

***Lithothamnion crispatum*: long-lasting species of non-geniculate coralline algae (Rhodophyta, Hapalidiales)**

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Abstract: The examination of fossil *Lithothamnion* specimens ranging in age from the Early Miocene to the Pleistocene revealed the occurrence of multiporate conceptacle chambers pitted with depressions. This is the diagnostic feature of *Lithothamnion crispatum*, a cosmopolitan hapalidiacean with a wide depth range in modern oceans. The comparison of the microscopic anatomy of both the fossil and modern *L. crispatum* confirmed that they are conspecific. Therefore, this species has a long stratigraphic distribution starting at least 20 My ago, without significant morphological changes in either reproductive or vegetative anatomy.

Key Words: Morphology; multiporate conceptacle roof; Tethys; Melobesioideae; Miocene.

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Résumé : *Lithothamnion crispatum* : une espèce d'algue coralline non-geniculée persistante dans le temps (Rhodophyta, Hapalidiales).- La révision d'exemplaires fossiles de *Lithothamnion* dont l'âge varie du Miocène inférieur au Pleistocène a montré la présence de chambres de conceptacles multiporés marquées par des dépressions, ce qui constitue le caractère diagnostique de *Lithothamnion crispatum*, une algue de la Famille des Hapalidiaceae, cosmopolite et vivant à une large gamme de profondeur dans les océans actuels. La comparaison des structures microscopiques de spécimens fossiles et modernes de *L. crispatum* confirme qu'ils doivent être considérés comme conspécifiques. Par conséquent, cette espèce a une répartition stratigraphique très grande, commençant il y a au moins 20 millions d'années, sans variation morphologique significative dans les parties reproductives ou végétatives de son anatomie.

Mots-clefs : Morphologie ; conceptacles à toit multiporé ; Téthys ; Melobesioideae ; Miocène.

Introduction

According to taxonomic uniformitarianism it is possible to extrapolate the ecological significance of modern species to their fossil counterparts. In most cases this core principle of paleoecology can be safely applied at least for paleoenvironments not older than the Miocene (HAUNOLD *et al.*, 1997; AGUIRRE *et al.*, 2000). To accomplish this goal, recent species need reliable identification in the fossil record achievable through the application of the same criteria used in biological classification. In this way, the biological and paleontological classifications can be made consistent as far as fossil preservation allows. For coralline algae this approach has been developed in various contributions (BRAGA *et al.*, 1993; BASSO *et al.*, 1996, 1997; RASSER and PILLER, 1999, 2000; BASSI *et al.*, 2009; IRYU *et al.*, 2009; AGUIRRE *et al.*, 2012; WOELKERLING *et al.*, 2014; HRABOVSKÝ *et al.*, 2015).

The aim of this paper is to contribute to this development by introducing a new diagnostic character never seen nor described before in the fossil material: the presence of pits surrounding the pore canals in the roof of the sporangial conceptacles. This is the key morphological feature of the non-geniculate coralline *Lithothamnion crispatum* HAUCK, 1878, unknown as a fossil until now. Fossils from the Pleistocene and the Miocene have been compared with modern Mediterranean specimens of *L. crispatum* to assess and study their relationship. The modern cosmopolitan distribution of *L. crispatum* includes the Mediterranean (BRESSAN and BABBINI, 2003; BASSO *et al.*, 2011), the Atlantic Ocean (KEATS *et al.*, 2000; NÓBREGA -FARIAS *et al.*, 2010), the Indian Ocean (SILVA *et al.*, 1996), the Pacific coast of Australia (HARVEY *et al.*, 2003) and the northern coast of New Zealand (NEILL *et al.*, 2015). Living specimens have been collected

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from 2 to 80 m of water depth, as rhodoliths or crusts attached on hard substrates, particularly in well lit waters (KEATS *et al.*, 2000; NÓBREGA-FARIAS *et al.*, 2010; BASSO *et al.*, 2011; PASCELLI *et al.*, 2013; NEILL *et al.*, 2015).

This species has a complex taxonomic history (details in BASSO *et al.*, 2011). In 2000 KEATS *et al.* established that *Lithothamnion superpositum* is conspecific with *Lithothamnion indicum*. Subsequently NÓBREGA-FARIAS *et al.* (2010) merged *L. superpositum* with *Lithothamnion heteromorphum*. Finally, in 2011, BASSO *et al.* synonymized *L. heteromorphum* with *L. crispatum*, the Mediterranean species with nomenclatural priority. As a consequence of these taxonomic revisions, the geographic and ecological distribution of the species has become very broad, encompassing all the oceans and both hemispheres. The key feature that allowed synonymizing these species is the occurrence of pits over the roof of the multiporate conceptacles, a unique feature among Hapalidiales and a diagnostic feature in the delimitation of this *Lithothamnion* species (WILKS and WOELKERLING, 1995). These roof pits are the consequence of the disintegration of the outermost cells in filaments surrounding the pore canals. In the resulting structure the pore canal is slightly raised in the center of a rosette of degenerate cells.

Material and methods

The specimens analyzed come from different localities, with some being studied in previous investigations (Table 1). Different specimens from the same sample or from the same outcrop were grouped together for analysis. Morphological data on Recent *L. crispatum* come from samples collected in the Egads Archipelago of the Western Mediterranean (Marettimo Island, BASSO *et al.*, 2011).

Fossil samples were cut with an abrasive rock saw, and the porosity was then filled with epoxy resin. The surfaces were polished with silicon carbide and glued onto standard glass slides. The excess material was subsequently cut off and the thickness of the sample was reduced to less than 30 µm. The thin sections of coralline algal samples were then observed under light microscope to study and measure their anatomical features. Growth-forms terminology follows WOELKERLING *et al.* (1993). Vegetative anatomy was measured along longitudinal radial sections (QUARANTA *et al.*, 2007; VANNUCCI *et al.*, 2008). The diameter of the cells was measured including the cell wall; cell length was measured as the distance between two primary pit connections including the cell wall (BASSO *et al.*, 1996). Conceptacles were measured along their axial section, *i.e.*, the longitudinal section that cuts a conceptacle medially so that the pore canal is completely visible (AFONSO-CARILLO *et al.*, 1984), in accor-

Table 1: Fossil samples analyzed.

Geographic Position	Age	N° of thin sections	Collector	Useful references
NW Italy-Tertiary Piedmont Basin-Villa San Bartolomeo	Burdigalian	1	VANNUCCI G.	VANNUCCI <i>et al.</i> , 1996, and COLETTI <i>et al.</i> , 2015, for further details regarding the outcrop, the geological setting and coralline algae flora
NW Italy-Tertiary Piedmont Basin-Uviglie	Burdigalian	3	GC	
NW Italy-Tertiary Piedmont Basin-Torre Veglio	Burdigalian	2	VANNUCCI G.	
S France-Sommières Basin-Souvignragues	Burdigalian	1	GC	REYNAUD and JAMES, 2012, for further details on the geological setting of the basin
Czech Republic-Central Parathethys- Židlochovice	upper part of Lower Langhian	1	JH	FILIPESCU and GÎRBACEA, 1997; KOVAČ <i>et al.</i> , 2005; SAINT-MARTIN <i>et al.</i> , 2007; DOLAKOVA <i>et al.</i> , 2008; HRABOVSKÝ <i>et al.</i> , 2015, for information regarding the geological setting and coralline algae flora
Romania-Central Parathethys-Lopadea Veche	upper part of Lower Langhian	1	JH	
Slovakia-Parathethys- Stupava-Vrchná Hora	Upper Langhian	1	JH	
Slovakia-Parathethys- Sandberg	Lower Serravallian	1	JH	
S Italy-Sicily- Castelluccio	Pleistocene	2	DB	

Table 2: Comparison of features and biometry of modern and fossil *L. crispatum*. The specimens of *L. crispatum* from the Lower Serravallian of Slovakia, Sandberg locality, were poorly preserved. Since it was not possible to make an appropriate number of measurements for most of the parameters, they are omitted from the table; VC= ventral core; PF=peripheral zone; s.d. =standard deviation; // = insufficient data cover.

