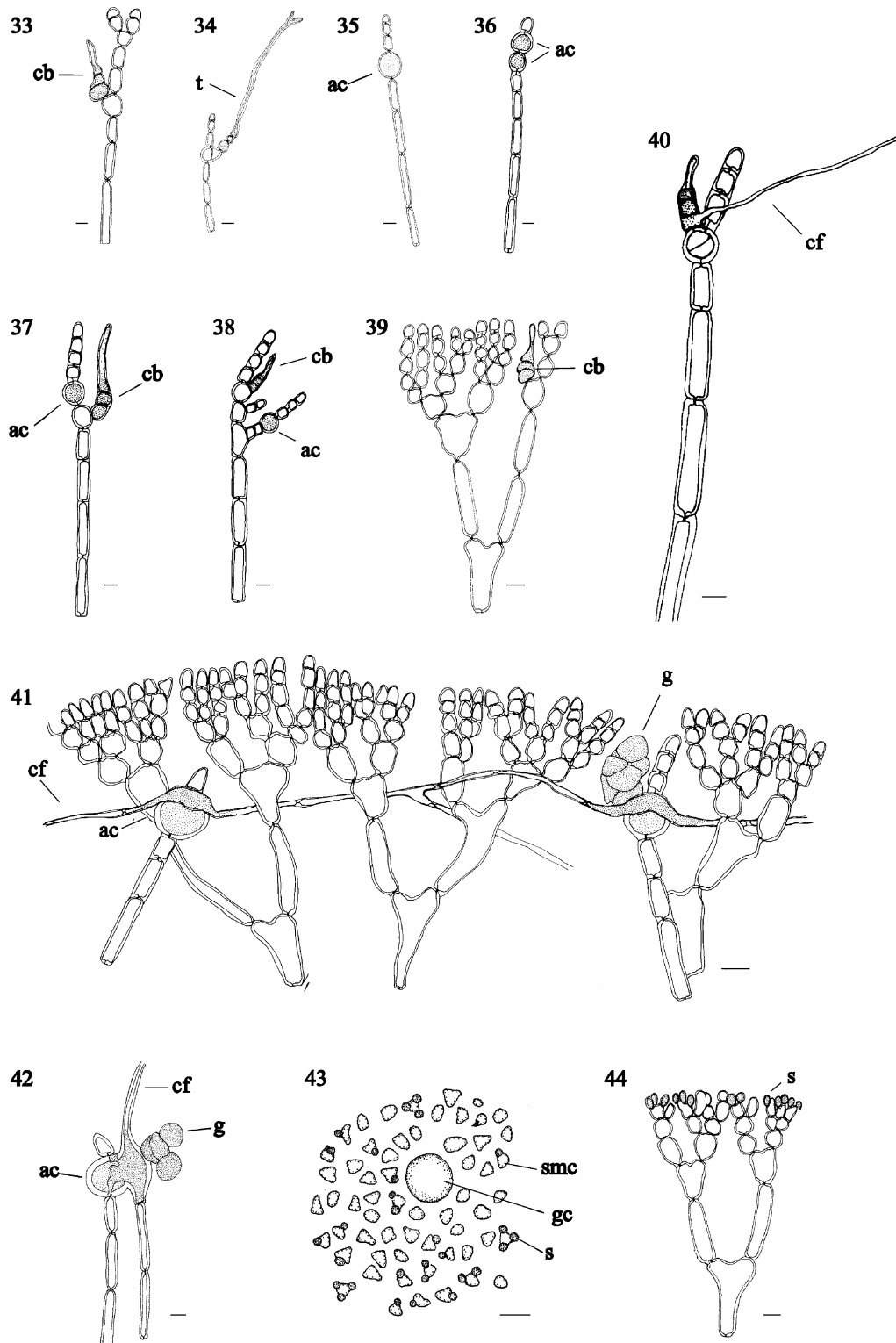
(24) Holotype (LD 22078, ♂), Marseilles (FR). (25) Specimen of *Gymnophlaea dichotoma* Kützinger described and illustrated by G. Berthold (1884) (Berthold Herbarium no. 001502, ♀), Gulf of Naples (IT). (26–27) Specimen HGI-A 5690 from the Columbretes Islands (ES) (*legit* C. Rodríguez-Prieto). Liquid-preserved (Figure 26) and pressed specimen (Figure 27). (28) Cross section of cortex of a slightly staining specimen (HGI-A 5692). (29) Cross section of a hardly staining and squashed specimen; cortex with elongated cortical cells (ecc) (H 3527). (30) Cross section showing a cortical fascicle with a medullary filament (mf), a rhizoidal filament (rf) and a gland cell (gc) (H 3527). (31) Branch tip of a specimen densely invested with 'glandular-like' hairs (gh) (H 3527). (32) Cross section of H 3527 showing a cortical fascicle with a 'glandular-like' hair (gh) (H 3527). Scale bars for Figures 24–27=1 cm; for Figures 28–30 and 32=100 μ m; for Figure 31=200 μ m.

