

Rethinking holotypes: how environmental silicon shapes sponge skeletons and their taxonomic interpretation

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Abstract

Routine taxonomic identification of sponges (phylum Porifera) relies largely on skeletal features and, despite the development of molecular markers, the skeletal approach persists because of its practicality. However, skeletal variability of uncertain origin often complicates species-level diagnoses. Using three common Mediterranean siliceous sponges, we experimentally demonstrate that environmental concentrations of silicic acid (dSi)—the raw material used by sponges for skeleton formation—is a major driver of skeletal diversity, significantly influencing the diversity of skeletal elements (spicules) produced and their dimensions. Most wild individuals of these three demosponges exhibit underdeveloped spicule inventories in their natural habitats, which are characterized by low dSi concentrations (~1 µM). When transferred to laboratory conditions with experimentally elevated environmental dSi (up to 100 µM), sponges develop more complex spicule inventories. The experimentally induced skeletal differences are so pronounced that low-dSi and high-dSi conspecifics could easily be misidentified as different species in skeletal-based studies. The findings further suggest that shallow-water siliceous demosponges, systematically outcompeted by diatoms in terms of dSi uptake, frequently encounter dSi concentrations in their habitats below the threshold required for full skeleton development. Importantly, this effect extends to museum-stored holotypes. Holotypes originating from sites with unusually high dSi relative to the mean across the species' distribution range are likely to produce fully developed skeletons that do not match the naturally underdeveloped skeletons prevailing in most conspecifics. Conversely, holotypes from habitats with average dSi concentrations across the species' natural distribution range (typically low dSi) will more accurately represent the skeleton found in most individuals, although this skeleton will probably correspond to an underdeveloped version of the species' full skeletal potential. Sponge biologists need to rethink the way in which sponge species are represented by their holotypes and how holotype skeletal traits can be better used for species' diagnosis. Holotype studies should be systematically contextualized through assessment of dSi concentrations at the type locality and across the species' distribution ranges.

Keywords holotype representativeness, skeletal variability, character reliability, skeleton-based taxonomy, taxonomy of Porifera

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Introduction

Every animal species formally described has a designated, single physical specimen—the holotype—that serves as the official reference for the traits defining the species and distinguishing it from others. In sponges (phylum Porifera), taxonomic identification has largely relied, in addition to the assessment of macroscopical body features, on the form and size of the skeletal elements (generically referred to as spicules) and their comparison with those of the holotype. Even today, with the advent of molecular tools for species' recognition, an initial study of the skeletal elements remains fundamental for validating the taxonomic identification of the individual that will provide molecular markers for the species.

Porifera comprise four taxonomic classes: one characterized by calcareous spicules (class Calcarea) and three forming siliceous spicules (Homoscleromorpha, Hexactinellida, and Demospongiae). By diversity, the class Demospongiae is the most complex, comprising 8150 species, which account for 82.5% of all known Porifera. For nearly two centuries, demosponge taxonomists have engaged in extensive debates about how to interpret and systematize skeletal diversity. In cases of similar external appearance, as occurs with many congeneric species, it often remains unclear whether the presence/absence of a spicule category or substantial differences in its dimensions, reflects distinct species or merely intraspecific variability. A paradigmatic example is provided by the demosponge *Petrosia* cf. *fici-formis*, a common Atlantic-Mediterranean species. Some individuals contain spicules in the form of only oxeas, while others contain oxeas, styles, and strongyles. Differences in the interpretation of such skeletal variability have led to long-standing discussions about whether this reflects within-species variation or a cryptic species-complex (Pulitzer-Finali 1983, Weerdt and Soest 1986, Bavestrello *et al.* 1994, Pansini 1996).

A major challenge in assessing the taxonomic value of skeletal variability is that its underlying causes remain poorly understood. Before molecular tools became widespread, the framework of Demospongiae classification was erected mostly on skeletal features (Sollas 1888, Topsent 1928, Lévi 1973), under the assumptions that both the number of spicule types a sponge can form and their dimensions were genetically predetermined and, therefore, reliable criteria for delineating species and higher-rank taxa. During the second half of the 20th century, pioneering experiments with freshwater sponges revealed that when silicic acid—the soluble compound demosponges use to build their siliceous skeletal elements—was available at high concentration in the water, the sponges formed spicules that were somewhat shorter and thicker (e.g. Elvin 1971, Pé 1973). These studies introduced the idea that environmental factors can modulate spicule size. Subsequent work on marine demosponges revealed that ambient concentrations of silicic acid can not only affect spicule size, but, more importantly, influence the formation of particular spicule types (Maldonado *et al.* 1999, Valisano *et al.* 2012). These studies suggested that below a threshold concentration of dissolved silicic acid (dSi),

the phenotypic expression (i.e. formation) of some spicule types may be inhibited. This connection between high dSi availability and formation of a more diverse spicule inventory aligns with observations that the kinetics of dSi consumption by every sponge species investigated to date (six demosponges and one hexactinellid; Fig. 1A) reach maximum rates only at experimental dSi concentrations at least 100 times higher than those typically available in sponges' natural habitats (Reincke and Barthel 1997, Maldonado *et al.* 2011, 2020, López-Acosta *et al.* 2016, 2018). When these different observations are brought together, concerns arise about whether sponges chronically exposed to ambient dSi concentrations far below those required for optimal dSi uptake may experience problems in building their siliceous skeleton. Could such a continued low dSi availability limit or inhibit the formation of certain spicule types, cause malformation in others, or reduce overall spicule size?

In this context, a study on Norwegian geodiids revealed that the cytochrome *c* oxidase subunit I (*COI*) sequence of *Geodia simplicissima*, a species originally described from only shallow waters, was identical to that of deep-water specimens of *Geodia barretti*, despite well-recognizable phenotypic differences in colour, external morphology, and spicule morphology (Cárdenas and Rapp 2013). In shallow-water specimens, several spicule types (i.e. microrhabds, sterrasters, and triaenes) were substantially smaller, irregular in shape, or underdeveloped; other spicule types (oxyasters and strongylasters) were normal in shape but smaller. In contrast, regular spicules consistently appeared in individuals from depths below 40 m, where dSi concentrations are at least twice higher ($> 8 \mu\text{M}$), depending on depth, than in shallow waters ($\sim 2.5 \mu\text{M}$). From these observations, it was hypothesized that both species are synonyms and that the lower dSi concentration in shallow waters disrupted spiculogenesis (Cárdenas and Rapp 2013). Yet, in this scenario, a major intriguing question remains: Why values of $2 \mu\text{M}$ dSi, which are typical in most shallow-water habitats of the modern ocean (Nelson *et al.* 1995, Tréguer *et al.* 1995, 2021), do not allow the correct formation of the sponge spicule complement?

The response may be that the ancient systems that sponges use to incorporate dSi are maladapted to function efficiently in the modern ocean (Maldonado *et al.* 1999, 2020, Shimizu *et al.* 2024, Leria and Maldonado 2026). This paradoxical situation may be better understood through a deep-time perspective. Fossil evidence (Botting *et al.* 2015, Chang *et al.* 2017, Turner 2021, Wang *et al.* 2024) and molecular data (Erwin *et al.* 2011, Ma and Yang 2016, Dohrmann and Wörheide 2017, Shimizu *et al.* 2024, Leria and Maldonado 2026) indicate that the silicification system of sponges became functionally assembled in the Early Cambrian, for both demosponges (515 Mya) and hexactinellids (around 528 Mya). At that time, ambient dSi concentrations exceeded $1000 \mu\text{M}$ (Maliva *et al.* 1989, Siever 1992, Jurkowska and Świerczewska-Gładysz 2024). Throughout the Palaeozoic (541–252 Mya), oceanic dSi concentration slowly declined due to proliferation of silicifiers, with a more dramatic decrease in the Mesozoic (251–66 Mya) driven by the emergence, diversification, and ecological expansion of diatoms (Maliva *et al.* 1989, 2005,

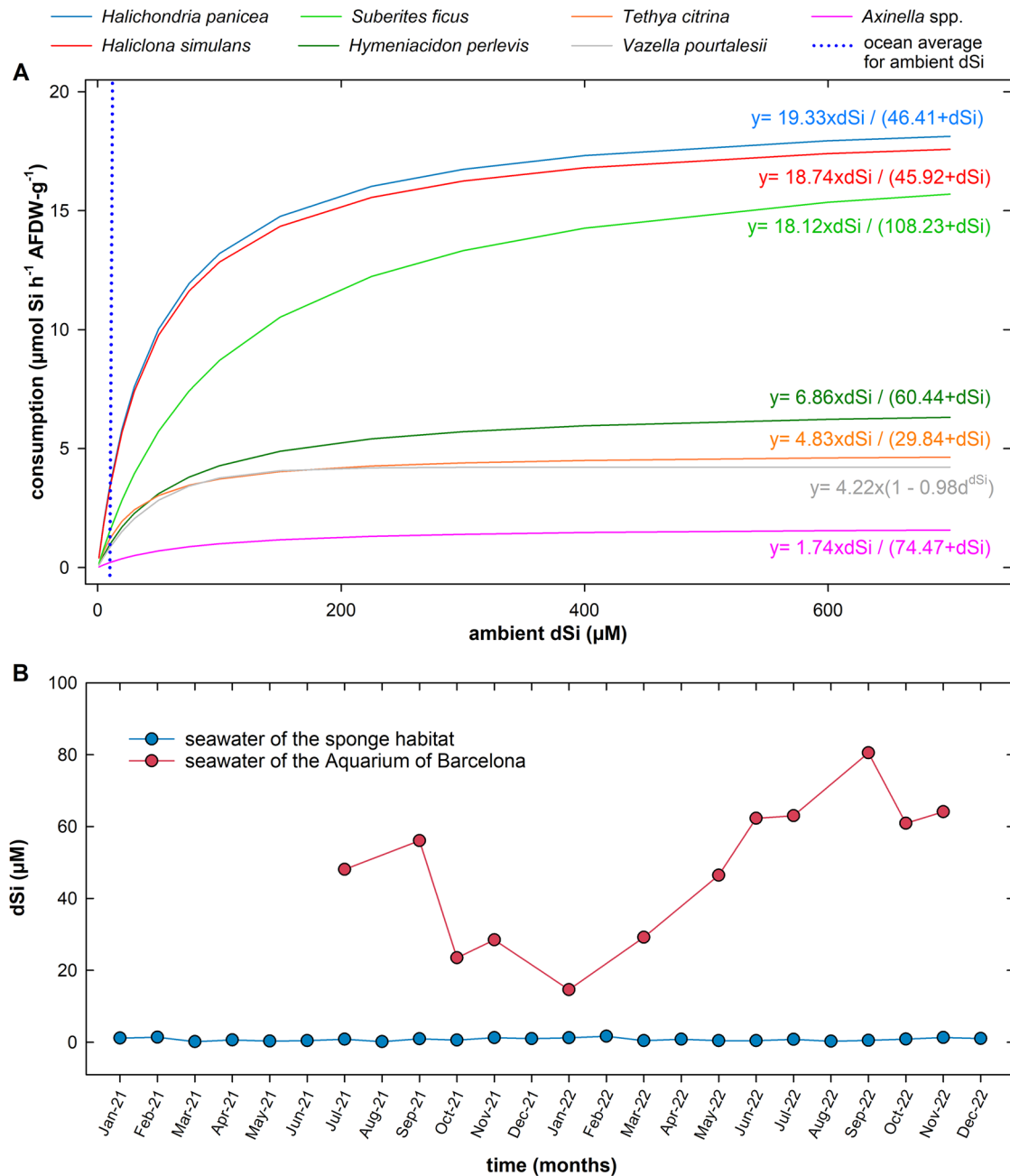


Figure 1 Consumption of silicic acid (dSi) by sponges and environmental availability of this nutrient. A, all six demosponges investigated to date follow a Michaelis–Menten kinetics, whereas the only hexactinellid investigated (*Vazella pourtalesii*) better fits an exponential rise to a saturable maximum, following data from Maldonado *et al.* (2020). In all cases maximum rate of dSi incorporation is reached at ambient dSi concentrations above 100 μM , i.e. a much higher than the dSi available in the natural sponge habitats (Fig. 1B) and higher than the average dSi concentration estimated for the global ocean (10 μM). B, concentration of dSi in the natural sponge habitat at the Santa Anna Point (Blanes, Spain; low-dSi condition) and in the experimental running-seawater conditions at the Aquarium of Barcelona (high-dSi condition).

Siever 1991, Conley *et al.* 2017). By the end of the Cretaceous (66 Mya), global ocean dSi levels had fallen close to the low range characterizing the modern ocean, which averages less than 10 μM in the photic zone and around 70 μM in the deep ocean (Nelson *et al.* 1995, Tréguer *et al.* 1995). Therefore, in most photic habitats of the modern ocean, dSi concentrations are probably too low for efficient utilization by the ancient silicification systems of sponges, particularly

lineages evolutionarily forged in pre-diatom, dSi-rich oceans (Maldonado *et al.* 1999, 2020, Leria and Maldonado 2026).

The potential consequences of a chronic dSi scarcity for siliceous skeleton formation in sponges remains poorly explored, particularly in the context of taxonomic practice. Importantly, it has not been considered that holotypes preserved in museum collections to be used as primary taxonomic references for species' comparisons may themselves reflect varying degrees

of environmentally induced skeletal underdevelopment, especially if they inhabited shallow-water, dSi-poor habitats. In this study, we experimentally generated skeletal diversity in three common Mediterranean demosponges to assess the extent and taxonomic implications of environmentally driven skeletal plasticity. Our findings provide an empirical basis to reassess the taxonomic weight assigned to skeletal traits, the interpretive status of holotype skeletal features, and the phenetic approaches in sponge taxonomy.

Materials and methods

Studied species

The experimental study involved three common species from Mediterranean sublittoral rocky habitats: *Crambe crambe* (Schmidt, 1862), *Agelas oroides* (Schmidt, 1864), and *Petrosia ficiformis* (Poiret, 1789). Swimming larvae of *C. crambe* and adult specimens of *A. oroides* and *P. ficiformis* were collected by scuba-diving at depths of 10 to 15 m at the Santa Anna Point (latitude 41.67, longitude 2.80; Blanes, Mediterranean coast of Spain) and taken alive to the laboratory for further experimental procedures.

The taxonomic identity of the local populations sampled for this study has been verified during previous studies that used specimens collected from these same populations to obtain a chromosomal genome sequence for the species. In the case of *C. crambe*, the *COI* barcoding fragment ('Folmer' fragment) of the sample was found to be identical to haplotype 1 of a previous individual studied from this same population (Maldonado *et al.* 2025). For *A. oroides*, there is no type material barcoded, but intergenic transcribed spacer (ITS) sequences were available from a different *A. oroides* specimen (ZMA POR 14435; INSD accession number: DQ075808, identified by F.J. Parra-Velandia, S. Zea, and R.W.M. van Soest), which proved to be 100% identical to the ITS sequences of the specimen from the herein studied population (Pita *et al.* 2025). In the case of *P. ficiformis*, sequences of type material were not available for taxonomic validation either, but the mitochondrial *COI* (Folmer region) of an individual collected from the herein studied population was found to be identical (Steindler *et al.* 2025) to *Petrosia ficiformis* reference material provided from NCB Naturalis, Leiden, identified by Nicole de Voogd (SNSB-BSPG.GW35178), also identical to the 'violet morph' in Burgsdorf *et al.* (2014).

Experimental setup

Several hundred swimming larvae of *C. crambe* were collected from the plankton using 50 mL plastic syringes during scuba dives in early August 2017 and 2018 (Supplementary Video S1). Larvae were transported to the Laboratory for Experimentation with Living Organisms (LEOV-CEAB) and immediately transferred into 5 mL, propylene Petri dishes commercially treated for cell culturing, previously filled with 3 mL of 0.1 µm filtered seawater. Larvae settled after several days of swimming, metamorphosing into juvenile sponges. At this stage, Petri dishes with settled juveniles (Fig. 2A) were immersed in plastic aquaria ($N = 15$), each filled with 3 L of

seawater filtered at 1 µm pore size. In five aquaria selected at random, the natural dSi concentration of the local coastal seawater was left unaltered (control group: 0.5–1.5 µM dSi; Fig. 1B). In a second set of five aquaria, dSi concentration was increased to 30 µM and, in a third set of aquaria, it was increased to 100 µM. The increase in dSi concentration was obtained by adding the corresponding amount of a 0.1 M solution of sodium metasilicate pentahydrate ($\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$; CAS: 10213-79-3; Fisher Scientific) to the natural, filtered seawater. Weekly, two-thirds of the seawater volume in each aquarium was replaced with new filtered natural seawater at the corresponding dSi concentration. The cultures were maintained over 6 months in a temperature-controlled ($18 \pm 0.5^\circ\text{C}$) and light-controlled (9 h light : 15 h dark) aquarium room (Fig. 2A–C). Sponges were fed once a week by adding to each aquarium 1 mL of a Si-free, peptone-based culture of wild marine bacteria, along with 5 mL of a high-concentration culture of *Nannochloropsis gaditana* and *Tetraselmis suecica*, both prepared anew from inoculum every 10–15 days. After 6 months of culturing, 10 juvenile sponges were collected at random from each of the three dSi treatments (1, 30, 100 µM) by scraping with a scalpel. Juveniles were placed on glass slides and dried. Slides were then placed on heat plates at 120°C and concentrated nitric acid carefully dropped on the little sponges to digest the soft parts and obtain cleaned spicules. When the acid drop evaporated, new drops were added. The process continued for several hours until cleaning was complete, as evidenced by the formation of a bright white mass of spicules on the slides. Slides were mounted with a cover slip using DPX mounting medium and studied under light microscopy. Following the same acid-cleaning procedure, but placing dry juveniles on glass coverslips rather than slides, we obtained clean spicule on circular coverslips, which were subsequently mounted on aluminium stubs and sputtered with carbon for observation under a HITACHI TM300 scanning electron microscope (SEM).