	<i>L. crispatum</i> Burdigalian Tertiary Piedmont Basin NW Italy	<i>L. crispatum</i> Burdigalian Sommières Basin S France	<i>L. crispatum</i> upper part of Lower Langhian Židlochovice Czech Republic	<i>L. crispatum</i> upper part of Lower Langhian Lopadea Veche Romania	<i>L. crispatum</i> Upper Langhian Stupava Slovakia	<i>L. crispatum</i> Pleistocene Castelluccio S Italy	<i>L. crispatum</i> Recent Marettimo S Mediterranean
Thallus organization	monomerous	monomerous	monomerous	monomerous	monomerous	monomerous	monomerous
Ventral core structure	non coaxial plumose	non coaxial plumose	non coaxial plumose	non coaxial plumose	non coaxial plumose	non coaxial plumose	non coaxial plumose
VC cells length	11-28 µm [16 mean; 3.2 s.d.; n=80]	//	10-28 µm [16.8 mean; 4.5 s.d.; n=30]	9-26 µm [16.7 mean; 3.6 s.d.; n=32]	10-26 µm [16.9 mean; 3.5 s.d.; n=42]	13-25 µm [18 mean; n=10]	11-34 µm [18.6 mean; 4.2 s.d.; n=258]
VC cells height	7-12 µm [9.6 mean; 1.6 s.d.; n=80]	//	7-12 µm [10.5 mean; 1.5 s.d.; n=30]	7-11 µm [9.6 mean; 1.2 s.d.; n=32]	6-11 µm [9 mean; 1.5 s.d.; n=42]	7-11 µm [8.5 mean; n=10]	5.5-15 µm [9.5 mean; 2.2 s.d.; n=258]
PF zonation	yes	yes	yes	yes	yes	yes	yes
PF cells length	8-22 µm [13.3 mean; 2.3 s.d.; n=160]	10-15 µm [12.5 mean; 1.5 s.d.; n=30]	9-20 µm [13.7 mean; 3 s.d.; n=50]	8-31 µm [14.3 mean; 4.5 s.d.; n=50]	11-20 µm [13.8 mean; 2.4 s.d.; n=42]	9-19 µm [13.2 mean; 2.5 s.d.; n=90]	7-25 µm [15 mean; 3.1 s.d.; n=332]
PF cells diameter	8-14 µm [10.2 mean; 0.9 s.d.; n=160]	9-12 µm [10.5 mean; 1.5 s.d.; n=30]	9-13 µm [10.5 mean; 0.9 s.d.; n=50]	9-15 µm [11.2 mean; 1.2 s.d.; n=50]	9-12 µm [10.4 mean; 0.9 s.d.; n=42]	8-13 µm [10.4 mean; 1 s.d.; n=90]	7-16 µm [12 mean; 1.7 s.d.; n=332]
Conceptacle chamber diameter	265-425 µm [340 mean; 55 s.d.; n=30]	//	280-510 µm [355 mean; 60 s.d.; n=15]	265-415 µm [350 mean; 50 s.d.; n=14]	260-495 µm [390 mean; 80 s.d.; n=14]	290-550 µm [425 mean; 70 s.d.; n=22]	270-720 µm [430 mean; 128 s.d.; n=18]
Conceptacle chamber height	90-135 µm [115 mean; 10 s.d.; n=30]	//	95-140 µm [115 mean; 15 s.d.; n=15]	110-145 µm [130 mean; 10 s.d.; n=14]	110-150 µm [125 mean; 15 s.d.; n=14]	105-190 µm [140 mean; 20 s.d.; n=22]	100-315 µm [177 mean; 66 s.d.; n=18]
Conceptacle H/D ratio	0.29-0.47 µm [0.34 mean; 0.05 s.d.; n=30]	//	0.25-0.49 µm [0.33 mean; 0.07 s.d.; n=15]	0.29-0.5 µm [0.38 mean; 0.06 s.d.; n=14]	0.27-0.44 µm [0.33 mean; 0.06 s.d.; n=14]	0.26-0.39 µm [0.33 mean; 0.05 s.d.; n=22]	0.15-0.57 µm [0.41 mean; 0.11 s.d.; n=19]
Roof thickness	27-48 µm [36 mean; 6 s.d.; n=30]	//	36-50 µm [42 mean; 5 s.d.; n=15]	40-62 µm [50 mean; 6 s.d.; n= 14]	34-51 µm [40 mean; 5 s.d.; n=14]	38-54 µm [46 mean; 6 s.d.; n=22]	20-45 µm [30 mean]
Number of roof cells	3 to 4	//	3 to 4	3 to 6	3 to 4	3 to 5	4 to 6
Diameter of the pits	19-36 µm [28 mean; 5 s.d.; n=20]	//	26-47 µm [31 mean; 5 s.d.; n=17]	43-59 µm [49 mean; 6 s.d.; n=18]	26-44 µm [32 mean; 4 s.d.; n=20]	26-38 µm [34 mean; 3.5 s.d.; n=20]	//
Length of the degenerate cells	9-20 µm [14 mean; 4 s.d.; n=20]	//	15-26 µm [20 mean; 3 s.d.; n=17]	20-38 µm [29 mean; 5 s.d.; n=18]	12-24 µm [18.5 mean; 3 s.d.; n=20]	15-24 µm [19.3 mean; 2.5 s.d.; n=20]	//
Number of rosette cells	6 to 7	//	//	6 to 7	//	//	5 to 7
Conceptacles position	slightly raised	slightly raised	slightly raised	slightly raised	slightly raised	slightly raised	slightly raised

dance to QUARANTA *et al.* (2007) and VANNUCCI *et al.* (2008). Very large conceptacle chambers, clearly resulting from the merging of two nearby conceptacles, were not considered in statistical analyses. The diameter of the roof pits was measured either in the axial section of conceptacles or in the equatorial section tangential to the roof-top layer of cells. The length of the degenerate cells (*i.e.*, the depth of the roof pit around the pore canal) was measured in the axial section of the conceptacles.

Analysis of variance (ANOVA) was employed to support the morphological comparison of the specimens and LEVENE'S test was used to assess the equality of variances. Since ANOVA can analyze only one character at time, the H/D ratio of asexual conceptacle chambers was used, because it summarizes both conceptacle dimensions. In order to analyze differences and similarities between the samples further, multivariate statistics were performed with PRIMER 6 (KRUSKAL, 1977; FIELD *et al.*, 1982; CLARKE and GORLEY, 2006). BRAY-CURTIS (B-C) samples similarity was calculated on average standardized values of the ventral core (hypothallium) and peripheral zone (perithallium) cell size, conceptacles diameter (D) and height (H), H/D ratio and roof thickness. The size of roof pits was not included in this analysis because of the small number of observations in the fossil and because no reference values was available for the modern specimens. Results, based on B-C similarity, were plotted as hierarchical agglomerative dendrograms.

Results

SYSTEMATIC PALEONTOLOGY

Class Florideophyceae CRONQUIST, 1960

Subclass Corallinophycidae LE GALL & SAUNDERS, 2007

Order Hapalidiales NELSON *et al.*, 2015

Family Hapalidiaceae (GRAY, 1864) HARVEY *et al.*, 2003

Subfamily Melobesioideae BIZZOZERO, 1885

Genus *Lithothamnion* HEYDRICH, 1897

Lithothamnion crispatum HAUCK, 1878

For the complete list of heterotypic synonyms refer to BASSO *et al.* (2011).