tion or fusion between auxiliary cell and neighboring carpogonia or other cells of the carpogonial branch never observed), or represent a plesiomorphy (i.e., a relic condition), as generally evident in the procarpic and primitive Ceramiales (see fig. 2 in Athanasiadis 2002), or, as well, a new type, homologous to that reported in the sister-taxon Schizymeniaceae where the connecting filament first fuses with proximal nutritive auxiliary cells before departing to meet remote ones (Masuda and Guiry 1995: 66).

Two species of *Nemastoma* co-exist in the Mediterranean Sea: *N. dichotomum* J. Agardh, recorded widely but infrequently in the Adriatic, Aegean and Ionian Seas, and *N. dumontioides* J. Agardh, restricted to the western Mediterranean but possibly extending eastward into the Adriatic Sea. They differ mainly in habit, the reproductive structures and the presumably exclusively apomictic reproduction in *N. dichotomum* (Table 3). Athanasiadis (1988) reported that plants of *N. dichotomum* are composed of one to several terete to slightly compressed fronds, but our findings show that this is equally true of *N. dumontioides* and cannot be considered a good tax-

onomic character. The two species may also differ in that *Nemastoma dichotomum* is presumed monoecious whereas *N. dumontioides* is regarded as dioecious in the present work, but this characteristic requires confirmation because male structures can be difficult to recognize, or may be protrandous.

Nemastoma dichotomum and *N. dumontioides* appear to be restricted to the Mediterranean Sea. Atlantic specimens from Spain of *Nemastoma dichotomum* conserved in L are misidentifications, as is probably the case with other previous Atlantic records: Channel Islands (Lyle 1920), regarded as a misidentification by Dixon and Irvine (1977); Madeira (Levring 1974), refuted by Athanasiadis (1987); and the report of an undescribed species of *Nemastoma* from Ireland (Guiry, pers. comm. in Athanasiadis 1996: 68). A little known species of *Nemastoma*, *N. canariense* (Kützinger) J. Agardh, occurs in the Canary Islands. Canarian specimens deposited in L showed that *N. canariense* differs from the Mediterranean taxa of *Nemastoma* in having a narrower cartilaginous thallus (1.5 mm broad), a thicker cortical layer and larger gland cells (see also Børgesen 1929).



Figures 33–44 Reproductive structures of *Nemastoma dumontioides* J. Agardh.