The utilization of larval cultures to study the newly produced skeleton by *C. crambe* in the different dSi treatments has important advantages over the use of adult specimens. A major difference is that, as larvae are either aspiculate or contain only few larval spicules, it can be assumed that the bulk of the skeleton developed by the juveniles has been produced entirely under the experimental dSi conditions. However, it is not always easy to obtain larvae for a given sponge species. The challenge is even larger for *A. oroides* and *P. ficiformis*, which, unlike *C. crambe*, are gonochoric broadcast spawners with externally developing embryos very difficult to obtain (Maldonado and Riesgo 2009a, b). For these reasons, the experiments with *A. oroides* and *P. ficiformis* were conducted by transferring collected adult sponges to the facilities of the Aquarium of Barcelona for subsequent culturing in high-dSi conditions for 12 months (Fig. 2D, E). Unlike in the *C. crambe* experiment, aquaria were provided with a continuous flow of running seawater. High-dSi concentration was achieved by filtering the incoming seawater through siliceous sand, which partially erodes sand grains, releasing soluble silicic acid interstitially (Aparicio *et al.* 2025). Because of technical difficulties to maintain an exact dSi concentration for long

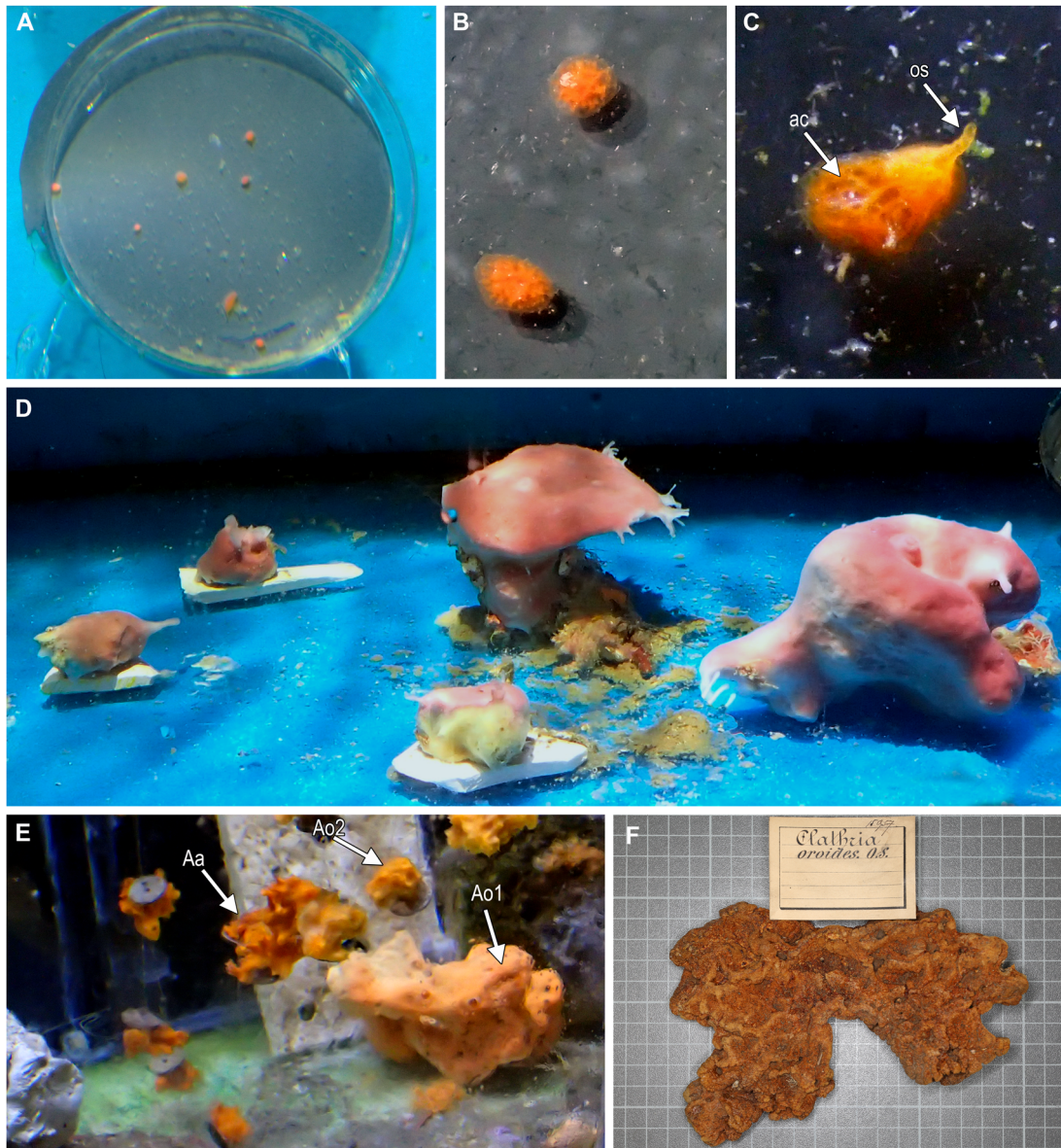


Figure 2 Views of studied specimens. A–C, views of *Crambe crambe* juveniles settled on Petri dishes after 3 months (A, B) and 6 months (C) of culturing at 100 μM dSi at the LEOV-CEAB. Note the oscular tube (os) and the subdermal aquiferous channels and spaces (ac) visible through the translucent layer of epithelial cells. D, individuals of *Petrosia ficiformis* after 6 months in high-dSi culturing conditions at the Aquarium of Barcelona. E, view of one of the individuals of *Agelas oroides* (Ao1) after 6 months in high-dSi culturing conditions at the Aquarium of Barcelona. The individual is sharing aquarium with explants of *Acanthella acuta* (Aa) and *Agelas oroides* (Ao2) used for different studies. F, view of the dry holotype of *Agelas oroides* stored at Landesmuseum Joanneum, Graz (LMJG).

periods in a running-water system, dSi values fluctuated over the duration of the experiment from 14.6 to 80.5 μM , averaging ($\pm$ SD) $48.1 \pm 20.1 \mu\text{M}$ (Fig. 1B). Yet, this range of values still provides dSi to the sponges in concentrations over one order of magnitude higher than the values available through the year in the natural habitat (range: 0.4–2.5 μM ; average: $0.8 \pm 0.4 \mu\text{M}$; Fig. 1B). During the experiment, sponges fed from the microplankton community developed in the seawater of the main exhibition tanks, but their food was also daily complemented by addition of 50 mL of a concentrated culture of *N. gaditana* and *T. suecica*.

After 12 months in this high-dSi treatment, tissue samples were collected for skeletal study. Samples were obtained only

from those body areas that had experienced a clear, net growth during the culturing period in the high-dSi treatment. Spicules of five cultured individuals per species were acid cleaned as explained above and compared with those obtained from 10 wild conspecific individuals sampled from the same location than the individuals taken to the Aquarium of Barcelona and that represented the natural, low-dSi, control condition.

Type specimens

Type material is available for the species *C. crambe* and *A. oroides*. Type material of *P. ficiformis* was searched for without success in the collections of the Muséum National

d'Histoire Naturelle, Paris (France); the Natural History Museum, London (UK); the Landes-Museum Joanneum, Graz (Austria); and the Museum für Naturkunde, Berlin (Germany). Based on this search, the type material for this species is presumed lost. Type material of *C. crambe* was not directly examined, as the skeletal features of the species were thoroughly re-described by Van Soest (2002) using both light and electron microscopy on two paralectotypes (LMJG15672 and LMJG15667), as detailed in the corresponding section of the Results. For *A. oroides*, we examined the skeleton of the holotype (LMJG15957; Fig. 1F) and a paratype (LMJG15988/124) using light and scanning electron microscopy, following protocols above explained.

Statistical analyses of skeletal data

Glass spicule slides for light microscopy and SEM stubs were examined by three different observers, and their data were pooled after exploratory trials confirmed the absence of statistically significant between-observer effects. Whenever possible, high numbers of spicules of each type per individual were measured to derive representative average (avg) and standard deviation (SD) values for the parameters of interest. For the skeletons developed by small juveniles of *C. crambe*, we aimed to obtain 100 measures per spicule type; if fewer than 100 spicules were present for a particular type, all available spicules were measured (Supplementary Table S1). In *A. oroides*, 200 spicules were measured for each spicule category found (Supplementary Table S2). In *P. ficiformis*, 200 oxeas per individual were measured for statistical analyses. Also, 200 additional spicules were randomly selected, and the ratios and dimensions of the various spicule types (styles, strongyles, and reniform strongyles) obtained (Supplementary Table S3). For all three sponge species, monaxonic spicules were measured for length and thickness. Thickness of diactines was measured at approximately the midpoint of the spicule, while thickness of monactines was measured at the subterminal region ('the neck') of the round end. Chelae were measured for their length and sphaeroclon desmas across their largest diameter.

Statistical tests examining the effects of dSi concentration on spicule length and width could only be conducted for spicule types that were formed at all the experimental levels of the dSi treatment. This criterion was met for the styles of *C. crambe* (Supplementary Statistics S1), the acanthostyles of *A. oroides* (Supplementary Statistics S2–S3), and the oxeas of *P. ficiformis* (Supplementary Statistics S4), but not for other spicule types that were formed only at some dSi concentrations. Spicule length (μm) and width (μm) for each spicule type were analysed as continuous response variables in separate models. Frequency distributions for length and width data were built to examine occurrence of size categories within a spicule type. Statistical significance for putative spicule categories were tested by methods based on mean comparison. All statistical analyses were conducted in R (v.4.5.2; R Core Team).

In *C. crambe*, the experimental design for data analysis consisted of three groups of 10 individuals (Factor 'Individual'), each group being exposed to one of three dissolved silicon (dSi) concentrations (1, 30, and 100 μM ; Factor 'dSi'). Because

multiple styles were measured from the same individual and in varying numbers across individuals, observations were neither statistically independent nor equally replicated. The experimental design is also nested, since individuals were exposed to only one of three dSi concentrations. To account for this hierarchical, nested, and unbalanced data structure, and to quantify inter-individual variability, we used linear mixed-effects models (LMMs), as implemented in the LME4 package in R. For each response variable, factor 'dSi' was included as a fixed effect, while factor 'Individual' was included as a random intercept, thereby modelling individual-specific deviations around the overall treatment means (Supplementary Statistics S1). This approach allows the estimation of treatment effects while correctly accounting for repeated spicule measurements within individuals and partitioning variance between treatment-level and individual-level sources. Model significance for fixed effects was assessed using Type III analysis of variance based on Satterthwaite's approximation of degrees of freedom, as implemented in the lmerTest package. Models were evaluated through visual inspection of residual plots and Q–Q plots. When a significant effect of dSi concentration was detected, post hoc pairwise comparisons among dSi levels were performed using estimated marginal means (EMMs), with Tukey's adjustment applied to control for multiple testing (using the EMMEANS package). To quantify the relative contribution of inter-individual variability to total phenotypic variation, variance components were extracted from each model, and intra-class correlation coefficients (ICCs) were calculated. ICC values represent the proportion of total variance attributable to differences among individuals within treatments (Supplementary Statistics S1).

In the case of *A. oroides*, only acanthostyles-1 could be compared across all sponge individuals and the two levels of 'dSi' factor (low and high concentration), while, in the case of *P. ficiformis*, only oxea size could be compared. In both species, sample size was also unbalanced across experimental factors and factor 'Individual' nested within factor 'dSi'. For both species, factor 'Individual' included 15 levels ('Individual 1' to 'Individual 15'). Individuals 1 to 10 were exposed only to the 'low' level of factor 'dSi', whereas Individual 11 to Individual 15 were exposed only to the 'high' level of factor 'dSi'. Because each individual sponge was exposed to a single dSi level, dSi was treated as a between-individual factor, and individual sponges were considered the true units of replication. To avoid pseudoreplication arising from multiple spicule measurements per individual, spicule measurements were first averaged at the individual level. Mean spicule length and width per individual were then analysed separately using linear models (LM) with factor 'dSi' (low vs. high) as a fixed effect. Model assumptions were assessed using residual diagnostic plots. As a complementary analysis, linear mixed-effects models (LMM) were fitted to the full spicule-level dataset to partition within- and among-individual variation, with individuals treated as random effects nested within dSi and heteroscedasticity between dSi levels accounted for using a variance function. Analyses were performed using the packages NLME, DPLYR, and EMMEANS (Supplementary Statistics S2, S4).

In the case of *A. oroides*, formation of a putative second size category of acanthostyles (acanthostyles-2) was detected

in only one of the two levels of factor 'dSi' (i.e. high dSi concentration). The dataset comprised 2000 individual spicules measured across five sponge individuals (Ag 11–Ag 15). Each individual contributed measurements for both spicule types, resulting in a hierarchical data structure in which multiple spicules were again nested within individuals. Because spicules produced by the same individual are not statistically independent, spicule type was considered a fixed factor with two levels (Acanthostyle-1 and Acanthostyle-2), while sponge individual was included as a random intercept to account for among-individual variability in mean spicule dimensions. Spicule length and width were analysed separately by LMMs (Supplementary Statistics S3).

Determination of silicic acid concentration

Samples of bottom water were collected monthly at the sponge habitat in the Santa Anna Point using a 60 mL sterile syringe during scuba dives over 2021 and 2022. Water samples were filtered through 0.22 µm polycarbonate membranes and stored refrigerated until determination of Si concentration ($\pm 1\%$) using an Alliance Futura autoanalyzer. Seawater samples were also taken from the experimental aquaria at the Barcelona Aquarium and mailed for nutrient determination through IPC mass analysis at Fauna Marin Lab (Aichaberg, Germany).

Results

Skeletal analysis of *Crambe crambe* (Demospongiae: Poecilosclerida)

Effects of dissolved silicon availability on skeleton formation

The skeleton formed by juvenile individuals after 6 months in three different dSi concentrations (1 µM: natural concentration; 30 µM; and 100 µM) varied drastically, in terms of spicule inventory and spicule size, as a function of dSi concentration (Figs 3, 4; Supplementary Table S1, Datafile S1). At 1 µM, only thin, needle-like spicules with one end round and the other sharp (i.e. styles/styloxeas) were formed (Figs 3A, 4A, B). This skeleton is in agreement with that formed in the wild populations of *C. crambe* established in the dSi-poor Spanish, Mediterranean coast, which also exhibit a simple skeleton formed by only styles. In contrast, in the high-dSi experimental conditions (i.e. to 30 and 100 µM), several additional spicule types were produced, as follows. First, the styles became stouter, and developed a terminal/subterminal thickening, becoming subtylostyles (Fig. 4A, B). Also, a moderate number of styles (approx. 8% to 10% across individuals) exhibited the two ends rounded, becoming strongyles (Figs 3C, 4C, D, J). Second, a category of much bigger styles/subtylostyles was formed, typically with the round end becoming slightly swollen (Figs 3D, 4E, F, J). While the small styles and strongyles occur through the entire sponge, the bigger subtylostyles were typically confined at the very base of the sponge (being referred to as choanosomal subtylostyles). Third, two categories of isochelae were formed: unguiferate-anchorate isochelae-1, being 25–43 µm in length,

with three to five teeth (Figs 3E, F, 4G, H), sometimes markedly underdeveloped (Fig. 3G); and isochelae-2 being 11 to 19 µm in length and often with malformed teeth (Fig. 3H). While isochelae-1 are abundant in all individuals, isochelae-2 are scarce or even absent, depending on the individual (Fig. 4G, H, J; Supplementary Table S1). Fifth, sphaeroclon desmas formed in all individuals at 100 µM dSi, ranging from three to 25 spicules per juvenile (Fig. 3I, J; Supplementary Table S1). In contrast, at 30 µM, only two individuals out of 10 formed these spicules, and in numbers of one each. As in the case of choanosomal subtylostyles, desmas were confined to the basal-most region of the sponges. Together, these findings indicate that the formation of large styles/subtylostyles, isochelae, and desmas require concentrations of 30 to 100 µM dSi, which are unlikely to occur naturally in most habitats within the distribution range of *C. crambe* populations.