Habit and vegetative structure

Fruticose to foliose growth form (Fig. 1). Protuberances from cylindrical to lamellate, up to 6 mm long and 3 mm wide and commonly club-shaped. Thalli with monomerous organization, with crustose portions 100 to 500 μm thick (Fig. 2.A-B). Ventral core up to 100 μm thick composed of plumose filaments that arch toward the thallus surface to form the peripheral zone (Fig. 2.C). Ventral core cells are rectangular, 9 to 28 μm in length and 6 to 12 μm in diameter (Table 2). Peripheral zone with alternation of larger cells and smaller cells (Fig.

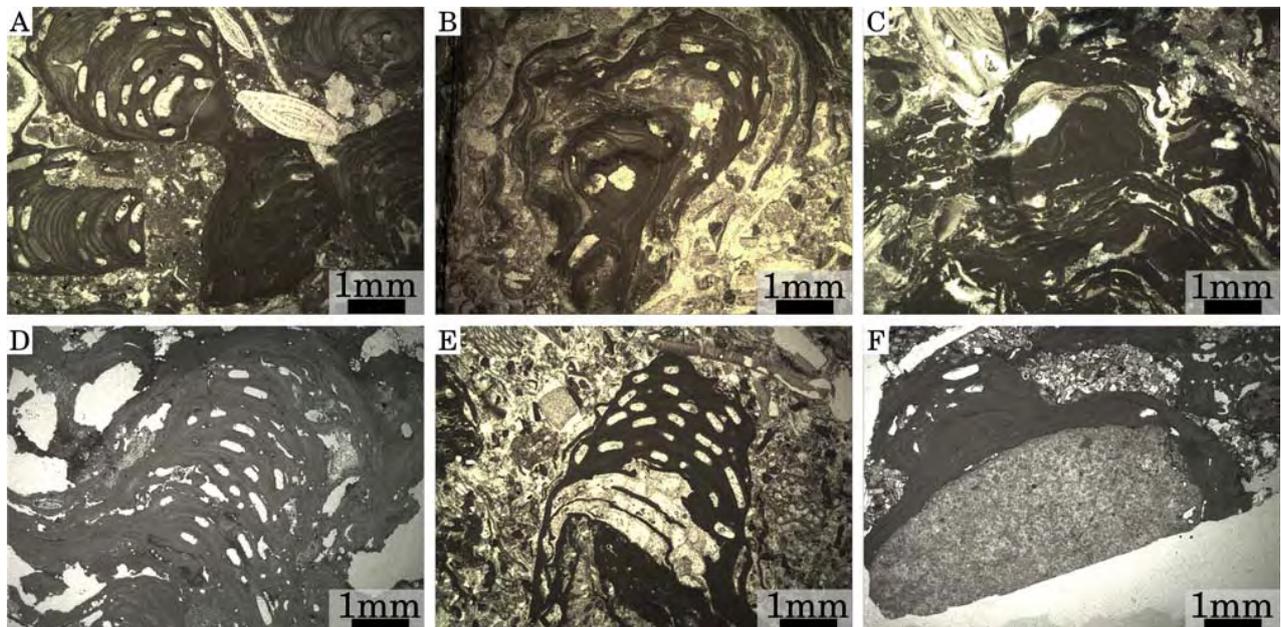


Figure 1: Growth-forms of fossil specimens. (A) Branches, Upper Langhian, Slovakia, Stupava-Vrchná Hora. (B) Wavy crusts, upper part of Lower Langhian, Czech Republic, Židlochovice. (C) Crusts in a rhodolith, Burdigalian, NW Italy, Tertiary Piedmont Basin, Villa San Bartolomeo. (D) Branch formed by stacked crusts, Pleistocene, S Italy, Castelluccio. (E) Branch formed by stacked crusts, upper part of Lower Langhian, Romania, Lopadea Veche. (F) Epilithic crust, Pleistocene, S. Italy, Castelluccio.

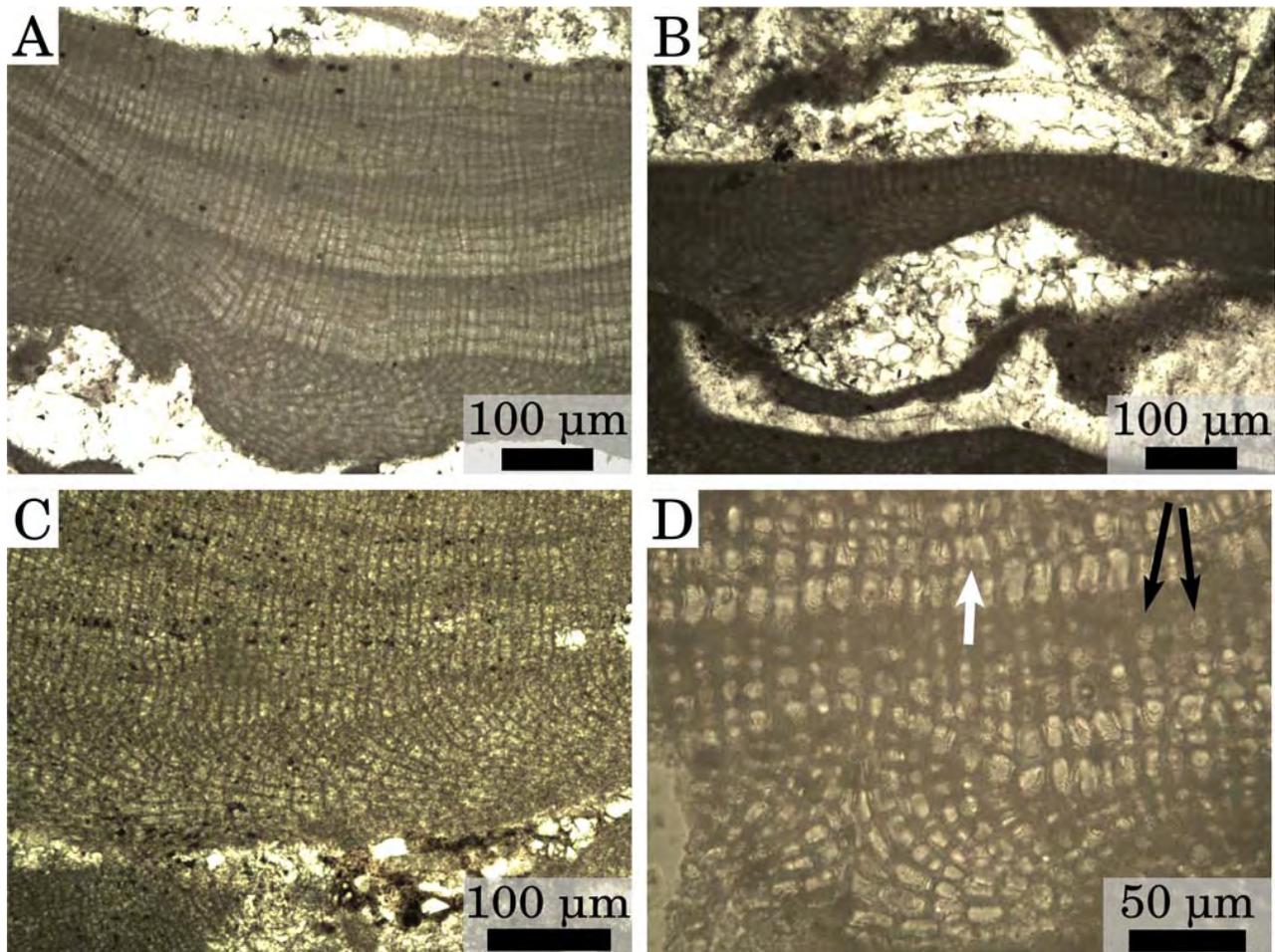


Figure 2: Vegetative anatomy. (A) Thick crust, upper part of Lower Langhian, Romania, Lopadea Veche. (B) Thin crust, upper part of Lower Langhian, Romania, Lopadea Veche. (C) Plumose ventral core, Burdigalian, NW Italy, Tertiary Piedmont Basin, Torre Veglio. (D) High-magnification detail of a secondary ventral-core and of the overlying peripheral zone, Burdigalian, NW Italy, Tertiary Piedmont Basin, Uviglie; white arrow=cell fusion; black arrows=cell filaments with visible primary pits.

