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SYMPOSIUM

The Integrative Taxonomic Approach Applied to Porifera: A Case Study of the Homoscleromorpha

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Synopsis The two main scientific tasks of taxonomy are species' delineation and classification. These two tasks are often treated differently, with classification accomplished by newly-developed phylogenetic methods, often based on molecular sequences, while delimitation of species is conducted by what is often considered to be an “old-fashioned” typological approach based on morphological description. A new “integrative taxonomy” has been proposed which maintains that species delimitation should be a multidisciplinary undertaking combining several independent datasets. Here we argue that the same principle is relevant to the classification of species. In the past 20 years, we assembled various datasets based on the external morphology, anatomy, cytology, spicule shapes, geography, reproduction, genetic sequences, and metabolomics of homoscleromorph sponges. We show how we used these datasets to describe new species of homoscleromorph sponges and to elucidate their phylogenetic relationships and their phylogenetic position within the phylum Porifera.

Introduction

Taxonomy is the science of identifying, describing, naming, and classifying organisms. The use of multiple and complementary sources of data to evaluate the status of species is called integrative taxonomy (Dayrat 2005; DeSalle et al. 2005; Padial et al. 2010). An integrative taxonomic approach is adopted in many recent α -taxonomic works in which several datasets are combined to differentiate species. Among the different datasets in use are the traditional characters based on morphology, life cycle, ecology, biogeography, allozyme electrophoresis, etc., as well as on mtDNA and rDNA sequences (Blanquer and Uriz 2007; Rützler et al. 2007; Gazave et al. 2010; Cárdenas et al. 2011; Ereskovsky et al. 2011; Rossi et al. 2011; Vargas et al. 2012) and on a new dataset of “metabolomic fingerprints” (Ivanišević et al. 2011; Pérez et al. 2011; Reveillaud et al. 2012). Congruence among multiple and complementary sources of data is used to propose phylogenetic classification.

Porifera is one of the last phyla of animals for which its position among other phyla, as well as its internal relationships, remain largely unresolved (Phillipe et al. 2009). The traditional view of sponge phylogeny has been summarized in *Systema Porifera* (Hooper and van Soest 2002). After the publication of this major work, the sponges' phylogeny has been largely rebuilt, thanks to the integration of multiple sources of data like molecular sequences and cytological or ecological characters. At the beginning of 21st century we are faced with a fascinating (r)evolution of building a novel classification of sponges based on new phylogenetic hypotheses (e.g., Cárdenas et al. 2012; Hill et al. 2013).

The goal of this article is to illustrate the evolution of sponge classification with a focus on a small clade, the Homoscleromorpha. This clade has the highest rate of description of new species: 40 having been described in the past 20 years, over 87 already known (van Soest et al. 2013), and many more awaiting description. The high rate of description

of new species in this group can be traced to the genetic studies initiated in the 1990s, which showed that the morphological variability observed in sympatric populations corresponded to high levels of genetic differentiation among them, suggesting an absence of gene flow (Boury-Esnault et al. 1992; Solé-Cava and Boury-Esnault 1999).

Homoscleromorpha are well-known dwellers of shady, rocky habitats. The Mediterranean species represent about 30% of the described world diversity of Homoscleromorpha, and most of them are found in semi-dark or dark submarine caves. The only exception is *Corticium candelabrum*, which—being rather euryecious—can be found both in shallow and deep water, either exposed to or hidden from light. Homoscleromorphs' overall presence in dark habitats seems to be confirmed around the world. For instance, although there is a good number of representatives of the genus *Plakortis* and *Plakinastrella* on coral reefs, our ongoing explorations of tropical submarine caves indicate that there is a much bigger reservoir of undescribed species there, including a number of representatives of the genus *Oscarella*.

History of the clade Homoscleromorpha from Schmidt 1862 to *Systema Porifera* (2002)

The first homoscleromorph described was *Corticium candelabrum* Schmidt 1862 (p. 42), a species with very characteristic spicules called “candelabra” (Fig. 1A) and allocated to the family Gumuninae with *Chondrilla nucula* Schmidt 1862 and *Chondrosia reniformis* Nardo 1847. The second one, in the same work, was *Oscarella lobularis* (Schmidt 1862, p. 80), a species without spicules, with a violet color and a soft consistency, and allocated to the genus *Halisarca* Johnston 1842 and the family Halisarcidae Schmidt 1862 (Fig. 1B).

Several years later, Schmidt (1868) described another species without spicules under the name *Chondrosia tuberculata* because its consistency was close to that of the genera *Chondrilla* and *Chondrosia*, even though its anatomy is close to *O. lobularis*, as well-illustrated by Fig. 4 of Schmidt's (1868) plate V (Fig. 1C). In 1877, Schulze synonymized *C. tuberculata* with *Halisarca lobularis*. This synonymy was not questioned for over a century and *O. lobularis* was considered as a cosmopolitan species reported from all oceans.

In 1880, Schulze described the genera *Plakina* (with three species), *Plakortis*, and *Plakinastrella* for which he created the family Plakinidae, allocated to Tetractinellida because of the shape of the spicules (Fig. 1D–F).