Our experimental dSi enrichment induced not only formation of additional spicule types, but also affected their size (Fig. 4A–I; Supplementary Table S1, Datafile S1). Analysis of styles, the only spicule type that can be compared across the three dSi levels, revealed a significant effect of dSi concentration on spicule length (LMM: $F_{2,28.1} = 10.36$, $P = .00043$; Supplementary Statistics S1). On average, spicules measured 161.4 ± 38.4 µm at 1 µM, 191.1 ± 31.4 µm at 30 µM, and 172.5 ± 22.0 µm at 100 µM (Fig. 4A). Post-hoc Tukey-adjusted comparisons (Fig. 4A) indicated that spicules at 30 µM were significantly longer than those at 1 µM (difference = 29.7 µm, $P > .001$), that length differences between 1 µM and 100 µM were not significant (difference = 11.1 µm, $P = .24$), and that 30 µM spicules were slightly longer than those in 100 µM (difference = 18.6 µm, $P = .026$). Spicule width was more strongly affected by dSi concentration (LMM: $F_{2,27.3} = 222.5$, $P < .0001$). Mean width was 1.75 ± 0.72 µm at 1 µM, 3.65 ± 0.72 µm at 30 µM, and 4.11 ± 0.72 µm at 100 µM. Post-hoc tests showed that all pairwise differences were significant (Fig. 4B): 30 µM > 1 µM (difference = 1.90 µm, $P < .0001$), 100 µM > 1 µM (difference = 2.36 µm, $P < .0001$), and 100 µM > 30 µM (difference = 0.46 µm, $P = .0021$). These results indicate a strong positive effect of increasing dSi concentration on spicule width, which resulted in a thickness increase of ~135% between 1 and 100 dSi.

In the model used for analysis, no interaction term was included beyond individual random effects, but the effect of dSi was consistent across individuals, as the fixed-effect estimates show. However, variance decomposition showed that inter-individual variability accounted for a moderate portion of total variance. For spicule length, the random effect of factor 'Individual' explained ~19% of variance (adjusted ICC = 0.192), with the remaining 81% attributable to intra-individual variation among spicules. For spicule width, the contribution of individual identity was smaller (adjusted ICC = 0.108), indicating that most variability (~89%) occurs among spicules within the same individual (Supplementary Statistics S1). These results highlight that, while dSi concentration drives overall spicule morphology, natural variation among individuals contributes meaningfully, especially for length. Post-hoc Tukey-adjusted comparisons clarified that the largest increases in spicule width occur progressively with

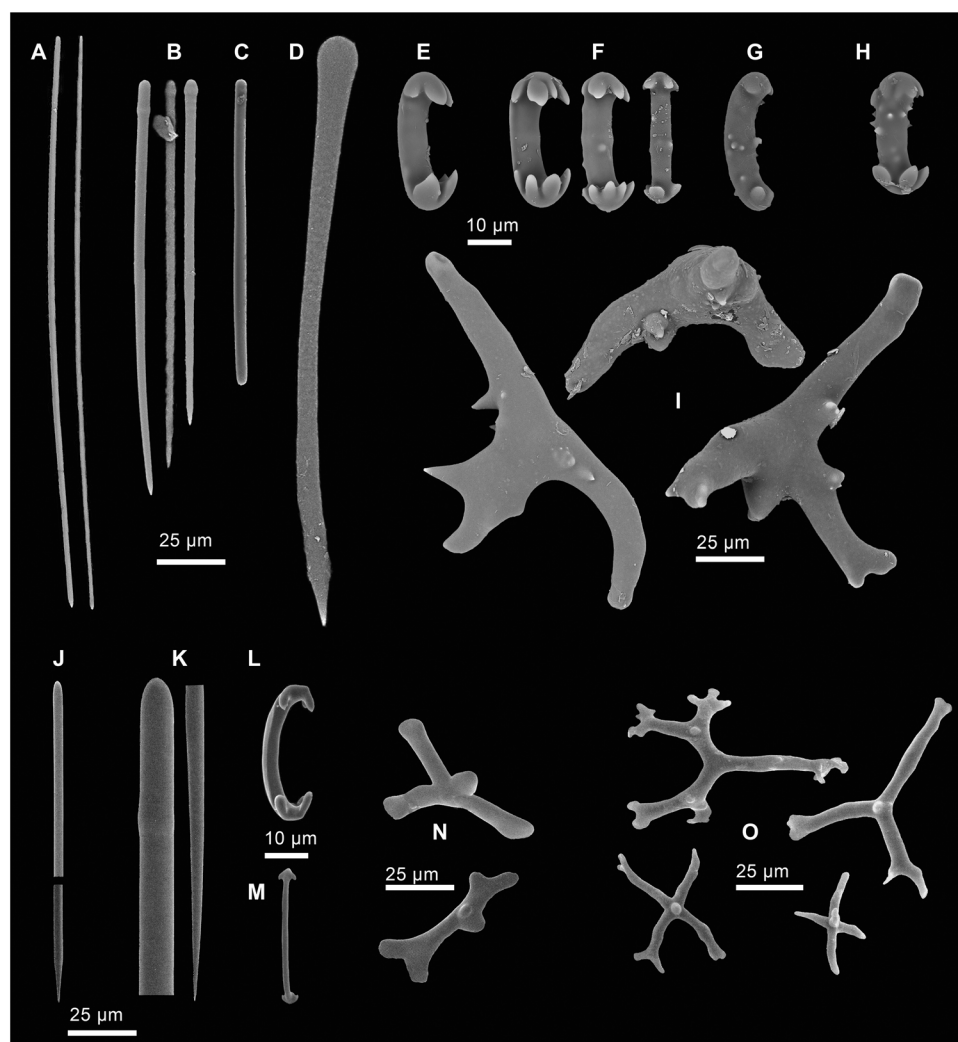


Figure 3 Scicule inventory of *Crambe crambe*. A, thin styles, the only spicule type formed by individuals maintained at 1 μ M dSi in the laboratory. B–I, additional spicule types formed by individuals maintained at 100 μ M dSi in the laboratory, which comprise styles-subtylostyles (B), strongyles (C), choanosomal subtylostyles (D), unguiferate isochelae-1 with three (E) or more teeth (F), or underdeveloped teeth (G), smaller isochelae-2 (H), and pseudo-astrose desmas (I). J–N, spicules of paralectotype LMJG15672, including styles (J), choanosomal styles-subtylostyles (K), three-toothed isochelae (L), which can be underdeveloped (M), and underdeveloped pseudo-astrose desmoids (N). O, pseudo-astrose desmoids of paralectotype LMJG15667. Images J–O were taken from [Van Soest \(2002\)](#) and modified and re-scaled for direct visual comparison with the spicule types produced under experimental dSi conditions. See [Figure 4](#) and [Supplementary Table S1](#) for numerical data.

increasing dSi, whereas spicule length shows a more complex pattern: length peaks at 30 μ M, with a slight reduction at 100 μ M ([Fig. 4A, B](#)). This suggests that width responds linearly to silica availability, while length may have a threshold or optimal concentration near 30 μ M. The significant pairwise differences among widths but more limited differences among lengths demonstrate that different dimensions of spicules are differentially sensitive to environmental silica, and that biological individuality further modulates this response.

Also, all other spicule types tended to be thicker at 100 μ M dSi than at 30 μ M dSi. A dSi concentration of around 100 μ M dSi also appears to be required for the formation of sphaeroclon desmas, which otherwise are not produced in full size and abundance ([Fig. 4I, J](#); [Supplementary Table S1](#)). Interestingly,

we also noticed that the first desmas were formed only after the juvenile sponges had been at least two months in high dSi concentrations. This pattern suggests that the formation of some spicule types may also be modulated, in addition to dSi availability, by ontogenetic controls.

The take-home message of these results is that the availability of dSi to the sponges drastically affects both the number of spicule types formed in the skeleton and their size, two features that have traditionally been considered reliable for deriving taxonomic decisions. The chronically low dSi concentration in natural populations of *C. crambe*—at least in those from most dSi-poor Mediterranean coastal regions—appears to hinder the full phenotypic expression of skeletal traits encoded in the genome.

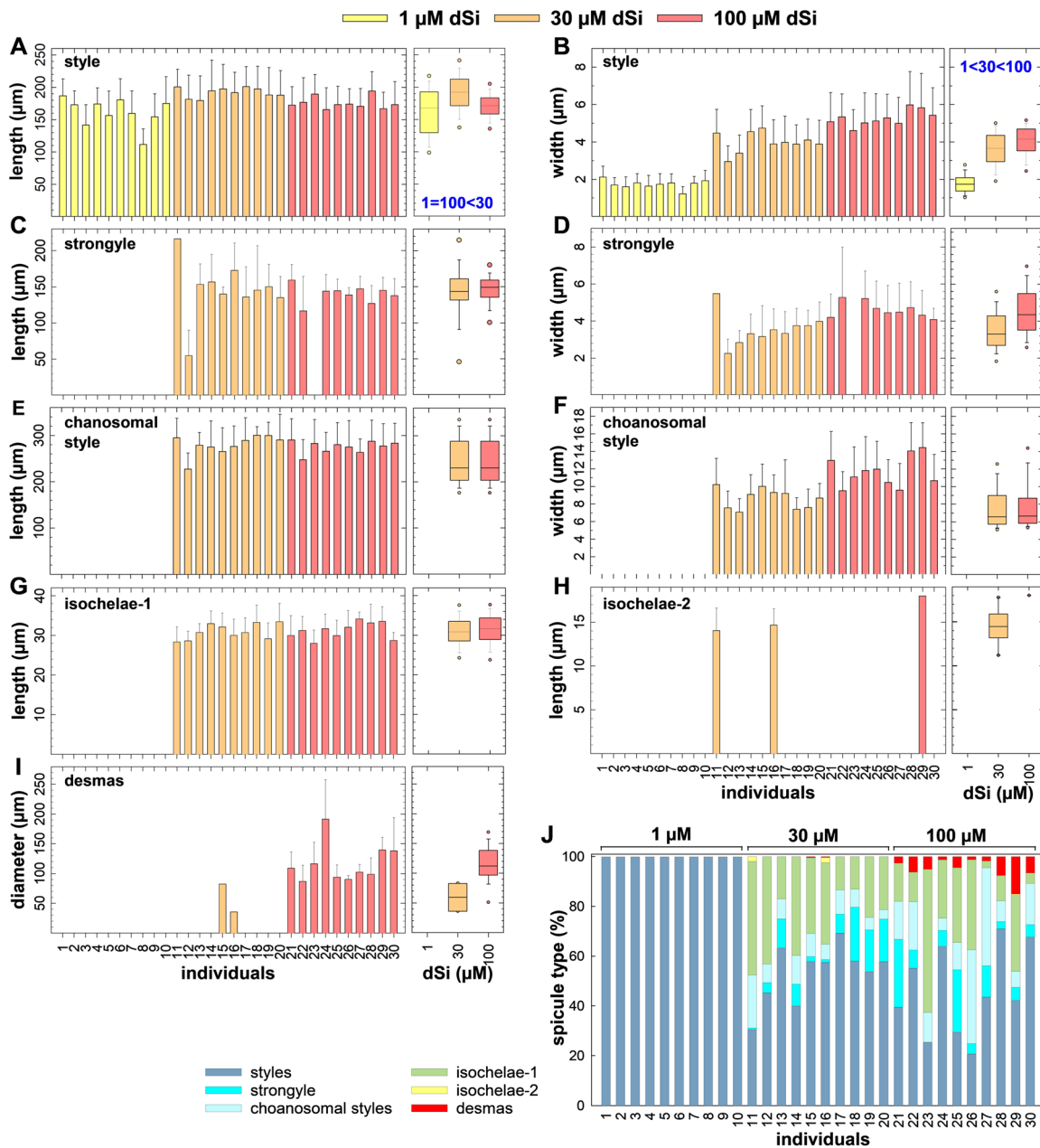


Figure 4 Summary of spicule inventory in *Crambe crambe* as a function of dSi concentration. Spicule measurements averaged ($\pm$ SD) by individuals (1–30) and dSi treatment (1, 30, 100 μ M) of styles (A, B), strongyles (C, D), and choanosomal styles (E, F), isochelae-1 (G), isochelae-2 (H), and desmas (I). The results of statistically significant differences detected in the length and width of styles across dSi levels are summarized graphically (length: 1 μ M = 100 μ M < 30 μ M; width: 1 μ M < 30 μ M < 100 μ M). J, Relative abundance (in %) of the different spicule types formed as a function of individual and dSi concentration. See [Supplementary Table S1](#) and [Datafile S1](#) for raw data of spicule size.

The skeleton of type specimens

The skeletal features of type specimens have been re-described in detail by [Van Soest \(2002\)](#), using two paralectotypes (LMJG15672 and LMJG15667) collected from the same Croatian area of the Adriatic Sea as the holotype (latitude: 43.13, longitude: 16.45; latitude: 43.69, longitude: 15.89). It is surprising that these specimens exhibit skeletal features ([Fig. 3J–O](#); [Supplementary Table S1](#)) resembling more those of the juveniles cultured in the laboratory under 100 μ M dSi than those of wild individuals. Such skeletal features are in

line with recent reports of previously undetected entries of dSi in the habitat where the individuals making the type series were collected (see Discussion). Those environmental dSi conditions would favour a skeleton that contains styles that do not become strongyles but subtylostyles, along with choanosomal styles-subtylostyles. In addition, both spicule types are even longer and thicker than the equivalent spicule categories produced by laboratory sponges cultured under 100 μ M dSi ([Supplementary Table S1](#)). Likewise, they contain isochelae-1 and desmas, although both of them are less robust than those

produced by sponges in the laboratory at 100 µM dSi and, in fact, the morphological features of their isochelae and desmas suggest that they both are slightly underdeveloped (Fig. 3L–O): the isochelae have underdeveloped teeth and the desmas comparatively thin arms (Van Soest 2002).

An important outcome is that the comparatively diverse spicule inventory of the Croatian specimens of the *C. crambe* type series do not closely match the spicule inventory commonly found in the bulk of the Mediterranean population of this species, in which only thin styles occur (Pulitzer-Finali 1978, Bibiloni 1990, Maldonado and Uriz 1996), with desmas and chelae reported from only a minority of individuals (Topsent 1925, Boury-Esnault 1971, Pulitzer-Finali 1978, 1983). This raises a dilemma applicable to other sponge species concerning the extent to which the skeleton of the holotype represents the species (see Discussion).

Skeletal analysis of *Agelas oroides* (Demospongiae: Agelasida)

Effects of dissolved silicon availability on skeleton formation

The microscopic study of the skeleton (Fig. 5A, B) and the size data of the spicules (Figs 6A, 7A, 8A–D; Supplementary Table S2, Datafile S2) revealed that all 10 wild specimens sampled from the dSi-poor, natural habitat had a skeleton containing a single spicule type: acanthostyles with very feeble spines arranged in verticils. Acanthostyles measure 97 ± 17 µm in length and 2.0 ± 0.9 µm in thickness. However, five additional individuals collected from the same wild population and maintained for 12 months in a flow-through aquarium under high-dSi conditions, showed two categories of acanthostyles. Acanthostyles-1 (Fig. 5C–E), which were feeble and nearly identical to those formed by the wild specimens, measuring 96 ± 16 µm in length and 3.2 ± 0.9 µm in width; and acanthostyles-2 (Fig. 5C, D, F), which were slightly longer (129 ± 24 µm) but much thicker (11.7 ± 2.8 µm) and spined than acanthostyles-1.

Comparison of frequency distribution of acanthostyle length and width between the 10 wild individuals grown in dSi-poor waters and the five individuals grown in dSi-high experimental conditions supports the occurrence of acanthostyles in a single size category in the wild individuals, but in two size categories in the dSi-high individuals (Figs 6A, B, 7A, B; Supplementary Table S2). The frequency distribution of acanthostyle length in the 10 wild individuals follows a unimodal, relatively symmetrical, normal function, with individual modes ranging from 89 to 105 µm across individuals (Fig. 7A). However, the frequency distribution of acanthostyle length in the five individuals cultured under high-dSi conditions, though roughly showing a unimodal pattern, become slightly asymmetrical due to the appearance of a low percentage of longer spicules (acanthostyles-2), which extend the upper tail of the distribution and raise the individual modal value of spicule length between 109 and 122 µm. The frequency distribution of spicule width data more clearly reveals the occurrence of two acanthostyle categories in the individuals cultured in high-dSi. In the wild individuals,

acanthostyle width distribution is unimodal, ranging from 2 to 7 µm across individuals (Fig. 7A). In contrast, it becomes clearly bimodal (Fig. 7B) in the individuals cultured under high-dSi conditions, revealing the formation of a second acanthostyle category, with a width mode ranging from 11 to 17 µm, and a maximum width in some spicules of up to 26 µm.