2.A). Peripheral zone cells rectangular, 8 to 31 μm in length and 8 to 15 μm in diameter (Table 2). Primary pit connections connect cells of the same filament while cells of adjacent filaments are connected by cells fusions (Fig. 2.D); in Recent specimens secondary pits connections have not been observed (see BASSO *et al.*, 2011). Trichocytes have not been observed. It was not possible to observe clearly the shape of epithallial cells either due to the abrasion of the upper layer of cells or the excessive thickness of the sections. No significant difference in length was observed between the alleged sub-epithallial initials and their inward derivatives, in agreement with the observations of Recent specimens of the species (NÓBREGA-FARIAS *et al.*, 2010, Fig. 4; BASSO *et al.*, 2011, Figs. 6 & 10-11).

REPRODUCTIVE ANATOMY

Only multiporate conceptacles have been observed in the specimens studied. Conceptacle chambers are generally sub-rectangular in shape, 260 to 550 μm in diameter and 90 to 190 μm in height (Table 2; Fig. 3.A-D). Concep-

tacles with very large diameter (generally 700 μm but up to 1000 μm) may result from the merging of two nearby reproductive chambers since such large structures were always observed close to adjacent couples of normal-sized conceptacles with the separating wall partially dissolved (Fig. 3.E-F). The conceptacles protrude slightly above the surrounding thallus surface (Fig. 3.A-F). The H/D (height/diameter) ranges between 0.25 and 0.5 (Table 2). The roof of the conceptacle is 27 to 62 μm in thickness and is composed of 3 to 6 cells (Table 2). The roof of the conceptacles is pitted with depressions, resulting from the disintegration of the uppermost cells of the filaments around the pore canals. The roof pits have a diameter ranging from 19 to 59 μm (Table 2; Fig. 3). The degenerate cells are 9 to 38 μm in length (Table 2). Rosettes of degenerate cells around the pore canal were observed in sections tangential to the roof of the conceptacles, with 6 to 7 cells counted around the pore canal (Table 2; Fig. 3.G-H).

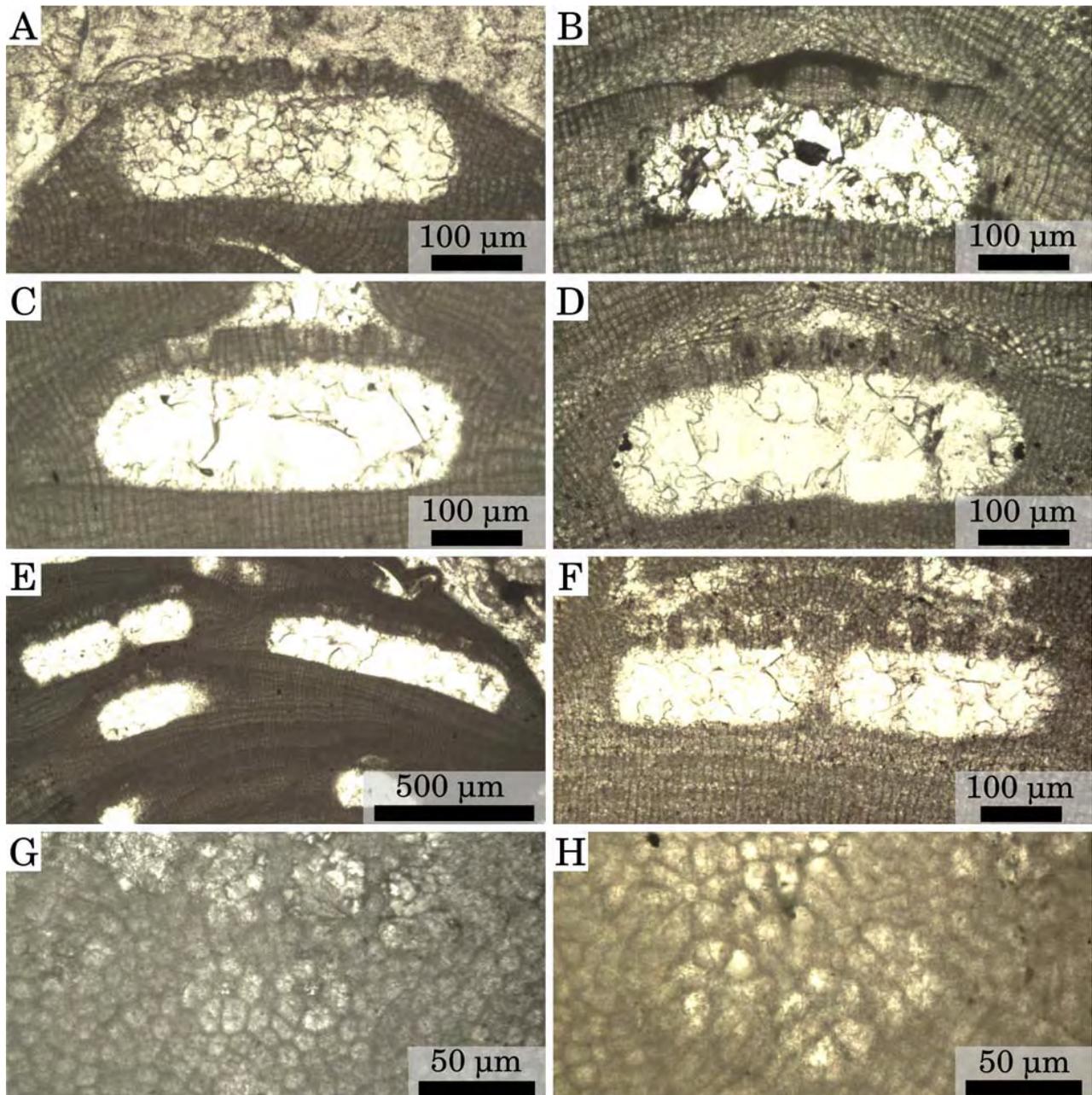
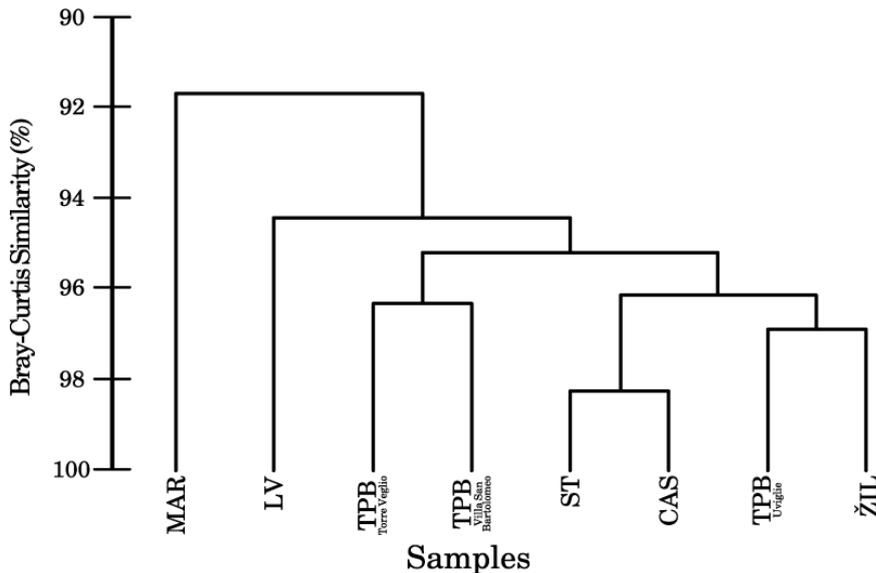