(33) Three-celled carpo-gonial branch (cb) with short trichogyne on a rhizoidal filament (CAT 870). (34) Three-celled carpo-gonial branch supported by a divided supporting cell and with a long and bifurcated trichogyne (t) (CAT 870). (35) Intercalary auxiliary cell (ac) on a rhizoidal filament (CAT 870). (36) Two auxiliary cells (ac) situated consecutively on the same rhizoidal filament (CAT 870). (37–38) Three-celled carpo-gonial branch (cb) and an auxiliary cell (ac) situated on the same rhizoidal filament. The auxiliary cell is situated intercalarily in the rhizoidal filament itself in Figure 38, and intercalarily in a lateral branch off the rhizoidal filament in Figure 39 (CAT 870). (39) Three-celled carpo-gonial branch (cb) borne by a cortical fascicle (CAT 870). (40) Connecting filament (cf) issuing from the basal cell of the carpo-gonial branch (CAT 870). (41) Connecting filaments (cf) connecting two auxiliary cells (ac) and young gonimoblast (g) issuing from the connecting filament (CAT 870). (42) Early development of the gonimoblast (g) arising from connecting filament (cf) near the contact to the auxiliary cell (ac) (H 2354). (43–44) Spermatangial structures. Up to three spermatangia (s) are produced from terminal spermatangia mother cells (smc). Note the gland cell (gc) (H 2355). Scale bars for Figures 33–44=10 μm .

Table 3 A comparison between the taxonomic characteristics of *Nemastoma dichotomum* and *N. dumontioides*.

	<i>N. dichotomum</i>	<i>N. dumontioides</i>
HABIT		
Height (cm)	7–8	8
Thallus section	Cylindrical in basal part, compressed or cylindrical in middle part, and usually compressed in upper part	Cylindrical
Texture	Cartilaginous except occasionally the upper part in pressed individuals	Gelatinous in fresh individuals and membranous in pressed individuals
Base	Occasionally caespitose	Often caespitose
Branching	Irregularly pseudodichotomous	Regularly pseudodichotomous with corymbose outline
Segments, diameter (mm)	1–5	4–5
Interdichotomy length	Constant, except for the upper ones	Decreasing gradually from the base to the apices
Terminal segments	Highly variable, digitate, var. <i>dichotomum</i> ligulate, var. <i>biasolettianum</i> wedge-shaped: var. <i>caulescens</i> spatulate: var. <i>incrassatum</i>	Cylindrical, short, occasionally ligulate
VEGETATIVE STRUCTURES		
Outer cortical cells	form length (μm) diameter (μm)	Ovoid, club-shaped, rounded or elongated (4–) 8 (–15) (4–) 6–8
Medullary cells	length (μm) diameter (μm)	Up to 440 (580, according to Athanasiadis 1988) 4–6
Cells of the rhizoidal filaments, length (μm)	5–12	6–10
Gland cells, length x diameter (μm)	Up to 70	Up to 70
'Glandular' hairs	10–28 (–38) × 8–22 (–30) Occasionally present	12–23 × 12–18 Observed in only one specimen
REPRODUCTIVE STRUCTURES		
Monoecious/dioecious	Monoecious	Dioecious
Type of reproduction	Apomictic	Sexual or apomictic
Carpogonial branch	number of cells carpogonium, length (μm) position	(2–) 3 5–240 Lateral or, occasionally terminal, on rhizoidal filaments, very rarely lateral on cortical fascicles
Auxiliary cells, diameter (μm)	8–18	14–17
Connecting filaments	Absent	Present in sexually reproducing individuals
Carpogonia diameter (μm)	10–24	12–23
Spermatangia	Single or in groups of 2–3	Single or in groups of 2–3
GEOGRAPHICAL DISTRIBUTION		
	Adriatic Sea, Aegean and Ionian Seas	Western Mediterranean, Adriatic Sea (?)

We have yet to find any specimen that fits the description of Reinsch's *N. inconspicuum* or any tetrasporangial plant, so the attribution of this entity is highly dubious. According to Cortel-Breeman and van den Hoek (1970) and van den Hoek et al. (1972), the development of carpospores of *N. dichotomum* gives rise to a tiny and filamentous stage producing monospores. As these authors did not describe nor give the origin of the gametophytic plants they studied, the filamentous stage obtained cannot be assigned to either *N. dichotomum* or *N. dumontioides*.