Importantly, when compared in terms of general morphology, ornamentation (Fig. 5), and dimensions (Figs 7D, 8A–D), acanthostyles-1 of the high-dSi cultured individuals (Ag 11 to Ag 15) are nearly undistinguishable from the only acanthostyle type formed in the wild individuals (Ag 1 to Ag 10). The LM and LMM analyses found only a tiny difference in length (6.7 µm, on average) and no differences in thickness between these two groups of acanthostyles (Supplementary Statistics S2). Also, although length differences are statistically significant (LM: $t = 2.34$, $P = .036$; $F_{1,13} = 5.46$), their magnitude is biologically unimportant: acanthostyle mean length was 96.7 ± 5.2 µm at low dSi and 103.4 ± 5.2 µm at high dSi. Such an average difference (6.7 µm) represents only about a 6% increment in length across dSi conditions. Acanthostyle width did not differ significantly between dSi levels: mean width was 2.93 ± 0.59 µm under low dSi and 3.23 ± 0.59 µm under high dSi, and the effect of dSi was not significant (LM: $t = 0.92$, $P = .373$; $R^2 = 0.062$). The complementary LMM approach, which explicitly accounted for the hierarchical structure of the data, supported these overall patterns. For acanthostyle length, the fixed effect of dSi was positive and of similar magnitude to that observed in the primary analysis (+6.7 µm). However, formal significance testing by level of dSi was limited by the very small number of effective degrees of freedom (2 dSi levels), resulting in undefined P -values, a known limitation of containment-based tests in such designs. Importantly, the random-effect structure revealed substantial variation attributable to the Individual factor ($SD \approx 5.07$ µm), indicating pronounced inter-individual differences in spicule length, as well as large residual (within-individual) variation ($SD \approx 16.64$ µm).

For acanthostyle width, mixed-effects models similarly showed no clear effect of dSi, while again highlighting a strong contribution of individual identity ($SD \approx 0.59$ µm) relative to the residual variance ($SD \approx 0.70$ µm). Post-hoc comparisons based on estimated marginal means, used here for descriptive purposes, were consistent with the model-based results. Estimated marginal means for length were higher under high dSi than under low dSi, whereas for width the estimated means for the two dSi levels largely overlapped, reinforcing the notion of absence of a detectable dSi effect. Overall, these analyses indicate that dissolved silicon availability minimally influenced acanthostyle length and had no effect on width, and that morphological variation among individuals represents a major component of total phenotypic variance, independent of environmental silicon levels. Therefore, these analyses strongly support that the acanthostyles found in the wild specimens and the acanthostyles-1 found in the individuals cultured at high dSi are the same spicule category.

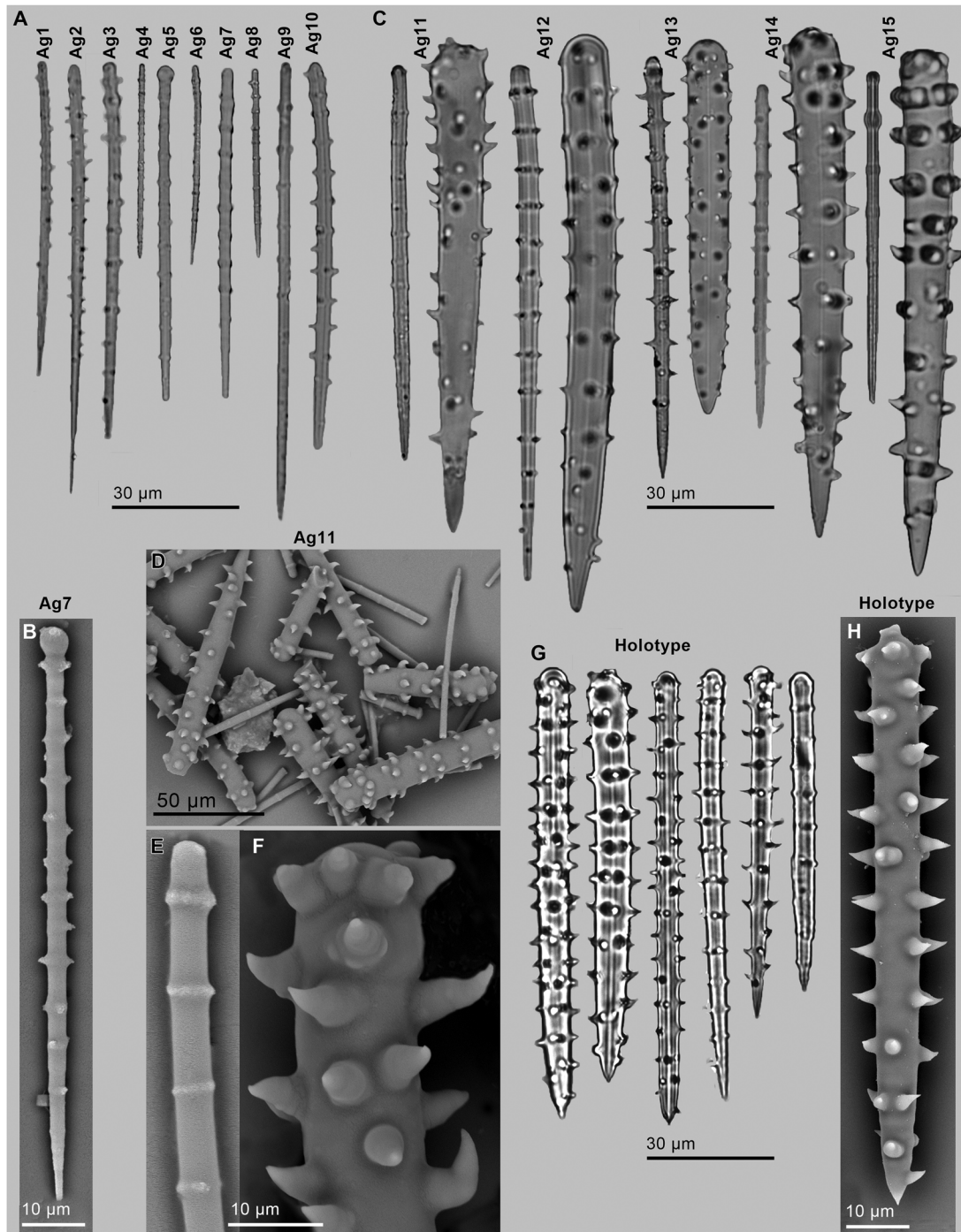


Figure 5 Spicule inventory of *Agelas oroides*. A, light-microscopy and SEM (B) views of acanthostyles produced by 10 wild individuals (Ag1–Ag10) from dSi-poor habitats in the Mediterranean Spanish coast. By size, these acanthostyles form a single category. C–F, comparative views of acanthostyles produced in two size categories by five wild conspecifics (Ag11–Ag15) subjected for one year to experimental conditions of high dSi ($\sim 48 \mu\text{M}$). G, H, views of acanthostyles from the holotype of *A. oroides* (LMJG 15957), which form a single size category. See [Figures 6 and 7](#) and [Supplementary Table S2](#) for numerical data. The holotype inhabited in an area of the Adriatic Sea where important groundwater discharges enrich the habitat in dSi.

In contrast, when we compared the size of acanthostyles-1 and acanthostyle-2 formed in the five individuals cultured at high dSi concentrations, marked differences in length and width were found between both categories ([Fig. 8A–D](#); [Supplementary Statistics S3](#)). Across individuals, acanthostyles-2 ($129.2 \pm 22.2 \mu\text{m}$)

were significantly longer (on average, $25.9 \mu\text{m}$) than acanthostyles-1 ($103.3 \pm 22.2 \mu\text{m}$), according to LMM model (Type III ANOVA: $F_{1,1994} = 678.0$, $P < .0001$). Among-individual variability accounted for a non-negligible component of total variance (random-intercept SD = $5.08 \mu\text{m}$), indicating consistent differences in mean spicule

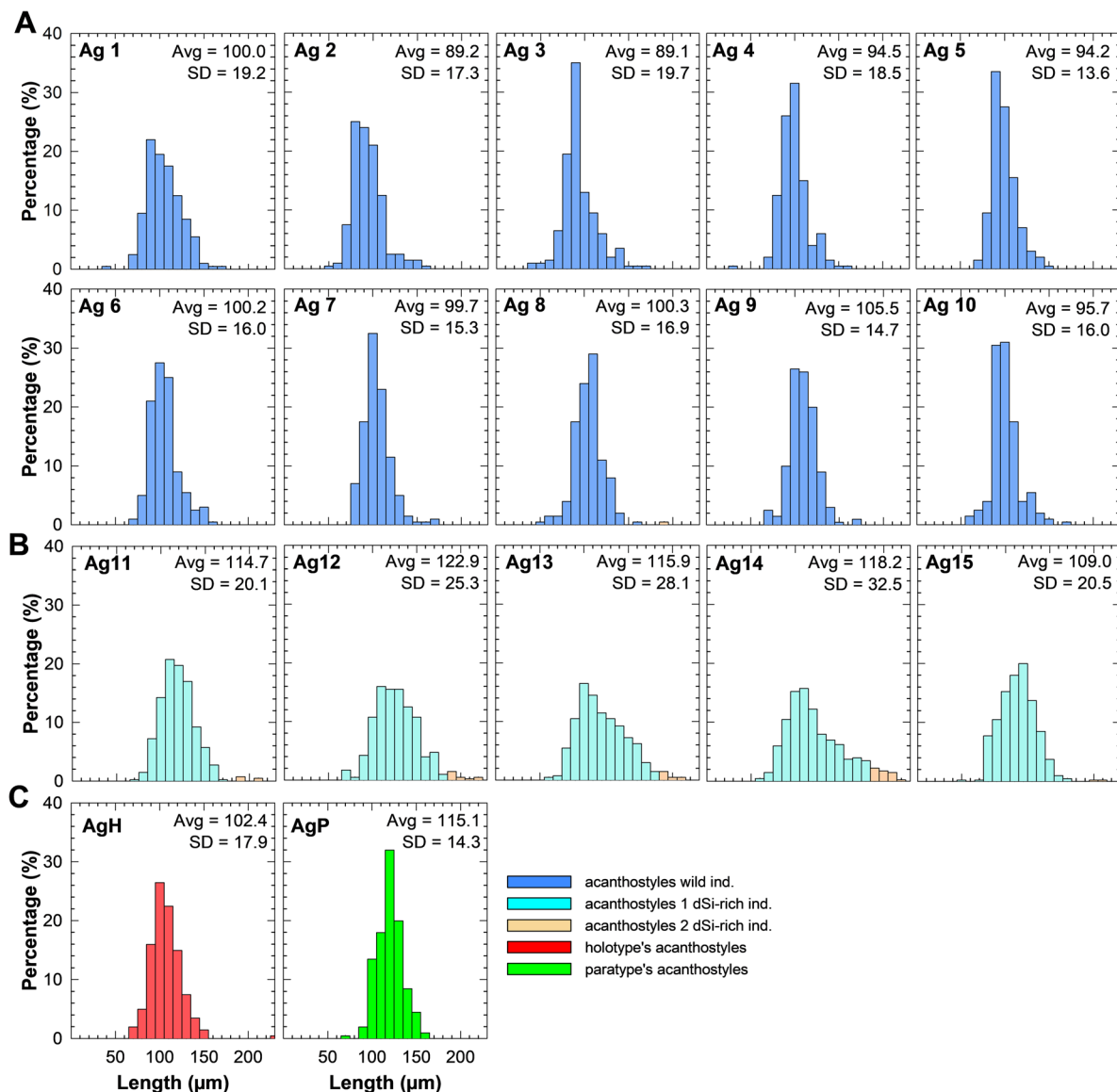


Figure 6 Frequency distribution of acanthostyle length in the individuals of *Agelas oroides* studied. See also [Supplementary Table S2](#) and [Supplementary Datafile S2](#) for numerical data. A, the 10 wild individuals show a unimodal, relatively symmetrical, normal distribution of acanthostyle length, with individual averages ranging from 89 to 105 μm. B, however, the five individuals cultured in high-dSi show unimodal, but slightly asymmetrical length distributions. This effect is mostly due to the appearance of a low percentage of slightly longer spicules (acanthostyles 2; See [Figs 4](#) and [5](#)), which increase the individual mean of spicule length to the range 109 to 122 μm. C, the holotype and paratype material, which come from an Adriatic Sea region where dSi can be risen due to important groundwater discharges, show unimodal distributions, but with average acanthostyle length being intermediate (102–115 μm) between that of the wild individuals grown in dSi-poor water and that of individuals cultured under experimental dSi-high conditions.

length among sponge individuals. Yet, allowing the effect of spicule type to vary among individuals (random-slope model) significantly improved model fit (likelihood-ratio test: $\chi^2 = 24.35$, d.f. = 2, $P = 5.17 \times 10^{-6}$), indicating that although acanthostyles-2 were consistently longer than acanthostyles-1, the magnitude of this difference varied among individuals. Pairwise post-hoc comparisons confirmed a strong and highly significant difference in length between both acanthostyle categories ($t = -26.04$, $P < .0001$).

Width analysis showed even greater differences between acanthostyles-1 and -2 (Type III ANOVA: $F_{1,1994} = 8801.5$, $P < 2.2 \times 10^{-16}$; [Fig. 8A–D](#); [Supplementary Statistics S3](#)).

Acanthostyles-1 had a mean width of $3.23 \pm 2.01 \mu\text{m}$, whereas acanthostyles-2 were markedly thicker, with a mean width of $11.66 \pm 2.01 \mu\text{m}$. The difference in width is $8.43 \pm 0.09 \mu\text{m}$, representing a 261% thickening with increasing dSi. Between-individual variance in width was comparatively small (random-intercept SD = $0.37 \mu\text{m}$), indicating that width differences were highly consistent across individuals. Pairwise post-hoc comparisons corroborated the large and highly significant difference between spicule types ($t = -93.82$, $P < .0001$).

The statistically significant differences in length and width observed between acanthostyles-1 and acanthostyles-2

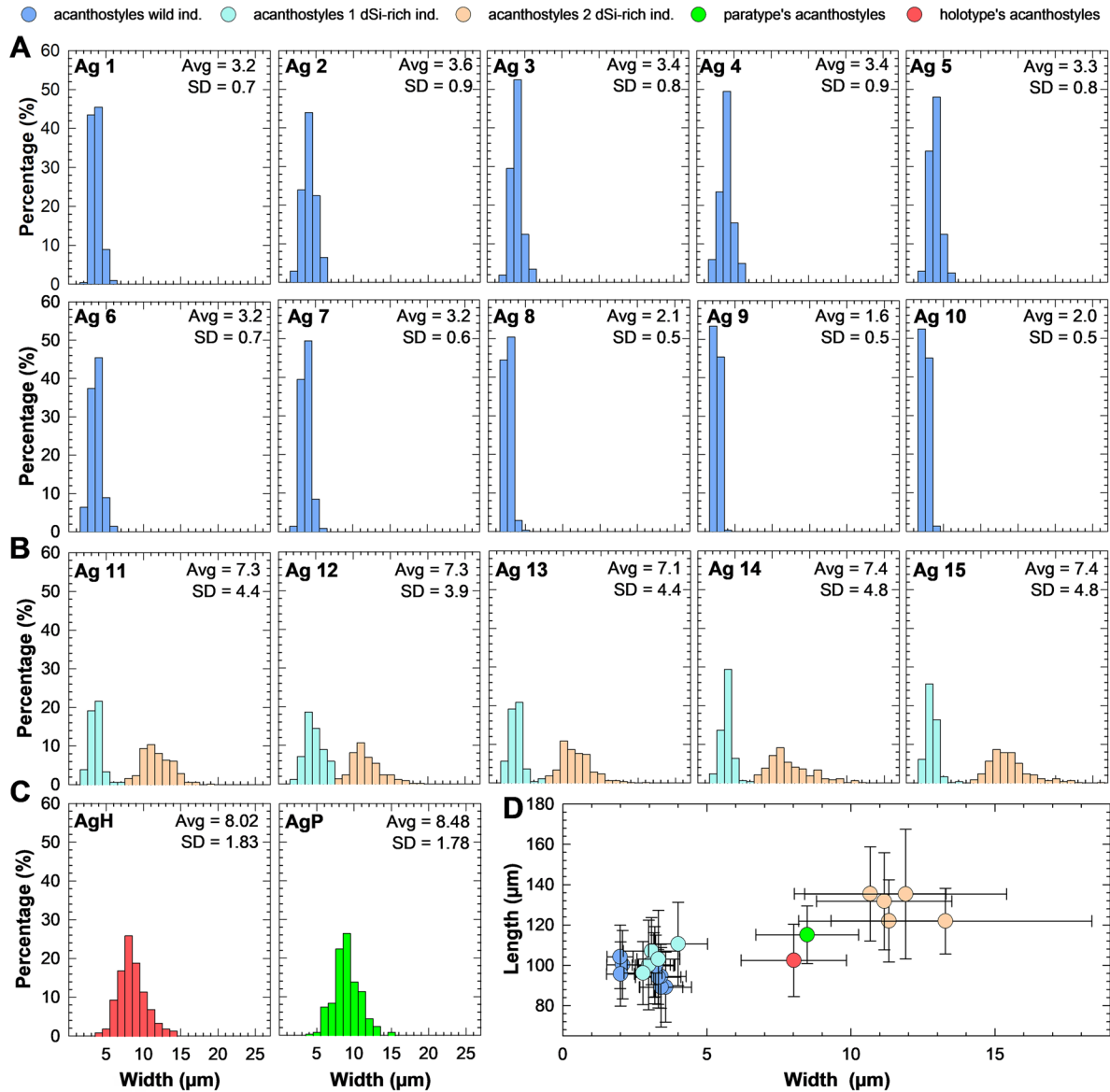


Figure 7 Frequency distribution of acanthostyle width in the various individuals of *Agelas oroides* studied. See also [Supplementary Table S2](#) and [Supplementary Datafile S2](#) for numerical data. A, the 10 wild individuals show a unimodal, relatively symmetrical normal distribution of acanthostyle width, with individual averages around 4 µm. B, however, the five individuals cultured in high-dSi conditions show a bimodal distribution. It reveals two spicule categories, due to formation of acanthostyles 2 (see [Figs 3](#) and [4](#)), which are substantially thicker. C, the holotype and paratype material, which come from an Adriatic Sea region where dSi concentration can be risen by important groundwater submarine discharges, show unimodal distributions, but with average acanthostyle width being intermediate between that in the wild individuals from dSi-poor habitats, and that of acanthostyles-2 of individuals cultured under experimental dSi-high conditions. D, when spicules in the various individuals are plotted by their average length and width, it becomes evident the occurrence of two well separated spicule categories only in the individuals cultured in high-dSi conditions. It is also seen that the acanthostyles-1 of individuals cultured in high-dSi conditions are indistinguishable from the only acanthostyles formed in the wild individuals. This graph also illustrates that the spicules of the type specimens exhibit intermediate sizes between the acanthostyles-1 and -2. For statistical comparisons, see [Figure 8](#) and [Supplementary Statistics S2](#) and [S3](#).