Figure 3: Reproductive anatomy. (A) Multiporate conceptacle, Burdigalian, NW Italy, Tertiary Piedmont Basin, Villa San Bartolomeo. (B) Multiporate conceptacle, Pleistocene, S. Italy, Castelluccio. (C) Multiporate conceptacle, upper part of Lower Langhian, Romania, Lopadea Veche. (D) Multiporate conceptacle, Upper Langhian, Slovakia, Stupava-Vrchná Hora. (E) Conceptacles almost merged (left) and conceptacles fully merged (right), upper part of Lower Langhian, Romania, Lopadea Veche. (F) Conceptacles almost merged, upper part of Lower Langhian, Czech Republic, Židlochovice. (G) Rosette of degenerate cells around the pore canals, Pleistocene, S. Italy, Castelluccio. (H) Rosette of degenerate cells around the pore canals, upper part of Lower Langhian, Romania, Lopadea Veche.

STATISTICAL ANALYSIS

LEVENE'S test verified that the variances of the H/D ratio of the samples are homogeneous, allowing the use of ANOVA (F distribution with 8 and 97 degrees of freedom). The calculated F-ratio is 1.64, so that we cannot reject the null hypothesis that the samples belong to a single population. That is, ANOVA demonstrates that there are no statistically significant differences between the mean H/D ratios of the conceptacles of the different samples. Multivariate statistical analysis of the main biometric variables

of vegetative and reproductive anatomy shows that all the samples are grouped together at a very high level of B-C similarity (>90%). The dendrogram displays two main clusters: fossil and modern *L. crispatum* (Fig. 4). Within the fossils, the largest difference is recognized between the Romanian sample of Lopadea Veche and the remaining fossil samples (Fig. 4). This latter group is characterized by a >95% of B-C similarity and encompasses samples of different age and different geographical origin (Fig. 4).



◀ **Figure 4:** Multivariate statistical analysis based on B-C similarity of the average value for each specimen of vegetative cell size and morphological characters of the multiporate conceptacles (see text for details); hierarchical agglomerative dendrogram. TPB=Tertiary Piedmont Basin, Burdigalian. ŽIL= Židlochovice, upper part of Lower Langhian, Czech Republic. LV=Lopadea Veche, upper part of Lower Langhian, Romania. ST=Stupava-Vrchná Hora, Upper Langhian, Slovakia. CAS=Castelluccio, Pleistocene, S. Italy. MAR=Marettimo, modern, Mediterranean.

PALEOECOLOGICAL DISTRIBUTION AND ASSOCIATED SKELETAL ASSEMBLAGES

The fossils occur in various depositional environments and are associated with different benthic organisms.

In the outcrops of Tertiary Piedmont Basin, coralline algae dominate the skeletal assemblages, with barnacles and larger benthic foraminifers also very common. These rhodalgal carbonates were deposited in a mesotrophic, high-energy environment at tropical latitudes (DERCOURT *et al.*, 2000; MOSBRUGGER *et al.*, 2005; COLETTI *et al.*, 2015).

The Sommières Basin was a small inlet of the Alpine Molasse Basin and located at the same latitude as the Tertiary Piedmont basin (DERCOURT *et al.*, 2000). Bryozoans, barnacles, echinoids and coralline algae were the main carbonate producers; larger benthic foraminifers were, in this case, very rare. These heterozoan carbonates were deposited in a tide-controlled environment under mesotrophic conditions as suggested by bryozoan dominance and the presence of authigenic phosphates (REYNAUD and JAMES, 2012).

Red algal limestones from Židlochovice were deposited in a stable shallow normal marine environment with low terrigenous input and seagrass meadows (DOLÁKOVÁ *et al.*, 2008, 2014). The sediments were deposited in a warm temperate to subtropical climate with marked seasonality (DOLÁKOVÁ *et al.*, 2014). Coralline algal to coralline algal-bryozoan limestones point to a cooler environment than the coral – coralline algal limestones of the southern sites of the Central Paratethys (KOVÁČ *et al.*, 2005; DOLÁKOVÁ *et al.*, 2008).

Limestones from Lopadea Veche correspond to the Gârbova de Sus Formation (Transylva-

nian Basin) that is characterized by nodular limestones (FILIPESCU and GÎRBACEA, 1997). The foraminiferal associations suggest that limestones and marls were deposited in a shallow water environment. Other common components are bivalves, bryozoans and rhodoliths (FILIPESCU and GÎRBACEA, 1997). The occurrence of patch reefs in the distinct reefal Podeni Member (limestone of Gârbova de Sus Formation) points to subtropical condition (SAINT-MARTIN *et al.*, 2007).

Sands, sandstones and limestones of the Stupava Member from the Vienna Basin contains the large benthic foraminifer *Amphistegina* (HRABOVSKÝ and FORDINAL, 2013) in high abundance. Sandy limestones were deposited within a dynamic environment of normal salinity at the proposed depth of 35-80 m, affected by terrigenous input. The studied site was located in a warm, temperate to subtropical climatic zone (HRABOVSKÝ and FORDINAL, 2013).

The Sandberg Member was proposed for marginal transgressive deposits of the Upper Langhian of the Vienna Basin (BARÁTH *et al.*, 1994). Limestones were deposited in a circalittoral or infralittoral environment, on rises protected from terrigenous input (BARÁTH *et al.*, 1994). Although terrestrial ecosystems suggest a subtropical climatic zone for these deposits, the marine microfauna indicates a cooling of the environment compared to the rest of the Langhian period (KOVÁČ *et al.*, 2005).

Heterozoan carbonates of the Pleistocene sequences of Castelluccio are largely dominated by bryozoans and coralline algae. The fossil association suggests a cool-temperate climate, probably just slightly colder than the modern Mediterranean's.