Three species described by Kützing are relegated to variety status as a result of the present study: (i) *N. dichotomum* var. *biasolettianum*, (ii) *N. dichotomum* var. *caulescens* and (iii) *N. dichotomum* var. *incrassatum* (Table 4). The last two are restricted to the Adriatic Sea, whereas the former is present both in the Adriatic Sea and in the Aegean and Ionian Seas. No relation can be hypothesized between the form and habitat of any of the varieties because the habitat information is, in most cases, not available. In addition, some specimens presumably collected from the same place, and sometimes even on

the same date, present very different morphologies. Athanasiadis (1988: 26) has emphasized the morphological differences existing between the lectotype and the syntypes of *N. dichotomum*, which all originate from Trieste, and our results show that all the syntypes belong to *N. dichotomum*, although some represent different varieties. *Nemastoma constrictum* and *N. constrictum* var. *longitrichogynum* are reduced to synonymy with *N. dichotomum* var. *caulescens*, as the characteristics listed by Ercegovic (1949) for distinguishing *N. constrictum* from *N. dichotomum* are not consistent: the frond constrictions are not evident in Ercegovic's specimen of *N. constrictum* that is deposited in the J. Feldmann Herbarium, and the '2-celled carpogonial branches lateral on cortical fascicles' that he described, are actually 'glandular-like' hairs. Likewise, *N. constrictum* var. *longitrichogynum* must also be rejected as a separate taxon because the structures identified and illustrated by Ercegovic as being 'carpogonial branches' up to 1000 μm long also appear to be 'glandular-like' hairs.

Nemastoma dichotomum and *N. dumontioides* are probably very closely related taxa, with the former pos-

Table 4 Recommended taxonomic changes.

Proposed taxa	Basionym	Heterotypic synonyms
<i>Nemastoma dichotomum</i> var. <i>biasolettianum</i> (Kützing) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov.	<i>Gymnophlaea biasoletiana</i> Kützing (1843)	–
<i>Nemastoma dichotomum</i> var. <i>caulescens</i> (Kützing) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov.	<i>Gymnophlaea caulescens</i> Kützing (1866)	<i>Nemastoma constrictum</i> Ercegovic (1949) <i>N. constrictum</i> var. <i>longitrichogynum</i> Ercegovic (1949)
<i>Nemastoma dichotomum</i> var. <i>incrassatum</i> (Kützing) Rodríguez-Prieto, Verlaque et Vergés comb. nov. et stat. nov.	<i>Gymnophlaea incrassata</i> Kützing (1843)	–

sibly derived from the latter by the acquisition of apomixis. The likelihood that *N. dichotomum* reproduces apomictically was first suggested by Athanasiadis (1988) based on a comparison of the lectotype (LD 22089) with recently collected plants from the Aegean and Adriatic Seas, in all of which gonimoblast initials arose directly from auxiliary cells, without any contact by connecting filaments. The absence of connecting filaments in all the female gametophytes of *N. dichotomum* studied here supports this hypothesis. Apomictic reproduction, which favors the preservation of random mutations, would also explain the high polymorphism displayed by *N. dichotomum*, unlike *N. dumontioides* in which sexual reproduction and little morphological variation are the norm. Consequently, until a molecular-genetic study of Mediterranean *Nemastoma* populations is carried out, we maintain *N. dumontioides* as distinct from *N. dichotomum*.

Key to the Mediterranean species and varieties of *Nemastoma* J. Agardh

1. Thallus gelatinous throughout, drying membranous; cylindrical, regularly pseudodichotomous with corymbose outline; interdichotomies decreasing gradually in length from the base to the apices; sexual or apomictic *N. dumontioides*
1. Thallus cartilaginous except distally; cylindrical basally, cylindrical or compressed medially, usually compressed distally; irregularly pseudodichotomous; interdichotomies not decreasing gradually in length from the base to the apices; apomictic *N. dichotomum* – 2
2. Interdichotomies increasing in length towards the upper parts; upper parts ligulate *N. dichotomum* var. *biasoletianum*
2. Interdichotomies decreasing in length towards the upper parts; upper parts not ligulate 3
3. Upper parts wedge-shaped; axis up to 5 mm wide *N. dichotomum* var. *caulescens*
3. Upper parts spatulate; axis up to 1.5–2 mm wide *N. dichotomum* var. *incrassatum*.

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