seemingly support the idea that *A. oroides* produces a second category of larger spicules only when environmental dSi availability is high. However, as it will be explained in the Discussion, this seemingly obvious conclusion is incorrect.

The skeleton of type specimens

We revised the holotype and a paratype of *A. oroides*. The holotype (LMJG 15957; [Fig. 2F](#)), originally described as *Clathria*

oroides Schmidt, 1864, was collected from the Adriatic Sea (43° 4' 0" N, 16° 12' 0" E) at 7.3 m. The morphometric study of these specimens shows that their skeleton consists of a single acanthostyle category in terms of both length and width ([Figs 6C](#), [7C](#)), with spicule morphology forming a continuum from thin forms with weak spines (i.e. similar to those produced under dSi-poor conditions) to relatively thick spicules with well-developed spines (i.e. similar to acanthostyles-2

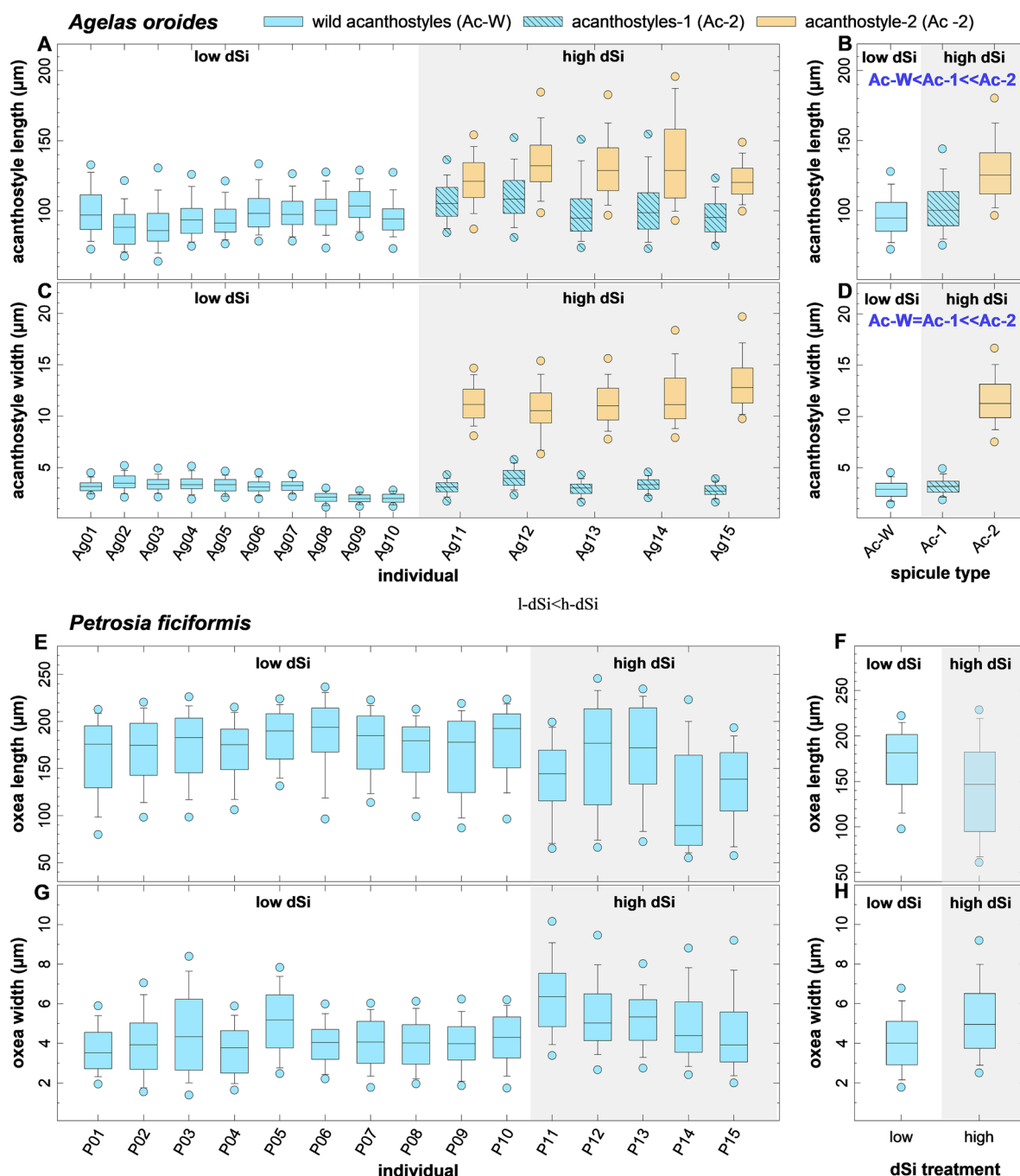


Figure 8 Comparisons of spicule length and width across dSi conditions. A, C, length and width of verticillate acanthostyles formed by *Agelas oroides* (see [Supplementary Datafile S2](#) for numerical values). Ten individuals collected from the wild populations (i.e. low dSi condition) formed only one category of acanthostyles (Ac-W), while all five individuals transferred to experimental high-dSi conditions formed two categories, namely acanthostyles-1 (Ac-1) and acanthostyle-2 (Ac-2). B, D, length and width of acanthostyles pooled by putative spicule category. By integrating the various statistical analyses (see main text and [Supplementary Statistics S2 and S3](#)), the concluding message is that the acanthostyles of wild individuals are only 6 μm shorter than acanthostyles-1 of individuals cultured at high-dSi (probably representing the same category), and both are much shorter than acanthostyles-2 (i.e. $Ac-W < Ac-1 < Ac-2$). Regarding width, the acanthostyles of wild individuals are indistinguishable from acanthostyles-1, being both much thinner than acanthostyles-2 (i.e. $Ac-W = Ac-1 < Ac-2$). E, G, length and width of oxea formed by 10 wild individuals of *Petrosia ficiformis* in natural, low-dSi conditions and by five other individuals transferred to experimental high-dSi conditions. F, H, length and width of *P. ficiformis* oxeas pooled by level of dSi treatment (low-dSi vs. high-dSi). The statistical analyses indicate that oxea length decreases slightly in high dSi conditions (l-dSi > h-dSi), while oxea thickness increases (l-dSi < h-dSi). See main text and [Supplementary Statistics S4](#) for statistics, and [Supplementary Datafile S4](#) for numerical values. Boxplots represent the first and third quartiles of data by their box size, the median value is given as an internal line, and the lower and upper limits as error bars. Outliers are shown in summarized form, as 5th and 95th percentiles.

of high-dSi conditions) (Fig. 5G, H). Holotype acanthostyle length ranges 62–221 μm and averages $102 \pm 16 \mu\text{m}$, and width ranges 3.6–13.6 μm and averages $8.0 \pm 1.8 \mu\text{m}$. Nevertheless, note that the holotype's spicules, despite being thicker and more spiny than those in the 10 wild individuals from the low-dSi Blanes population, never become as thick as acanthostyles-2 produced under experimental dSi-enriched conditions (Supplementary Table S2, Datafile S2).

From the current analysis of *A. oroides* skeletal data, a plausible take-home message is that the species' holotype does not adequately represent the full spicule skeleton encoded in its genome, which, according to our experimental results, would comprise two categories of acanthostyles when dSi is abundant. However, this preliminary conclusion is misleading (see Discussion), because, as in the case of *C. crambe*, spicules can lie to sponge taxonomists.

Skeletal analysis of *Petrosia ficiformis* (Demospongiae: Haplosclerida)

Effects of dissolved silicon availability on skeleton formation

The microscopy study of the skeleton (Fig. 9) and the analyses of spicule size (Figs 8E–H, 10 and 11; Supplementary Tables S3, S4, Datafile S3) revealed that all 10 wild specimens sampled from the Mediterranean Spanish coast had a skeleton mostly consisting of only one spicule type: curved oxeas in a single size category. In contrast, the five individuals collected from the same location and maintained under high-dSi conditions over one year formed, in addition to oxeas, three other spicule types: styles, strongyles, and reniform strongyles. These additional spicule types were not casual occurrences, since they collectively accounted for about 30% to 80% of the total spicules found in the slides, depending on the individual (Figs 9C, 11D).

Importantly, visual inspection of the frequency distribution of oxea length (Fig. 10A, B) and width (Fig. 11A, B, D) reveals a tendency in many wild individuals to form a kind of bimodal distribution, but such a pattern never emerges clearly, not even among the individuals cultured under high dSi. However, LM and LMM analyses confirmed that mean oxea length and width differed between low-dSi and high-dSi conditions with statistical significance (Fig. 8E–H; Supplementary Datafile S4, Statistics S4). Individuals exposed to high dSi exhibited a mean spicule length of $143.59 \pm 54.55 \mu\text{m}$ compared to $172.90 \pm 39.45 \mu\text{m}$ for individuals under low dSi (LM: $F_{1,13} = 14.24$, $P = .00232$). Post-hoc comparisons confirmed such a $-29.32 \pm 7.77 \mu\text{m}$ shortening with increasing dSi ($t = -3.77$, $P = .00232$). In contrast, spicule width increased slightly—but with statistical significance (LM: $F_{1,13} = 16.80$, $P = .00126$)—in the individuals of the high dSi group ($5.35 \pm 0.26 \mu\text{m}$) relative to those of the low dSi group ($4.13 \pm 0.20 \mu\text{m}$). A posteriori mean comparisons supported this pattern ($t = 4.10$, $P = .00126$). The observed spicule shortening connected to a thickening with increasing dSi availability suggests occurrence of a trade-off during the spicule formation process between elongation and thickening.

However, it must be noticed that, when analysed in magnitude, the changes in oxea size with increasing dSi are less important in length ($\sim 18\%$ decrease) than in width (27% increase) and, in general, they both are smaller than those observed in spicules of *C. crambe* and *A. oroides*. Complementarily, the LMM analysis indicated that a substantial proportion of variation in both spicule length and width was structured among individuals rather than within individuals.

The summary message of the dSi effects on *P. ficiformis* skeleton is that an increased dSi availability exerts only moderated changes in spicule dimensions, while inducing more important changes in the composition of the spicule inventory, with formation of styles, strongyles, and reniform strongyles, in addition to the oxeas (Figs 9C, D, 11D). Such changes in the spicule inventory are not taxonomically trivial, since individuals containing only oxeas fits the diagnosis of *Petrosia clavata* (Esper, 1794), a rare Atlantic-Mediterranean species currently accepted as a valid taxon. In contrast, specimens forming styles, strongyles, and reniform strongyles, in addition to oxeas, better fit the diagnosis of *Petrosia ficiformis*, another valid Atlantic-Mediterranean species. Traditionally, the occurrence of reniform strongyles—which in our experiment were only formed under high dSi availability—has been considered a diagnostic character for the species *P. ficiformis* (Pulitzer-Finali 1983). Our findings rather support that, although *P. ficiformis* and *P. clavata* are currently considered valid taxa (World Porifera Database on February 2026 at <https://www.marinespecies.org/porifera/>), *P. clavata* should not be a valid species. It appears to represent the dSi-poor skeletal condition of *P. ficiformis* (see Discussion). Likewise, reniform strongyles cannot make a reliable diagnostic character for *P. ficiformis*, since they are formed only when dSi is available in abundance in the environment.

The skeleton of *Petrosia* spp. type material

The holotypes of *P. ficiformis* and *Petrosia clavata* are unfortunately lost and cannot help in this study.

Discussion

Availability of dissolved silicon and chronic skeletal underdevelopment

Our results support the emerging view that many siliceous sponges inhabiting marine shallow waters chronically experience dSi concentrations below the levels required for efficient dSi utilization (Reincke and Barthel 1997, Maldonado *et al.* 1999, 2012, 2020, Cárdenas and Rapp 2013, Shimizu *et al.* 2024, Leria and Maldonado 2026). The consequences are that the siliceous skeleton of many sponges is underdeveloped and incomplete to varying degrees, depending on the species. This skeletal underdevelopment appears to be generalized among temperate and tropical sponges, primarily driven by chronic dSi scarcity in these regions of the modern ocean, a situation exacerbated by competition with diatoms for this essential nutrient.