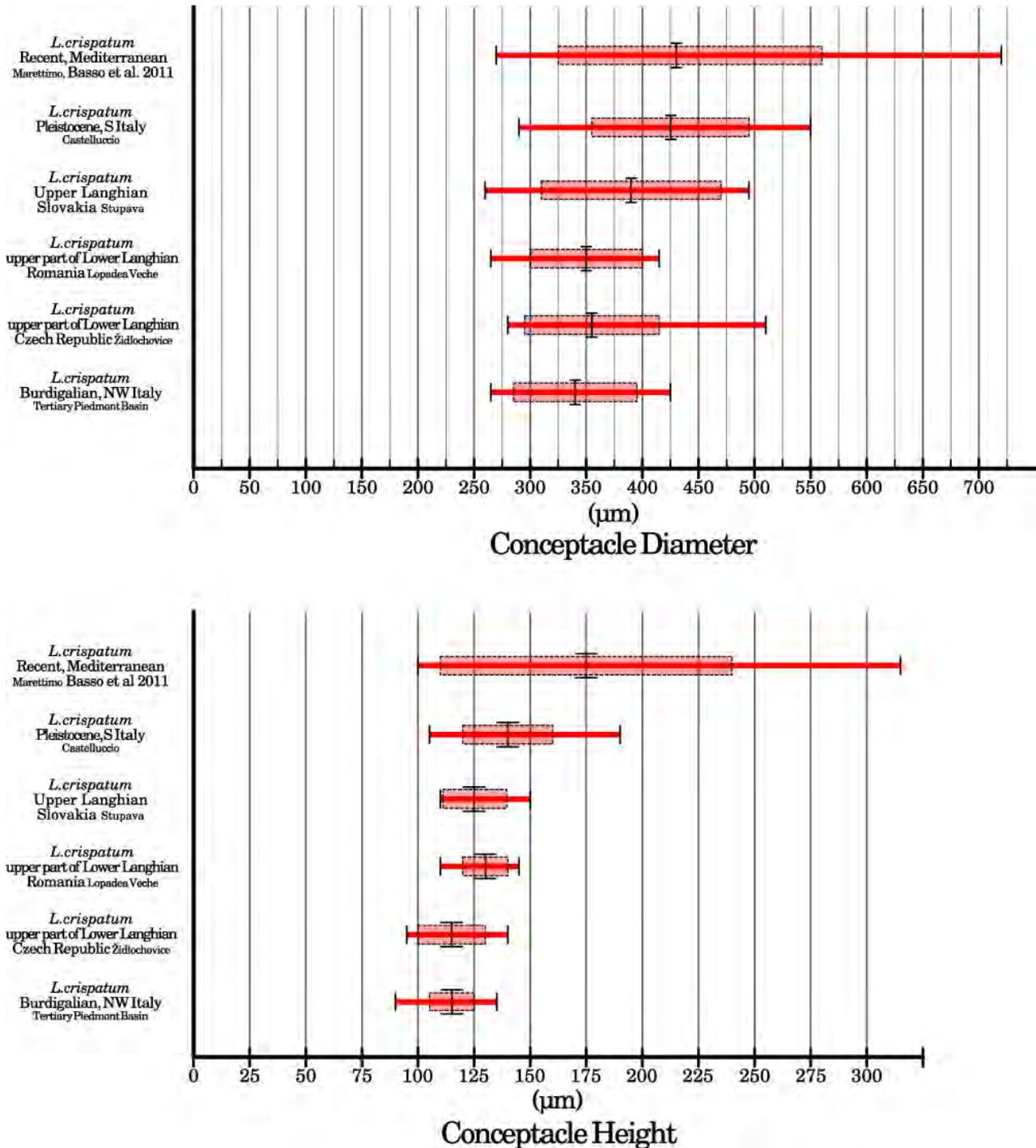


Figure 5: Comparison of conceptacle chamber dimensions between fossil and recent samples. The long red bar indicates total range; the pale red rectangle indicates the standard deviation around the average which is represented by the large vertical black bar.

Discussion

COMPARISON BETWEEN FOSSIL SAMPLES

Fossil samples have a remarkably similar vegetative anatomy, without significant variation in thallus organization or the range and average size of vegetative cells (Table 2; Fig. 2).

Reproductive anatomy is also rather uniform among fossil samples (Table 2; Fig. 3). Both the total range and the average (\pm s.d.) interval overlap consistently, for both conceptacle

diameter and conceptacle height (Fig. 5). Pleistocene specimens have the largest conceptacles among all the fossils, while those from the Tertiary Piedmont Basin have the smallest (Table 2; Fig. 5). Conceptacle H/D ratio is very consistent among the samples and most of the average values lie between 0.33 and 0.34, *i.e.*, conceptacle with diameter three times their height (Table 2). Only the sample from the upper part of the Lower Langhian of Lopadea Veche (Romania) is characterized by a

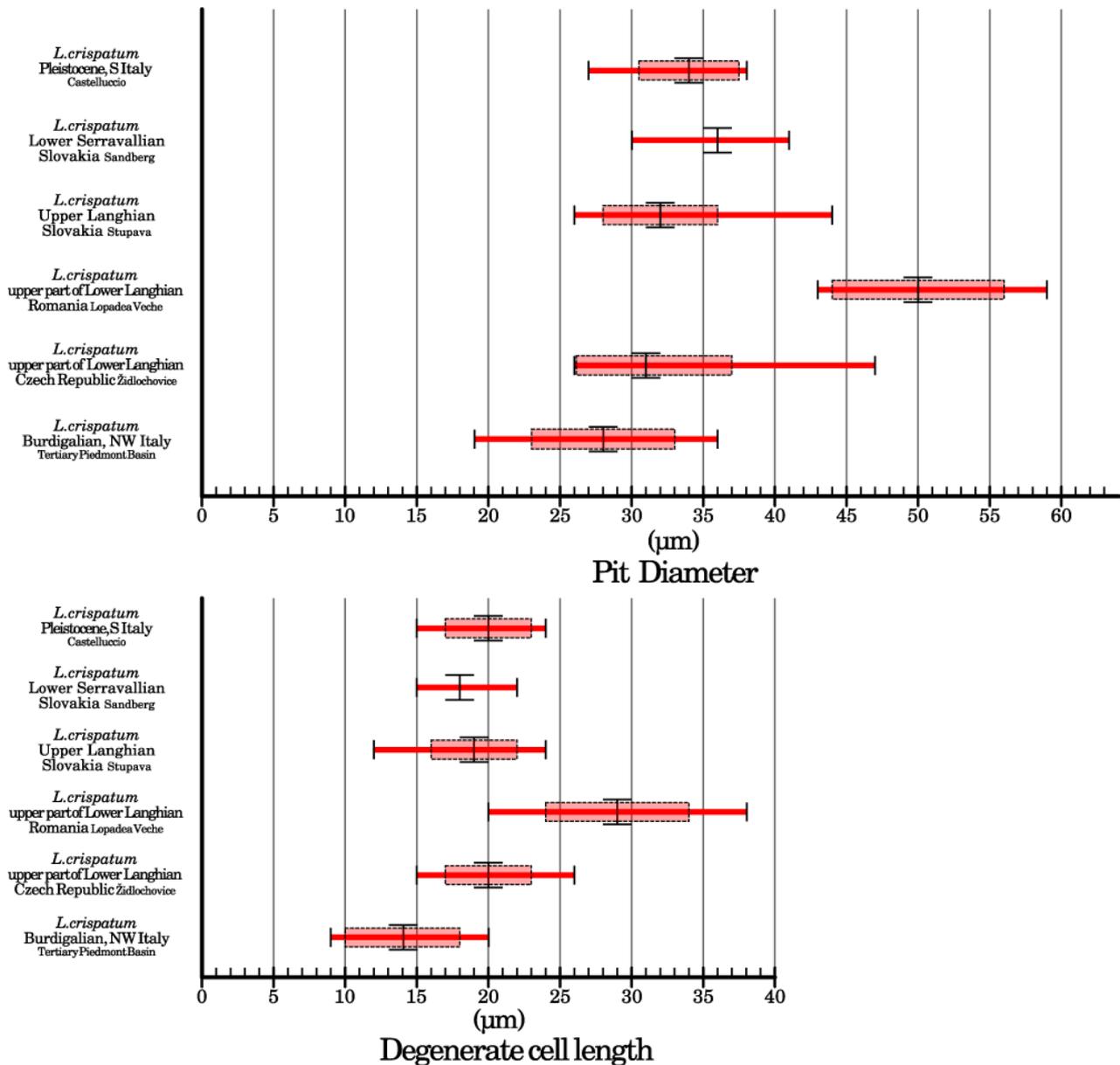


Figure 6: Comparison of the size of roof pits between fossil samples. The long red bar indicates total range; the pale red rectangle indicates the standard deviation around the average which is represented by the large vertical black bar.

slightly higher value of H/D ratio, due to its slightly higher than average conceptacle chambers (Table 2). ANOVA testing of this morphological parameter confirms that there is very little variation among the different samples and that they probably belong to the same statistical population.