Vosmaer (1881, 1884, 1887) was the first to recognize the unique characters of the species “*lobularis*” of Schmidt 1862 and he allocated it to a new genus *Oscarella* in order to emphasize the differences in anatomical and reproductive characters between this species and *Halisarca dujardini* Johnston 1842. However, this new genus was still placed in the family Halisarcidae. In his book on sponge classification, Vosmaer (1887) recognized two groups: (1) Oligosilicina for Chondrosiidae, with *Chondrosia* Nardo 1847 and *Chondrilla* Schmidt 1862, and Halisarcidae with *Halisarca* and *Oscarella*; (2) Tetractinellida for Geodiidae Gray 1867, Ancorinidae Schmidt 1870, Plakinidae Schulze 1880, and Corticiidae Vosmaer 1887.

Lendenfeld (1887) considered “*Oscarella* as an askeleton form of *Plakina*” and “*Halisarca* as an askeleton form of *Aplysilla*”. On the basis of its reproduction and the organization of its aquiferous system, and following the suggestion of Schulze, he created the new family Oscarellidae for *Oscarella*, which was allocated to Choristida in a tribu “Macrocamerae” together with Plakinidae. The family Corticiidae with *Corticium* and *Thrombus* was allocated to Choristida too, but in a tribu “Microcamerae” together with Pachastrellidae. In the meantime, Halisarcidae was allocated to Keratosa together with Aplysillidae.

In the “Challenger” report on Tetractinellida, Sollas (1888) considered three sub-orders within the order Choristida: Sigmatophora, Astrophora, and Microsclerophora. Among them, the Microsclerophora were defined as “Choristida in which megascleres are absent; the characteristic microscleres are either tetractinose asters, candelabra, or minute triaenes.” (p. cl). This order was composed of Plakinidae (*Plakina*, *Plakortis*), Corticiidae (*Corticium*, *Calcabrina* Sollas 1888, *Corticella* Sollas 1888, and *Rhachella* Sollas 1888) and Thrombidae Sollas 1888 (*Thrombus* Sollas 1886).

In his “*Etude monographique des Spongiaires de France II. Carnosa*,” Topsent (1895) followed in part the previous authors and also considered three sub-orders: Microtriaenosa, Oligosilicina, and Microsclerophora, the latter with the three families Plakinidae, Corticiidae, and Oscarellidae. By including the family Oscarellidae in Microsclerophora, he followed the hypothesis of Lendenfeld (1887). The family Plakinidae corresponded exactly to the description by Schulze (1880); the family Corticiidae included the genus *Corticium* only. The family Thrombidae was removed from this sub-order along with the genera *Calcabrina*, *Rhachella*, and *Corticella*.

The name Microsclerophora was then replaced by Homosclerophora by Dendy (1905) on the assumption