Our experiments show that providing chronically dSi-limited sponges with higher dSi availability produces substantial changes in both spicule diversity and size, with the magnitude

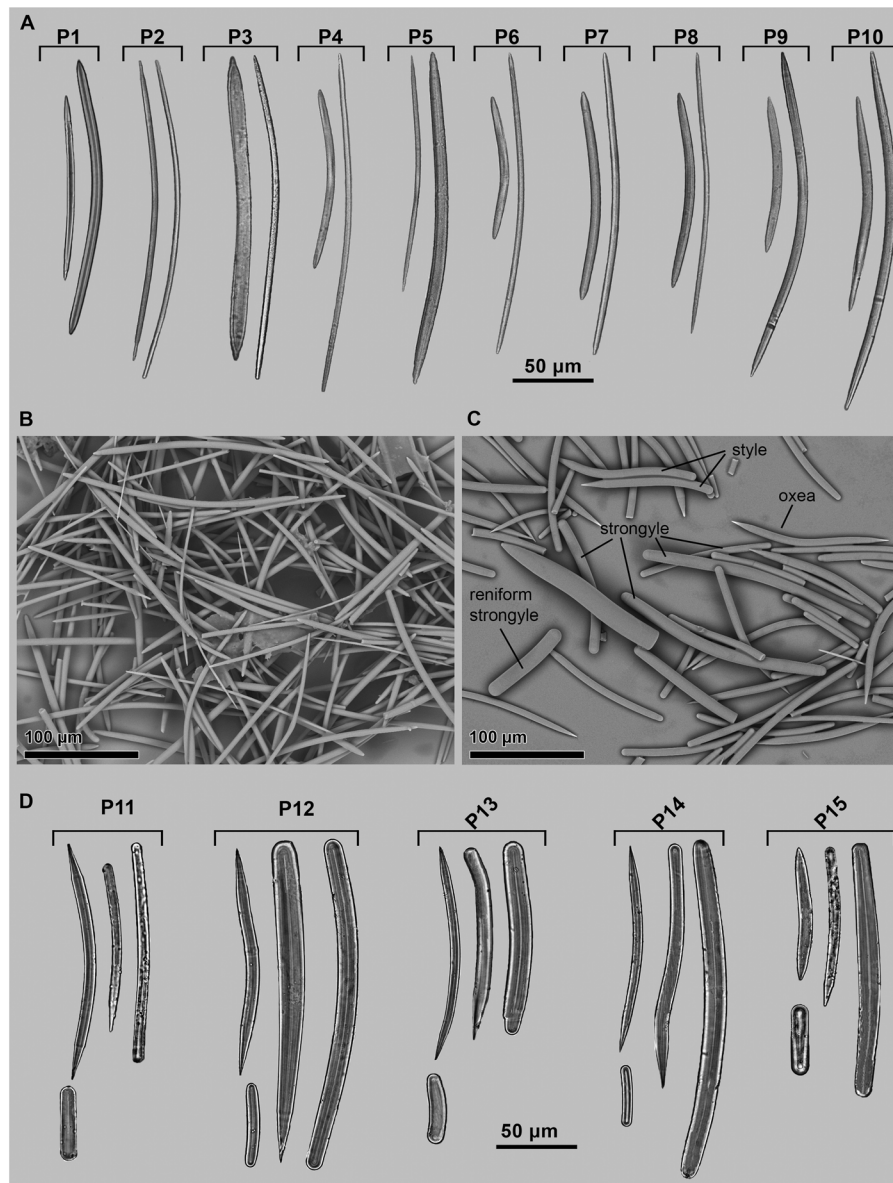


Figure 9 Spicule inventory of *Petrosia ficiformis*. A, light-microscopy view of the only spicule type (oxeas) produced by 10 wild individuals (P01–P10) in the dSi-poor ($1\ \mu\text{M}$), natural, Mediterranean habitat. Note that there is a wide variety in the length and thickness of these oxeas, although the size frequency distribution does not show a clear cut in two categories (see Figs 10 and 11). B, C, SEM general view comparing the skeleton of a wild individual formed by only oxeas and that of a high-dSi cultured individual containing additional strongyles, styles, and reniform strongyles. D, light-microscopy view of the various spicule types produced by five conspecific individuals (P11–P15) when maintained for 1 year under experimental conditions of high dSi ($48.1\ \mu\text{M}$), which include—in addition to the oxeas—styles, strongyles, reniform strongyles.

of these effects being species-dependent. Exposure to high dSi concentrations promotes the formation of spicule types that are absent in wild individuals, probably suppressed under the naturally low dSi conditions typical of many shallow-water habitats at tropical and temperate latitudes. For instance, *C. crambe* formed five spicule types under high-dSi experimental conditions (strongyles, choanosomal styles, isochelae-1 and -2, and desmas), in addition to feeble styles, which are the only spicule type observed in the wild specimens from the low-dSi Blanes population (Figs 3, 4J). In *P. ficiformis*, three spicule types were produced under high-dSi conditions (styles, strongyles, and reniform strongyles), in addition

to the thin oxeas, which are the only spicule type found in the wild specimens from the Blanes population (Fig. 9). In *A. oroides*, only one additional spicule category was formed under high-dSi conditions, acanthostyles-2, which are somewhat longer and much thicker than the acanthostyles with feeble spines present in the wild specimens (Fig. 5).

These similar skeletal responses to dSi enrichment in three species from distinct lineages (orders Poecilosclerida, Agelasida, and Haplosclerida) suggest that the relatively low concentrations of this nutrient in most modern shallow-water ecosystems (excluding polar regions) are driving chronic and generalized skeletal underdevelopment among demosponges.

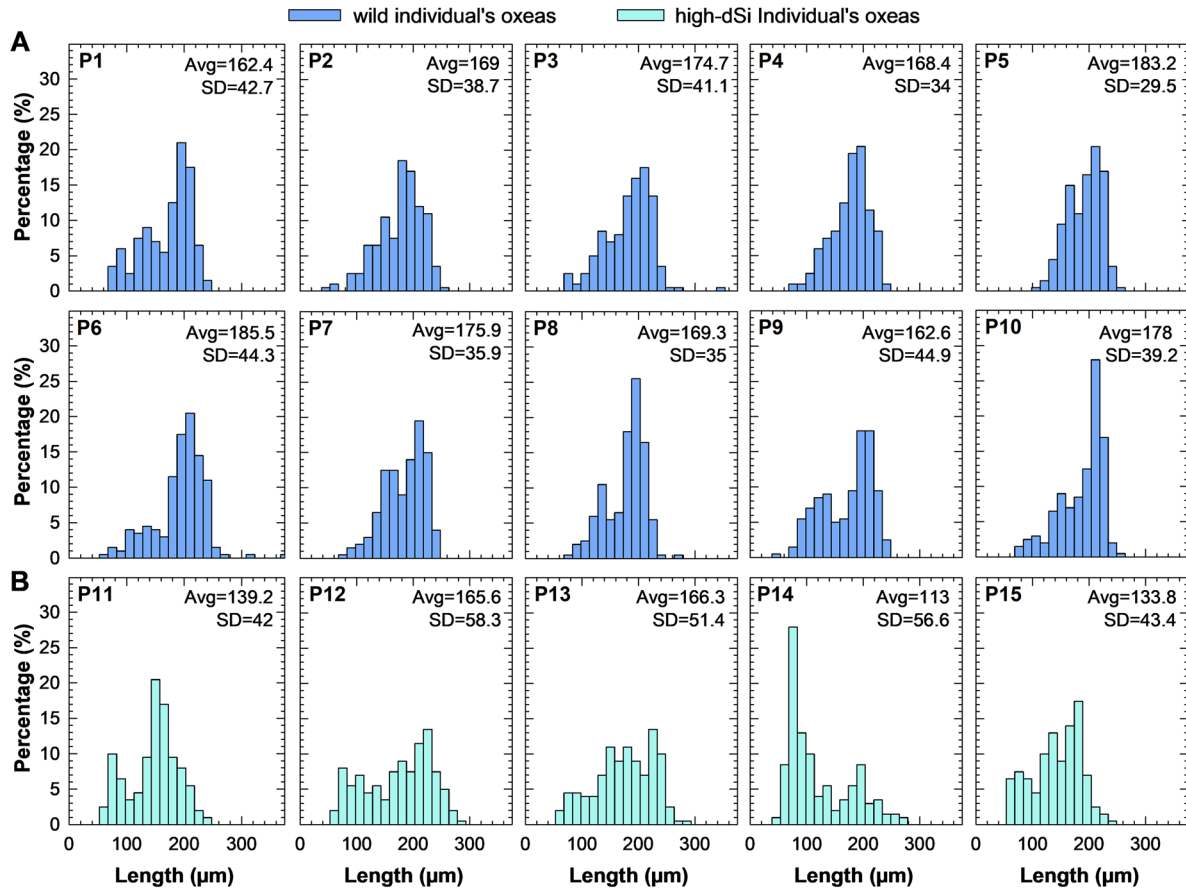


Figure 10 Frequency distribution of oxa length in the individuals of *Petrosia ficiformis* studied. See also [Supplementary Table S3](#) and [Supplementary Datafile S4](#) for numerical values. Percentages are calculated on 200 spicules measured at random. A, the 10 wild individuals show a skewed distribution of oxa length, with a trend toward a bimodal pattern, but without clearly discriminating two separate length categories. B, the five individuals cultured in high-dSi show a similarly skewed distribution and, importantly, maintain a range of length similar to that of the wild conspecifics. Additional statistical tests for oxa comparison are in [Figure 8E–H](#) and [Supplementary Statistics S4](#).

Remarkably, despite their underdeveloped skeletons, these species maintain structural integrity and survive under environmental pressures, indicating that the functional role of certain spicule types (e.g. chelae) and certain spicule features (e.g. spination) may not be as critical for survival as previously assumed.

Under high dSi availability, spicule dimensions also change, with width generally more responsive than length. Increased dSi consistently induces substantial spicule thickening, while length can either increase or decrease slightly, depending on the species. This opposing response suggests a trade-off between elongation and thickening during spicule formation, potentially reflecting differential allocation of silicon under varying environmental availability. The length of spicules is thought to be largely determined during an intracellular phase of silica deposition aiming axial growth, while thickening occurs primarily during a still poorly-understood phase of extracellular silica deposition. Under low dSi, silicon may be preferentially invested in axial growth, allowing spicules to become longer at the expense of thickness, whereas higher silicon availability enables increased deposition in the radial direction, producing more robust spicules. Such plasticity in spicule morphology may have important functional and

evolutionary implications, influencing mechanical resistance, structural support, and ecological performance. These findings highlight the importance of considering multiple morphological dimensions when assessing the environmental effects on skeletal traits and underscore the value of mixed-effects modelling for disentangling treatment effects from individual-level variability in complex biological datasets.

Taxonomic implications of chronic and periodic dissolved silicon limitation

The skeletal variability we have experimentally generated through changes in environmental dSi availability is qualitatively significant in all cases. In the absence of molecular markers, artificially induced intraspecific skeletal differences could easily be misinterpreted as evidence for new species, as appears to be the case for *P. ficiformis* versus *P. clavata*. In cases of taxonomic uncertainty, the recommended approach is to compare the skeletons of the specimens in question with that of the holotypes.

In this context, we have determined that the type material is probably lost for both of *P. ficiformis* and *P. clavata*, but it remains available for *C. crambe* and *A. oroides*. However, our

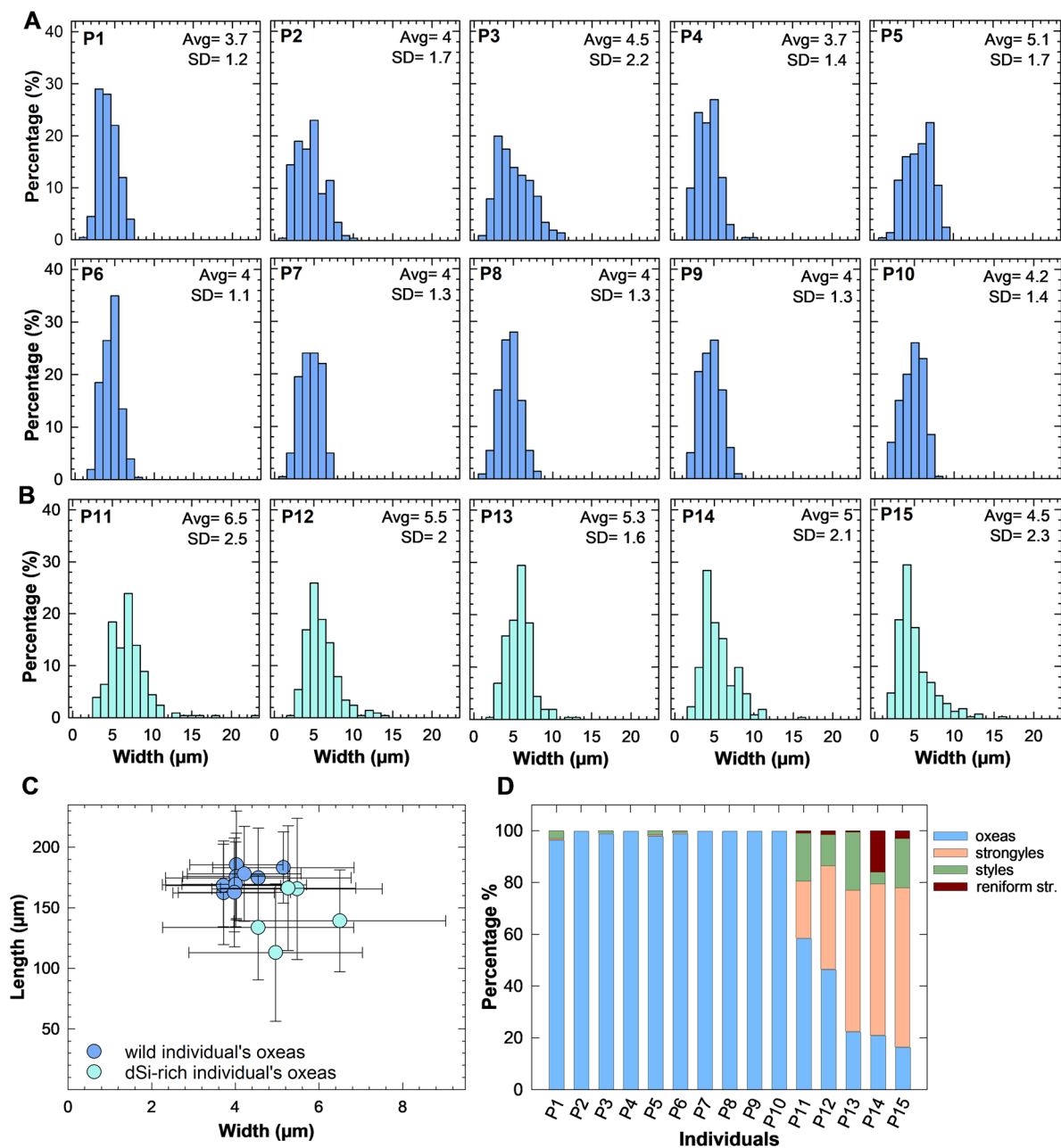


Figure 11 Frequency distribution of oxea width in the individuals of *Petrosia ficiformis* studied. See also [Supplementary Table S3](#) and [Supplementary Datafile S4](#) for numerical values. Percentages are calculated on 200 spicules measured at random. A, the 10 wild individuals show a unimodal distribution of oxea width. B, the five individuals cultured in high-dSi still show a unimodal distribution but skewed by occurrence of a low number of somewhat thicker spicules. However, a bimodal distribution of spicule width never emerges. C, when oxeas are plotted by their average length and width in the individuals studied, the spicules of the individuals cultured in high-dSi conditions show a trend to be slightly shorter and slightly thicker than those of the wild individuals, but, indeed, there is not a clear discrimination between both groups of individuals. Additional statistical tests for oxea comparison are in [Figure 8E–H](#) and [Supplementary Statistics S4](#). D, the main differences in skeleton between the wild individuals and the individuals cultured in high-dSi conditions is that the former ones produce essentially only oxeas, whereas the latter form styles, strongyles, and reniform strongyles in addition to the oxeas.

results show that the skeleton of the specimens forming the type series of *C. crambe* do not match the skeletal features of the bulk of the wild population, which is characterized by the presence of only thin styles. Surprisingly, the skeleton of the type specimens more closely resembles the more complex skeletons produced under experimental dSi concentrations as high as 100 μM (including choanosomal styles, isochelae,

and desmas; [Fig. 3](#)). This observation likely indicates that type specimens were collected from a region of the Croatian Adriatic Sea where dSi must be relatively higher than in the rest of the Mediterranean. The interpretation is further supported by the skeletal features of the holotype and the para-type of *A. oroides*, which were collected from the same region of the Adriatic Sea as the type material of *C. crambe*. In the

holotype of *A. oroides*, the verticillate acanthostyles (Fig. 5G, H), occurring in a single category, exhibit dimensions and a degree of spine development that do not closely match those of the acanthostyles found in the bulk of the low-dSi Blanes population (Fig. 5A, B). Instead, the holotype acanthostyles display features intermediate between the feeble, poorly-spined acanthostyles typical of low-dSi wild populations and the more robust acanthostyles produced experimentally under high-dSi conditions (Fig. 5C, D).

The 'atypical' skeletal features observed in type material of *C. crambe* and *A. oroides*—which do not match the bulk of the populations but resemble skeletons produced under experimentally high dSi—are consistent with recent evidence of previously underestimated dSi inputs in the northern and eastern Adriatic Sea. Contrary to the traditional view of the Adriatic as an oligotrophic Mediterranean region with consistently low dSi, it has been shown that dSi concentrations fluctuate seasonally, reaching averages of 10 μM and occasional peaks up to 80 μM in the northern Adriatic due to discharges from the Po River and other fluvial systems (Grilli *et al.* 2020). More importantly, in the vicinity of the estuary of the Krka River, close to the collection sites of the type specimens of *C. crambe* and *A. oroides* by Schmidt (1862, 1864), important discharges of underground water with high dSi concentrations have been documented (Liu *et al.* 2019). Although these inputs may diffuse rapidly and dilute in the water column, sponges attached to the seabed are likely to experience periodically elevated dSi concentrations within the deep boundary layer. Such localized dSi enrichment probably explains why the holotype skeletons resemble those formed under experimentally high-dSi conditions, rather than matching the underdeveloped skeletons typical of most wild individuals in naturally low-dSi habitats across the Atlantic-Mediterranean region.

This raises an important taxonomic question. Is it preferable to have a holotype representing with fidelity the phenotypic expression of all the skeletal characters encoded in the genome of the species, even when such a complete spicule inventory is very rarely found among the individuals of the populations, or, alternatively, to select a holotype that does not reflect the full phenotypic expression of the species genome but that closely matches the underdeveloped skeletons in the bulk of the wild population. Regardless of the approach, assessing the dSi concentration at the type locality, whenever possible, may be crucial to evaluating the degree of skeletal completion or underdevelopment in the holotype and to better interpret its taxonomic significance.