Besides conceptacle size, all the other features of the reproductive anatomy of the fossil samples are remarkably similar. The number of cells that compose the roof and roof thickness are uniform. Large conceptacles, created by the merging of two nearby conceptacles, were observed in all the samples (Table 2).

Some differences may be observed in the morphology of roof pits (Fig. 3.G-H). While the number of cells composing the rosette around the pore canals in the examined specimens is always 6 to 7, the diameter of the pits and the length of the degenerate cells are quite variable (Table 2; Fig. 6). Most of the samples, especially those of the Tertiary Piedmont Basin, have small and shallow pits (Fig. 3.A, G), but the one from the upper part of the Lower Langhian of Romania has larger and deeper pits (Figs. 3.C, H & 6). These differences cannot be related to differential preservation of the structures. Although mechanical and biological abrasion of coralline surfaces are common, the Romanian sample shows preserved walls bet-

ween the cells forming the rosettes (Fig. 3.H), so that abrasion processes must be ruled out. The internal details of these structures are very well preserved and it is possible to distinguish between the cavity of the central pore channel and the cavities of the surrounding degenerate cells (Fig. 3.C). Differential dissolution would have resulted in blurred structures and therefore this process is also unlikely.

In the dendrogram (Fig. 4) the Romanian sample is separated from all other fossils indicating that, even setting aside the roof pits, this sample is somewhat different from the others. However, taking in to account the other morphological variables, there is still an almost complete overlap between the ranges and the averages of this sample and those of the remaining fossils (Table 1). Since the differences are largely overwhelmed by the similarities and no comprehensive information exists on roof-pit morphological variability in modern *L. crispatum*, the Romanian specimens are considered conspecific with the other fossils examined.

In addition to the fossils discussed above, another specimen studied by Harlan JOHNSON (1962, 1964) deserves attention. The specimen is Eocene in age and was collected in the island of Ishigaki (Ryukyu, southern Japan). It was initially misidentified as *Lithothamnion vaughanii* HOWE, 1919 (JOHNSON, 1962), and later as *Mesophyllum vaughanii*. The published picture shows two conceptacles with clearly pitted roof (JOHNSON, 1962, Pl. 13, fig. 1; JOHNSON, 1964, Pl. 6, fig. 6). According to JOHNSON's description, the specimen has perithallial cells 10 to 19 μm in length and 8 to 11 μm in diameter, and conceptacles are 191 to 330 μm in diameter and 102 to 132 μm in height (JOHNSON, 1964). The vegetative anatomy is similar to that of the other fossils considered here, but its conceptacles have a significantly smaller diameter. Since a revision of JOHNSON's material is beyond the scope of this work it is impossible to assess with confidence its conspecificity with *L. crispatum* as circumscribed in this paper. However, we suggest that multiporate conceptacles with pitted roofs could have appeared as early as the Eocene.

COMPARISON WITH MODERN *LITHOTHAMNION CRISPATUM*

According to WILKS and WOELKERLING (1995), KEATS *et al.* (2000), NÓBREGA-FARIAS *et al.* (2010) and BASSO *et al.* (2011), the presence of pore canals bordered by a rosette of degenerate cells is a diagnostic feature that is unique to the genus *Lithothamnion*. It allows the identification

of *Lithothamnion crispatum*, so that the fossil specimens examined must be included in this species. Setting aside this key feature, an accurate and direct comparison with the Mediterranean specimens (Marettimo, Egadi Islands, southern Tyrrhenian Sea) reveals that the fossil and modern groups are morphologically extremely similar (Figs. 7 - 8). The vegetative anatomy is almost identical in both ranges and average values (Table 2; Fig. 7). Reproductive anatomy is also similar: the conceptacles of modern *L. crispatum* are similar in size to the fossil ones, and so are the H/D ratio and the roof characteristics (Table 2; Fig. 8). The multivariate statistical analysis further emphasizes the similarity between Recent and fossil *L. crispatum*, since the two groups are clustered at more than 90% of B-C similarity. On the other hand, it must be recognized that the multivariate analysis separates the sample from Marettimo from its fossil counterparts (Fig. 4). This separation is caused by the larger average size of the conceptacles of the Recent specimens. The largest difference occurs between the lower Burdigalian samples of the Tertiary Piedmont Basin and the Marettimo sample, while the Pleistocene sample from Castelluccio is the closest to the modern one (Table 2; Fig. 5). However, since the total ranges of the two groups overlaps completely, these differences should be considered within the natural variability of the species (Fig. 5). The outcome of the analysis of variance performed on the H/D ratio also strengthen this hypothesis. The Marettimo specimens and the fossils also share similar morphology and a common diameter of the roof pits, length of the degenerate cells, and number of cells in the rosette (Fig. 8.C-D). Large roof pits, as large as those of the sample from Romania, were observed in the lectotype from the Adriatic Sea, Mediterranean (BASSO *et al.*, 2011, Figs. 13-14), suggesting that the size of the roof pits is a quite variable feature.

A general comparison with data from other papers on modern *L. crispatum* is more difficult since biometric data are usually provided just as total range, and not all the variables are measured every time. However, it should be pointed out that although all the modern specimens are grouped together by the presence of roof pits, minor morphological differences seem to exist amongst them (BASSO *et al.*, 2011, Table 1). Therefore, the variability displayed by the different populations of *L. crispatum* in the world's oceans is comparable to, if not greater than, the variability observed amongst the various fossil populations.

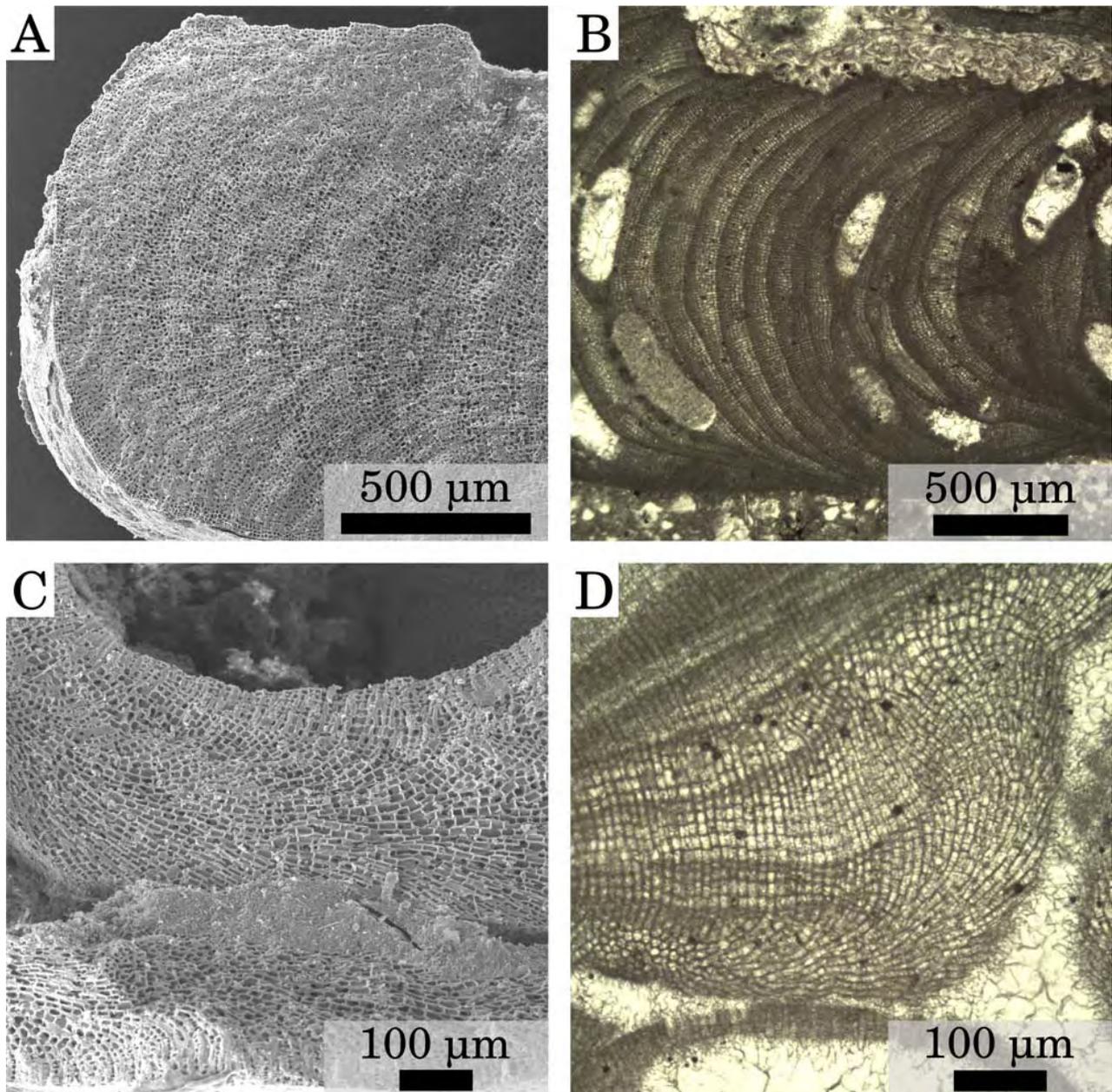


Figure 7: Comparison of the vegetative anatomy of fossil and Recent *L. crispatum*. (A) Zoned branch, Recent, Mediterranean, Egadi Islands, Marettimo. (B) Zoned branch, Upper Langhian, Slovakia, Stupava-Vrchná Hora. (C) Plumose ventral-core, *L. crispatum* lectotype, Recent, Rovigno, Mediterranean, Northern Adriatic, modified after Basso *et al.* (2011). (D) Plumose ventral-core, Upper Langhian, Slovakia, Stupava-Vrchná Hora.

Since no morphological evidence can be used to separate modern *L. crispatum* from the fossil corallines analyzed in this work, they must be considered conspecific, and consequently the stratigraphic range of *L. crispatum* is extended at least to the Burdigalian.

Conclusions

Fossil and modern specimens share the same vegetative and reproductive anatomy and are characterized by the same unique diagnostic feature: the presence of a rosette of degenerate cells (a pit in axial sections) around the multiporate conceptacle pore canals. Both qualitative comparison and statistical analyses

show that their morphology is remarkably similar. The fossil specimens analyzed here are thus conspecific with the modern *Lithothamnion crispatum*, whose stratigraphic range is extended back at least to the Burdigalian. The species has been found associated with markedly different skeletal assemblages and in very varied climatic conditions throughout the Cenozoic. The fossil record, consistent with its modern cosmopolitan distribution, suggests a remarkable adaptability. Although the morpho-functional significance of *L. crispatum* roof pits remains unknown, they seem to be a successful and persistent feature.

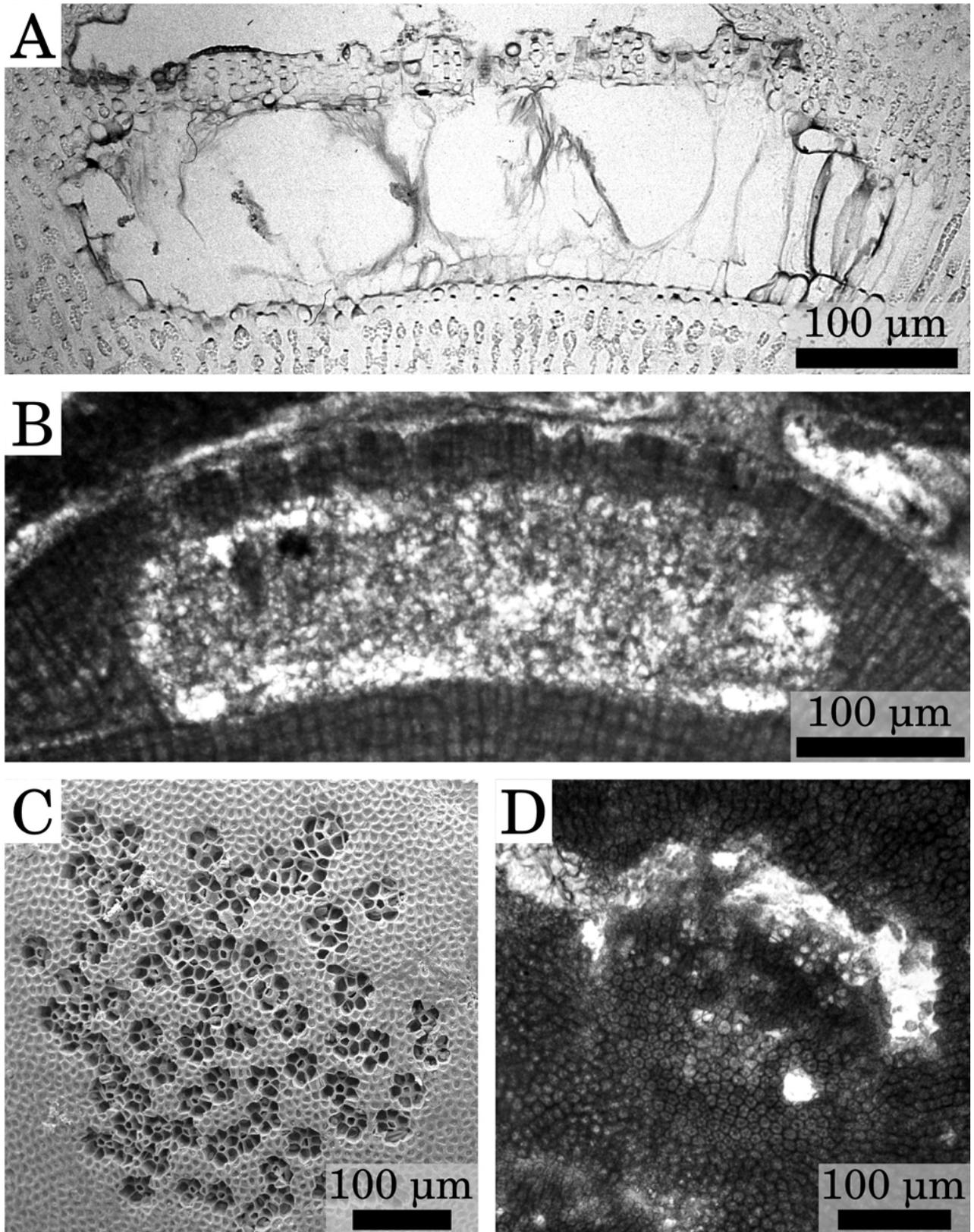


Figure 8: Comparison of the reproductive anatomy of fossil and recent *L. crispatum*. (A) Conceptacle chamber, Recent, Mediterranean, Egadi Islands, Marettimo. (B) Conceptacle chamber, Burdigalian, NW Italy, Tertiary Piedmont Basin, Villa San Bartolomeo. (C) Rosette of degenerate cells around pore canals, Burdigalian, NW Italy, Tertiary Piedmont Basin, Torre Veglio. (D) Rosette of degenerate cells around pore canals, Recent, Mediterranean, Egadi Islands, Marettimo.

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