The results of our experiments also highlight the risk that taxonomists may misinterpret certain spicule size categories that form only under high-dSi conditions as genetically encoded traits, when they are in fact 'physiological skeletal artefacts' resulting from periodic shifts in dSi availability in the sponge's habitat. This appears to be the case for the acanthostyles-2 produced by *A. oroides* only under high-dSi conditions.

Morphological and statistical analyses indicate that acanthostyles-2 differ significantly from acanthostyles-1 (Fig. 7A, B, D; Supplementary Statistics S3), which would lead most taxonomists to conclude that *A. oroides* forms only one

acanthostyle category under low-dSi conditions and two categories under high-dSi conditions. However, the feeble acanthostyles of wild individuals are nearly identical in size and ornamentation to acanthostyles-1 of individuals transferred to high-dSi conditions. This suggests that *A. oroides* may form only a single acanthostyle category: thin, poorly spined acanthostyles (acanthostyles-1) under low-dSi conditions, which are formed as thicker spicules with larger spines (acanthostyles-2) when dSi is abundant. Both 'false' spicule categories coexist in the bodies of the experimental *A. oroides* specimens because the sponges were transferred from their natural low-dSi habitat (where they had formed only poorly developed acanthostyles) to high-dSi experimental conditions (where they produced thick, spiny acanthostyles). Although we sampled only peripheral body zones known to have grown during the high-dSi culturing period, it was unavoidable to collect some spicules formed earlier in the natural habitat (acanthostyles-1). Avoiding this spicule overlap entirely would require initiating experiments with aspiculate larvae rather than adult spiculate individuals, but the larval approach is not always feasible.

This example of a 'ghost' spicule size category—one not encoded in the species' genome—conveys an important message for sponge taxonomists: extreme caution is needed when interpreting skeletal traits in specimens from habitats where dSi concentrations may fluctuate seasonally or follow other periodic patterns. A paradigmatic natural scenario is provided by anchialine submarine caves with intermittent groundwater discharges. In such habitats, significant dSi inputs can occur during the rainy season, enabling sponges to produce fully developed skeletal elements, which then coexist in the same body with underdeveloped spicules formed during the dry season, when dSi becomes limiting.

The scenario can be even more extreme in caves lacking groundwater input and with restricted seawater exchange. In these habitats, dSi values may be very low, which probably explains reports of rare cave sponge species exhibiting unusually underdeveloped skeletal features (Labate 1964, 1965, Labate and Narena 1964, Harmelin *et al.* 1985). In some caves, even spicule dissolution has been reported (Bavestrello *et al.* 1996), probably because the water is highly undersaturated with dSi, chemically favouring silica dissolution to restore equilibrium. At the opposite extreme, some caves receive significant dSi-enriched groundwater and, due to limited exchange with the coastal ocean, maintain high dSi availability. In these habitats, sponge species that require hypersilicified skeletons, such as lithistid demosponges, may proliferate extensively (Pisera and Gerovasileiou 2021). Serious taxonomic challenges arise when the same sponge species can colonize both dSi-rich and dSi-poor caves. Our results predict that the same species will form markedly different skeletons in each habitat, potentially rendering conspecific individuals unrecognizable to phenetic taxonomists and highlighting the frequent need for DNA-based identification.

Although anchialine caves provide a paradigmatic example of habitats where the effects of dSi availability on sponge skeletons—and, consequently, on taxonomic practice—can be pronounced, they are not the only environments experiencing large dSi fluctuations. Significant changes in dSi

concentrations have also been reported in shallow Antarctic habitats, where sponges can form extensive aggregations (Dayton 1989, Barthel and Gutt 1992, Gutt *et al.* 2013, Maldonado *et al.* 2017). In these regions, winter dSi concentrations can rise to very high levels (80–100 μM) because low light limits diatom dSi consumption, whereas during the warm season, diatom proliferation reduces dSi values in the water column to near zero (Leynaert *et al.* 1993). Likewise, in mangroves, where sponges can establish important communities on the roots (Rützler *et al.* 2000, Maldonado *et al.* 2010, 2017, Diaz 2012, Becking *et al.* 2013), dSi can fluctuate significantly due to a variety of factors such as plant uptake, tidal cycles, rainfall patterns, benthic efflux from sediments, and phytoplankton consumption (Elizondo *et al.* 2021).

The skeletal effects of dSi fluctuations are expected to be most pronounced in shallow-water ecosystems for two main reasons: (i) diatoms can chronically maintain low dSi levels only in the photic zones of tropical and temperate oceans, and (ii) the main inputs of dSi into the ocean occur at the surface, through riverine discharge, groundwater input, and mineral dust deposition (Tréguer *et al.* 2021). In contrast, deep-sea dSi concentrations fluctuate less in magnitude and more slowly over time. Hexactinellid sponges, which primarily inhabit deep-water environments where dSi concentrations exceed 10 μM (Alvarez *et al.* 2017), are, therefore, unlikely to experience the chronic dSi limitation affecting shallow-water demosponges. Nevertheless, specific deep-sea habitats can influence sponge skeletal formation. For example, hydrothermal vents release fluids highly enriched in dSi (Tréguer *et al.* 2021), potentially enhancing dSi availability for skeletal growth. Conversely, some hydrothermal vents also discharge germanium (Ge), such as certain Pacific black smokers, where Ge/Si ratios in vent plumes are approximately 10-fold higher than in river water (Mortlock *et al.* 1993). Near these Ge-rich plumes, skeletal growth may be impaired, as Ge—owing to its chemical similarity to Si—can enter the silicification pathway, producing aberrant spicules or even inhibiting spicule formation (Elvin 1972, Simpson *et al.* 1979, Simpson 1981).

In summary, sponges inhabiting environments with pronounced dSi fluctuations—whether in shallow waters or in the deep sea—are likely to exhibit greater skeletal variability than conspecifics from habitats with more stable dSi concentrations. Understanding these habitat-specific features is essential for interpreting skeletal variability and its taxonomic implications.

Environmental dissolved silicon and sponge evolution through palaeontological time

Recent studies suggest that the elevated dSi concentration characterizing the Ediacaran and Early Cretaceous oceans, which was probably toxic to the early skeletal sponges, shaped the evolution of silicon-transport systems in Hexactinellida, Demospongiae, Homoscleromorpha, and Calcarea (Leria and Maldonado 2026), and triggered the independent emergence of silicification in each sponge class (Shimizu *et al.* 2024). After this initial skeletonization, the environmental availability of

silicate continued to influence the evolutionary and ecological history of siliceous sponges throughout palaeontological time.

It is now well established that the original benthic communities of the Cambrian oceans have been drastically modified through the successive mass extinctions of the Ordovician (444 Mya), Late Devonian (372 Mya), End-Permian (252 Mya), End-Triassic (201 Mya), and End-Cretaceous (66 Mya). Although each extinction had different causes, most of them included combinations of shifts in temperature, oxygen availability, ocean pH, and/or water chemistry. These are all environmental stressors to which many modern sponges are known to be relatively resilient (Maldonado and Young 1998, Bell *et al.* 2013, 2018, 2026, Micaroni *et al.* 2022, Maldonado *et al.* 2026).

After revising the palaeontological literature, we have identified independent studies suggesting, for each of the extinctions, that some sponge groups were probably relatively resilient to the harsh conditions associated with the extinction, since the sponge fauna tended to a more rapid post-extinction recovery than other benthic groups. This pattern has been discussed for the End-Ordovician (Botting *et al.* 2017), Late Devonian (Wood 2004), End-Permian (Reinhardt 1991, Mehl 1992, 1999, Wiedenmayer 1994, Wu *et al.* 2024), and End-Triassic (Ritterbush *et al.* 2015). In these after-extinction expansions there were important contributions by siliceous sponges, which had access to substantial dSi concentrations to fuel their skeletal growth. It is thought that the processes of continental spreading, weathering of the new floor, and submarine volcanism sourced the required dSi.

In the Late-Triassic extinction, which was in part driven by ocean acidification, calcified organisms (mollusks, corals, and calcareous sponges) were more impacted. Again, in the aftermath, Early Jurassic benthic assemblages became dominated first by siliceous demosponges and later by hexactinellids (Mehl 1992, Wiedenmayer 1994). This proliferation was facilitated because, despite a progressive increase in the biological dSi consumption since the Cambrian (Conley *et al.* 2017), Early-Jurassic dSi concentrations were still quite high, probably above 800 μM (Racki and Cordey 2000, Ritterbush *et al.* 2015). During the Jurassic and Early Cretaceous, the still modest abundance of diatoms, along with the intense creation of new floor and marine vulcanism, kept sourcing the dSi required for the growth of the hypersilicified skeletons. These circumstances supported an exceptional proliferation of highly silicified hexactinellids and lithistid demosponges, which allied with microbiolites to form reefs on the warm continental shelves of the Late Mesozoic Tethys Sea, typically growing at shallower depths than their modern descendants (Moret 1925, Mostler 1976, Ghiold 1991, Mehl 1992, Wiedenmayer 1994).

Importantly, the post-Cretaceous scenario for the after-extinction recovery differed markedly from the one of previous extinctions. The progressive evolutionary diversification and ecological expansion of diatoms during the Jurassic and Cretaceous had reduced silicate concentrations in the global ocean by the end of the Cretaceous. This scarcity of dSi, which had no precedent in the global ocean, prevented reef-forming sponges from developing their massive siliceous

skeletons, which constituted the structural core of the reefs. Many of those sponge species and genera became extinct; some survived to our days despite being unable to complete their hypersilicified, complex skeletons. The populations of the surviving hexactinellids and lithistids appear also to have progressively migrated towards the aphotic zone (typically to the epibathyal zone), where they persist today because in the absence of sunlight the competition with diatoms is reduced and the environmental dSi availability is higher than in well-illuminated, shallower waters. Therefore, unlike in previous extinctions, the faunal recovery (which led to the modern sponge fauna) was driven mostly by sponges capable of surviving under low silicate availability and even with underdeveloped skeletons, as our results indicate.

Conclusion

Our experimental results demonstrate that environmental dSi availability strongly influences skeletal development in shallow-water demosponges, with both the magnitude and nature of the effects varying among species. A consistent pattern emerges: the naturally low dSi concentrations prevailing in most shallow-water habitats of modern oceans—maintained by intense diatom uptake—are insufficient to support full formation of demosponge siliceous skeletons. Consequently, these sponges persist with skeletons that are underdeveloped to varying degrees. Crucially, our findings highlight that such skeletal underdevelopment may also affect holotypes preserved in museum collections, which traditionally have been considered as standard references for species. Sponge taxonomists need to reconsider the way in which sponge holotypes represent species and how their skeletal traits can be used for species diagnosis, particularly in comparative skeletal analyses.

To improve the assessment of skeletal completeness in the holotype of siliceous sponges, we propose two recommendations. (i) For holotypes described in previous studies, every effort should be made to identify dSi concentrations from the original sponge habitat using available literature or databases. When such information is unavailable, dSi values from the nearest comparable habitat, including a similar bathymetric range, should be used. Knowledge of bottom-water silicate concentrations will enable more accurate interpretation of the skeletal characteristics of historical holotypes. (ii) For newly described holotypes of siliceous sponges, reporting the silicate concentration of the bottom seawater at the collection site should be considered standard practice, if not a mandatory requirement. This particularly applies to species from shallow-waters (less than 200 m). Whenever possible, these measurements should be obtained directly from bottom waters rather than inferred from mid-water column data, which are more commonly available from oceanographic surveys but may not accurately represent the chemical conditions experienced by the sponges.

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Supplementary material

Supplementary material is available at *Zoological Journal of the Linnean Society* online.

Conflicts of interest

The authors declare no conflict of interest.

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Data availability

All the original data necessary to reproduce the analyses reported in this study can be accessed through the article content and its associated supporting information. For the review process, the reviewer sharing link is: http://datadryad.org/share/LINK_NOT_FOR_PUBLICATION/2TiZQDwctxkXuct0xn33zu1YaDfrLtYUQKri_MAVbTU

References

- Alvarez B, Frings PJ, Clymans W *et al.* Assessing the Potential of Sponges (Porifera) as Indicators of Ocean Dissolved Si Concentrations. *Frontiers in Marine Science* 2017;**4**. <https://doi.org/10.3389/fmars.2017.00373>
- Aparicio M, Le Bihan A, Jeandel C *et al.* Contribution of sandy beaches to the global marine silicon cycle. *Nature Geoscience* 2025;**18**:154–9. <https://doi.org/10.1038/s41561-024-01628-6>
- Barthel D, Gutt J. Sponge associations in the eastern Weddell Sea. *Antarctic Science* 1992;**4**:137–50.
- Bavestrello G, Pansini M, Sarà M, *et al.* The variability and taxonomic status of different Petrosia-like sponges in the Mediterranean sea. In: Soest RWM, van Kempen ThMG, Braekman JC (eds), *Sponges in Time and Space*. Rotterdam: A.A. Balkema, 1994, 83–92.
- Bavestrello G, Cattaneo-Vietti R, Cerrano C *et al.* Spicule dissolution in living *Tethya omanensis* (Porifera: Demospongiae) from a tropical cave. *Bulletin of Marine Science* 1996;**58**:598–601.

- Becking LE, Cleary DFR, de Voogd NJ. Sponge species composition, abundance, and cover in marine lakes and coastal mangroves in Berau, Indonesia. *Marine Ecology Progress Series* 2013;**481**:105–20. <https://doi.org/10.3354/meps10155>
- Bell JJ, Davy SK, Jones T *et al.* Could some coral reefs become sponge reefs as our climate changes? *Global Change Biology* 2013;**19**:2613–24. <https://doi.org/10.1111/gcb.12212>
- Bell JJ, Rovellini A, Davy SK *et al.* Climate change alterations to ecosystem dominance: how might sponge-dominated reefs function? *The Bulletin of the Ecological Society of America* 2018;**99**:e01434. <https://doi.org/10.1002/BES2.1434>
- Bell JJ, Shaffer M, Luter HM *et al.* Assessing sponge resilience to ocean acidification in natural reef environments. *Journal of Experimental Marine Biology and Ecology* 2026;**595**:152164. <https://doi.org/10.1016/J.JEMBE.2026.152164>
- Bibiloni MA. Fauna de Esponjas de las Islas Baleares. In: *Variación Cualitativa y Cuantitativa De La Población De Esponjas En Un Gradiente Batimétrico. Comparación Baleares-Costa Catalana*. Ph.D. Thesis, Universidad de Barcelona. Departamento de Ecología, 1990.
- Botting JP, Cárdenas P, Peel JS. A crown-group demo-sponge from the early Cambrian Sirius Passet Biota, North Greenland. *Palaeontology* 2015;**58**:35–43. <https://doi.org/10.1111/pala.12133>
- Botting JP, Muir LA, Zhang Y *et al.* Flourishing sponge-based ecosystems after the end-ordovician mass extinction. *Current Biology: CB* 2017;**27**:4:556–62. <https://doi.org/10.1016/j.cub.2016.12.061>
- Boury-Esnault N. Spongiaires de la zone rocheuse littorale de Banyuls-sur-Mer. I. Ecologie et Répartition. Vie et Milieu. Bulletin du Laboratoire Arago, Université de Paris 1971;**22**:159–92.
- Burgsdorf I, Erwin PM, López-Legentil S *et al.* Biogeography rather than association with cyanobacteria structures symbiotic microbial communities in the marine sponge *Petrosia ficiformis*. *Frontiers in Microbiology* 2014;**5**:529. <https://doi.org/10.3389/fmicb.2014.00529>
- Cárdenas P, Rapp HT. Disrupted spiculogenesis in deep-water Geodiidae (Porifera, Demospongiae) growing in shallow waters. *Invertebrate Biology* 2013;**132**:173–94. <https://doi.org/10.1111/ivb.12027>
- Chang S, Feng Q, Clausen S *et al.* Sponge spicules from the lower Cambrian in the Yanjiahe Formation, South China: the earliest biomineralizing sponge record. *Palaeogeography, Palaeoclimatology, Palaeoecology* 2017;**474**:36–44. <https://doi.org/10.1016/j.palaeo.2016.06.032>
- Conley DJ, Frings PJ, Fontorbe G *et al.* Biosilicification drives a decline of dissolved Si in the Oceans through geologic time. *Frontiers in Marine Science* 2017;**4**. <https://doi.org/10.3389/fmars.2017.00397>
- Dayton PK. Interdecadal variation in an Antarctic sponge and its predator from oceanographic climate shifts. *Science (New York, N.Y.)* 1989;**245**:1484–6. <https://doi.org/10.1126/science.245.4925.1484>
- Diaz MC. Mangrove and coral reef sponge faunas: untold stories about shallow water Porifera in the Caribbean. *Hydrobiologia* 2012;**687**:179–90. <https://doi.org/10.1007/s10750-011-0952-5>
- Dohrmann M, Wörheide G. Dating early animal evolution using phylogenomic data. *Scientific Reports* 2017;**7**:3599–6. <https://doi.org/10.1038/s41598-017-03791-w>
- Elizondo EB, Carey JC, Al-Haj AN *et al.* High productivity makes mangroves potentially important players in the tropical silicon cycle. *Frontiers in Marine Science* 2021;**8**:652615. <https://doi.org/10.3389/fmars.2021.652615>
- Elvin D. Growth rates of the siliceous spicules of the fresh-water sponge *Ephydatia muelleri* (Lieberkühn). *Transactions of the American Microscopical Society* 1971;**90**:219–24.
- Elvin DW. Effect of germanium upon development of siliceous spicules of some fresh-water sponges. *Experimental Cell Research* 1972;**72**:551–3. [https://doi.org/10.1016/0014-4827\(72\)90027-4](https://doi.org/10.1016/0014-4827(72)90027-4)
- Erwin DH, Laflamme M, Tweedt SM *et al.* The Cambrian conundrum: early divergence and later ecological success in the early history of animals. *Science (New York, N.Y.)* 2011;**334**:1091–7. <https://doi.org/10.1126/science.1206375>
- Ghiold J. The sponges that spanned Europe. *New Scientist* 1991;**129**:58–62.
- Grilli F, Accoroni S, Acri F *et al.* Seasonal and interannual trends of oceanographic parameters over 40 years in the Northern Adriatic Sea in relation to nutrient loadings using the EMODnet chemistry data portal. *Water* 2020;**12**:2280. <https://doi.org/10.3390/w12082280>
- Gutt J, Böhmer A, Dimmler W. Antarctic sponge spicule mats shape macrobenthic diversity and act as a silicon trap. *Marine Ecology Progress Series* 2013;**480**:57–71. <https://doi.org/10.3354/meps10226>
- Harmelin JG, Vacelet J, Vasseur P. Les grottes sous-marines obscures: un milieu extrême et un remarquable biotope refuge. *Téthys* 1985;**11**:214–29.
- Jurkowska A, Świerczewska-Gładysz E. The evolution of the marine Si cycle in the Archean–Palaeozoic—an overlooked Si source? *Earth-Science Reviews* 2024;**248**:104629. <https://doi.org/10.1016/J.EARSCIREV.2023.104629>
- Labate M. Poriferi di grotta superficiale del litorale Adriatico Pugliese. *Annali del Pontificio Istituto Superiore di Scienze e Lettere “S. Chiara”* 1964;**14**:319–42.
- Labate M. Ecologia dei Poriferi della Grotta della Regina. (Adriatico meridionale). *Bolletino di Zoologia* 1965;**32**:541–53.
- Labate M, Narena P. La fauna dei Poriferi nei laghi di Ganzirri e Faro (Messina). *Archivio Zoologico Italiano* 1964;**49**:249–80.
- Leria L, Maldonado M. Innovations in silicon transport shaped the rise of biosilicification and skeletal evolution in sponges. *Molecular Biology and Evolution* 2026;**43**:1–24. <https://doi.org/10.1093/molbev/msag047>
- Lévi C. Systématique de la Classe des Demospongiaria (Demosponges). In: Grassé PP (ed.), *Spongiaires. Anatomie, Physiologie, Systématique, Ecologie*. Paris: Masson et Cie, 1973, 577–631.
- Leynaert A, Nelson DM, Quéguiner B *et al.* The silica cycle in the Antarctic Ocean: is the Weddell Sea atypical? *Marine Ecology Progress Series* 1993;**96**:1–15.

- Liu J, Hrutić E, Du J *et al.* Net submarine groundwater-derived dissolved inorganic nutrients and carbon input to the oligotrophic stratified karstic estuary of the Krka River (Adriatic Sea, Croatia). *Journal of Geophysical Research: Oceans* 2019;124:4334–49. <https://doi.org/10.1029/2018JC014814>
- López-Acosta M, Leynaert A, Maldonado M. Silicon consumption in two shallow-water sponges with contrasting biological features. *Limnology and Oceanography* 2016;61:2139–50. <https://doi.org/10.1002/lno.10359>
- López-Acosta M, Leynaert A, Coquille V *et al.* Silicon utilization by sponges: an assessment of seasonal changes. *Marine Ecology Progress Series* 2018;605:111–23.
- Ma JY, Yang Q. Early divergence dates of demosponges based on mitogenomics and evaluated fossil calibrations. *Palaeoworld* 2016;25:292–302. <https://doi.org/10.1016/j.palwor.2015.03.004>
- Maldonado M, Riesgo A. Gametogenesis, embryogenesis, and larval features of the oviparous sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). *Marine Biology* 2009a;156:2181–97. <https://doi.org/10.1007/s00227-009-1248-4>
- Maldonado M, Riesgo A. Reproduction in the phylum Porifera: a synoptic overview. In: *Biology of Reproduction*. Barcelona: Treballs de la Societat Catalana de Biologia, 2008;59:29–49. <https://doi.org/10.2436/20.1501.02.56>
- Maldonado M, Uriz MJ. Skeletal morphology of two controversial Poecilosclerid genera (Porifera, Demospongiae): *Discorhabdella* and *Crambe*. *Helgoländer Meeresuntersuchungen* 1996;50:369–90. <https://doi.org/10.1007/bf02367110>
- Maldonado M, Young CM. Limits on the bathymetric distribution of keratose sponges: a field test in deep water. *Marine Ecology Progress Series* 1998;174:123–39. <https://doi.org/10.3354/meps174123>
- Maldonado M, Carmona MC, Uriz MJ *et al.* Decline in Mesozoic reef-building sponges explained by silicon limitation. *Nature* 1999;401:785–8. <https://doi.org/10.1038/44560>
- Maldonado M, Riesgo A, Bucci A *et al.* Revisiting silicon budgets at a tropical continental shelf: silica standing stocks in sponges surpass those in diatoms. *Limnology and Oceanography* 2010;55:2001–10. <https://doi.org/10.4319/lo.2010.55.5.2001>
- Maldonado M, Navarro L, Grasa A *et al.* Silicon uptake by sponges: a twist to understanding nutrient cycling on continental margins. *Scientific Reports* 2011;1:30. <https://doi.org/10.1038/srep00030>
- Maldonado M, Ribes M, van Duyl FC. Nutrient fluxes through sponges. Biology, budgets, and ecological implications. *Advances in Marine Biology* 2012;62:113–82. <https://doi.org/10.1016/B978-0-12-394283-8.00003-5>
- Maldonado M, Aguilar R, Bannister RJ *et al.* Sponge grounds as key marine habitats: a synthetic review of types, structure, functional roles, and conservation concerns. In: Rossi S, Bramanti L, Gori A *et al.* (eds), *Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots*. Cham, Switzerland: Springer International Publishing, 2017, 145–83. https://doi.org/10.1007/978-3-319-21012-4_24
- Maldonado M, López-Acosta M, Beazley L *et al.* Cooperation between passive and active silicon transporters clarifies the ecophysiology and evolution of biosilicification in sponges. *Science Advances* 2020;6:eaba9322. <https://doi.org/10.1126/sciadv.aba9322>
- Maldonado M, Pita L, Hentschel U *et al.* Aquatic Symbiosis Genomics Project Leadership. The chromosomal genome sequence of the sponge *Crambe crambe* (Schmidt, 1862) and its associated microbial metagenome sequences. *Wellcome Open Research* 2025;10:275. <https://doi.org/10.12688/wellcomeopenres.24154.1>
- Maldonado M, Escarré C, Lloveras A. In search of resilient sponges as candidate habitat engineers for the renaturalization of polluted harbor environments. *Frontiers in Marine Science* 2026;13:1875098. <https://doi.org/10.3389/fmars.2026.1875098>
- Maliva RG, Knoll AH, Siever R; New Collective Author. Secular change in chert distribution: a reflection of evolving biological participation in the silica cycle. *Palaios* 1989;4:519–32. <https://doi.org/10.1130/11539810>
- Maliva RG, Knoll AH, Simonson BM. Secular change in the Precambrian silica cycle: insights from chert petrology. *Geological Society of America Bulletin* 2005;117:835–45. <https://doi.org/10.1130/b25555.1>
- Mehl D. Die Entwicklung der Hexactinellida seit dem Mesozoikum. *Paläobiologie, Phylogenie und Evolutionsökologie. Berlin Geowissenschaftliche Abhandlungen* 1992;E:1–164. <https://doi.org/10.23689/figeo-5877>
- Mehl D. The phylogenetic history of sponges in palaeozoic times. *Memoirs of the Queensland Museum* 1999;44:410.
- Micaroni V, Strano F, McAllen R *et al.* Adaptive strategies of sponges to deoxygenated oceans. *Global Change Biology* 2022;28:1972–89. <https://doi.org/10.1111/gcb.16013>
- Moret L. Contribution à l'étude des spongiaires siliceux du Crétacé supérieur français. *Mémoires de la Société Géologique de France* 1925;5:1–327.
- Mortlock RA, Froelich PN, Feely RA *et al.* Silica and germanium in Pacific Ocean hydrothermal vents and plumes. *Earth and Planetary Science Letters* 1993;119:365–78. [https://doi.org/10.1016/0012-821X\(93\)90144-X](https://doi.org/10.1016/0012-821X(93)90144-X)
- Mostler H. Poriferenspiculae der alpinen Trias. *Geologische-Paläontologische Mitteilungen Innsbruck* 1976;6:1–42.
- Nelson DM, Tréguer P, Brzezinski MA *et al.* Production and dissolution of biogenic silica in the ocean: revised global estimates, comparison with regional data and relationship to biogenic sedimentation. *Global Biogeochemical Cycles* 1995;9:359–72. <https://doi.org/10.1029/95GB01070>
- Pansini M. *Petrosia pulitzeri* n.sp. (Porifera, Demospongiae) from Mediterranean caves. *Italian Journal of Zoology* 1996;63:169–72. <https://doi.org/10.1080/11250009609356126>
- Pé J. Étude quantitative de la régulation du squelette chez une éponge d'eau douce. *Archives de biologie (Liege)* 1973;84:147–73.
- Pisera A, Gerovasileiou V. Lithistid demosponges of deep-water origin in marine caves of the North-Eastern Mediterranean Sea. *Frontiers in Marine Science* 2021;8:20. <https://doi.org/10.3389/fmars.2021.630900>

- Pita L, Maldonado M, Erpenbeck D *et al.*; Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory Team. The chromosomal genome sequence of the Maltese sponge, *Agelas oroides* (Schmidt, 1864), and its associated microbial metagenome sequences. *Wellcome Open Research* 2025;10:464. <https://doi.org/10.12688/WELLCOMEOPENRES.24745.1>
- Pulitzer-Finali G. Report on a collection of sponges from the Bay of Naples. III. Hadromerida, Axinellida, Poecilosclerida, Halichondrida, Haplosclerida. *Bollettino dei Musei e degli Istituti Biologici dell' Università di Genova* 1978;45:7–89.
- Pulitzer-Finali G. A collection of Mediterranean Demospongiae (Porifera): with, in appendix, a list of the Demospongiae hitherto recorded from the Mediterranean Sea. *Annali Del Museo Civico Di Storia Naturale Giacomo Doria* 1983;84:445–621.
- Racki G, Cordey F. Radiolarian palaeoecology and radiolarites: is the present the key to the past? *Earth-Science Reviews* 2000;52:83–120. [https://doi.org/10.1016/S0012-8252\(00\)00024-6](https://doi.org/10.1016/S0012-8252(00)00024-6)
- Reincke T, Barthel D. Silica uptake kinetics of *Halichondria panicea* in Kiel Bight. *Marine Biology* 1997;129:591–3. <https://doi.org/10.1007/s002270050200>
- Reinhardt JW. Eastern Tethyan sponge buildups at the close of the Palaeozoic (Uppermost Permian, Sichuan/China). In: Reitner J, Keupp H (eds.), *Fossil and Recent Sponges*. Berlin: Springer-Verlag, 1991, 456–64.
- Ritterbush KA, Rosas S, Corsetti FA *et al.* Andean sponges reveal long-term benthic ecosystem shifts following the end-Triassic mass extinction. *Palaeogeography, Palaeoclimatology, Palaeoecology* 2015;420:193–209. <https://doi.org/10.1016/J.PALAEO.2014.12.002>
- Rützler K, Díaz MC, van Soest RWM *et al.* Diversity of sponge fauna in mangrove ponds, Pelican Cays, Belize. *Atoll Research Bulletin* 2000;467:229–48.
- Schmidt O. *Die Spongien des Adriatischen Meeres*. Leipzig: ed. W. Engelmann, 1862.
- Schmidt O. *Die Spongien des Adriatischen Meeres*. Leipzig: Engelmann, W, 1864.
- Shimizu K, Nishi M, Sakate Y *et al.* Silica-associated proteins from hexactinellid sponges support an alternative evolutionary scenario for biomineralization in Porifera. *Nature Communications* 2024;15:181. <https://doi.org/10.1038/s41467-023-44226-7>
- Siever R. Silica in the oceans: biological-geochemical interplay. In: Schenider SH, Boston PJ (eds), *Scientists on Gaia*. Cambridge, MA: MIT Press, 1991, 287–95.
- Siever R. The silica cycle in the Precambrian. *Geochimica et Cosmochimica Acta* 1992;56:3265–72.
- Simpson TL. Effects of germanium on silica deposition in sponges. In: Simpson TL, Volcani BE (eds), *Silicon and Siliceous Structures in Biological Systems*. New York: Springer, 1981, 527–49.
- Simpson TL, Refolo LM, Kaby ME. Effects of germanium on the morphology of silica deposition in a freshwater sponge. *Journal of Morphology* 1979;159:343–53. <https://doi.org/10.1002/JMOR.1051590305>
- Sollas WJ. Report on the Tetractinellida collected by H.M.S. *Challenger*, during the years 1873–76. 1888;25:1–458.
- Steindler L, Maldonado M, Pita L *et al.*; Aquatic Symbiosis Genomics Project Leadership. The chromosomal genome sequence of the stone sponge *Petrosia ficiformis* (Poiret, 1789) and its associated microbial metagenome sequences. *Wellcome Open Research* 2025;10:450. <https://doi.org/10.12688/WELLCOMEOPENRES.24743.1>
- Topsent E. Étude des Spongiaires du Golfe de Naples. *Archives de Zoologie Expérimentale et Générale* 1925;63:623–725.
- Topsent E. Spongiaires de l'Atlantique et de la Méditerranée, provenant des croisières du Prince Albert I de Monaco. *Résultats des Campagnes Scientifiques Accomplies sur son yacht par Albert I, Prince souverain de Monaco* 1928;74:1–376.
- Tréguer P, Nelson DM, Bennekom AJV *et al.* The silica balance in the world ocean: a reestimate. *Science (New York, N.Y.)* 1995;268:375–9. <https://doi.org/10.1126/science.268.5209.375>
- Tréguer PJ, Sutton JN, Brzezinski M *et al.* Reviews and syntheses: the biogeochemical cycle of silicon in the modern ocean. *Biogeosciences* 2021;18:1269–89. <https://doi.org/10.5194/bg-18-1269-2021>
- Turner EC. Possible poriferan body fossils in early Neoproterozoic microbial reefs. *Nature* 2021;596:87–91. <https://doi.org/10.1038/s41586-021-03773-z>
- Valisano L, Pozzolini M, Giovine M *et al.* Biosilica deposition in the marine sponge *Petrosia ficiformis* (Poiret, 1789): the model of primmorphs reveals time dependence on spiculogenesis. *Hydrobiologia* 2012;687:259–73.
- Van Soest RWM. Family Crambeidae Lévi, 1963. In: Hooper JNA, Van Soest RWM (eds), *Systema Porifera. A Guide to the Classification of Sponges*. New York: Kluwer Academic/Plenum Publisher, 2002, 547–55. https://doi.org/https://dx.doi.org/10.1007/978-1-4615-0747-5_58
- Wang X, Liu AG, Chen Z *et al.* A late-Ediacaran crown-group sponge animal. *Nature* 2024;630:905–11. <https://doi.org/10.1038/s41586-024-07520-y>
- Weerdt WHde, Soest RWM van. Marine shallow-water Haplosclerida (Porifera) from the South-Eastern part of the North Atlantic Ocean. *Zoologische Verhandelingen. Leiden* 1986;225:1–49.
- Wiedenmayer F. Contribution to the knowledge of post-Paleozoic neritic and archibental sponges (Porifera). *Schweizerische Paläontologische Abhandlungen* 1994;116:1–147.
- Wood R. Palaeoecology of a post-extinction reef: Famennian (Late Devonian) of the Canning Basin, north-western Australia. *Palaeontology* 2004;47:415–45. <https://doi.org/10.1111/J.0031-0239.2004.00373.X>
- Wu S, Reitner J, Harper DAT *et al.* New keratose sponges after the end-Permian extinction provide insights into biotic recoveries. *Geobiology* 2024;22:e12582. <https://doi.org/10.1111/GBI.12582>

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