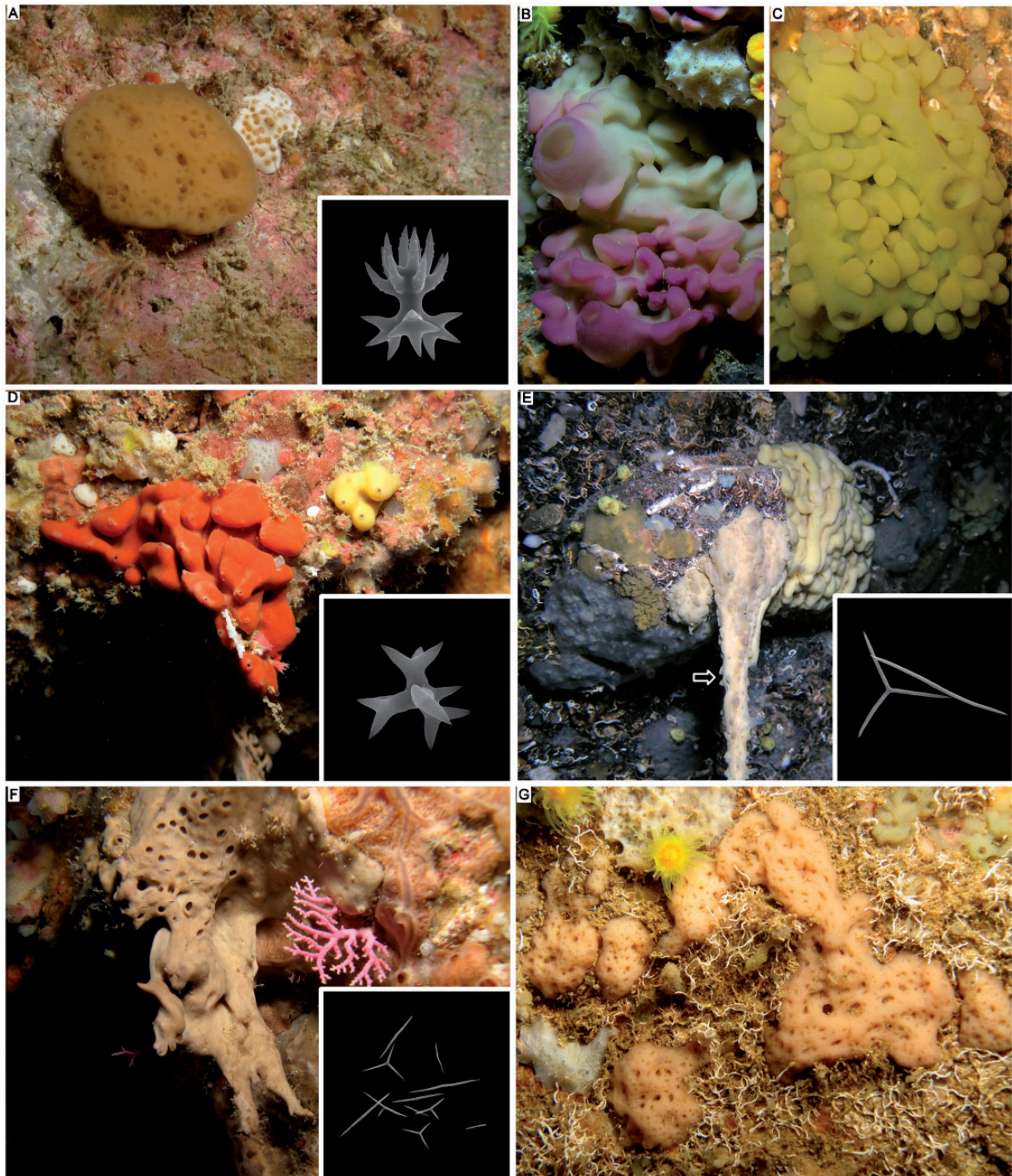


Fig. 1 *In situ* pictures of homoscleromorphan species. (A) *Corticium candelabrum*, type species of the genus *Corticium* from the northwestern Mediterranean coast (depth 15 m); inset: its typical spicule, called “candelabrum” (MEB micrograph). (B) *Oscarella lobularis*, type species of the genus *Oscarella*, from the northwestern Mediterranean coast (depth 16 m), without skeleton. (C) *Oscarella tuberculata*, from northwestern Mediterranean coast (depth 10 m), without skeleton. (D) *Plakina* sp., from a submarine cave in southwestern Pacific, New Caledonia (depth 15 m); inset: a lophose clathrop from the skeleton (MEB micrograph). (E) *Plakortis simplex* (arrow), type species of the genus *Plakortis*, from a submarine cave in the northwestern Mediterranean coast (depth 18 m); inset: diods and triods from the skeleton (MEB micrograph). (F) *Plakinastrella* sp., from a submarine cave in the Caribbean sea (Martinique Island; depth 18 m); inset: diods and triods of the skeleton (MEB micrograph). (G) *Pseudocorticium jarrei*, type-species of the genus *Pseudocorticium*, from the southwestern Mediterranean coast (Algeria; depth 15 m), without skeleton.

that the plakinid spicules are not microscleres but rather spicules of a single size category. Following Sollas, Dendy reallocated Homosclerophora to Tetractinellida on the basis of the shape of the spicules and the presence of four actines (Fig. 1A, D–F).

Later, Topsent (1928) adopted the name Homosclerophora with the same composition as indicated in his previous work of 1895: three families, two of them, Oscarellidae and Corticiidae, remaining monogeneric, and the third one, Plakinidae, including four genera *Plakina*, *Plakortis*, *Plakinastrella*, and *Placinolopha* Topsent 1897. He contested the primitiveness of this group, a view supported by Dendy and other authors, and thought, on the contrary, that Homosclerophora evolved independently. However, he still placed the group within Tetractinellida.

Laubenfels (1936, p. 60) thought Oscarellidae to be particularly difficult to allocate and left this family close to Haplosclerina “to call attention to the extent of our ignorance of its phylogeny”! He split off the other genera to the families Halinidae, Corticiidae, and Plakinastrellidae within Carnosa. The great embryologist Meewis (1938) described the reproduction of *H. lobularis* and contested the synonymy of *Halisarca* and *Oscarella*. These two authors were not followed by subsequent researchers. Indeed, Topsent (1944) wrote a very critical article about the old-fashioned classification used by Meewis.

Lévi (1953) discussed the classification of Laubenfels (1936), resurrected the family Plakinidae, and finally added to this family the genus *Oscarella* that he considered as an askeletal *Plakina*, as had Lendenfeld earlier. This family was thus composed of *Oscarella*, *Plakina*, *Plakortis*, *Placinolopha*, and *Plakinastrella*. Lévi also included the Corticiidae with the genus *Corticium* within Homosclerophora *sensu* Topsent.

At this stage, all the previous authors mainly considered Homosclerophora as part of the Tetractinellida because of the shape of the spicules. However, when Lévi (1956) completed his work on the Embryology and Systematics of Demospongiae, he clearly separated Homosclerophora from Tetractinellida (p. 160) based on a comparison of spicules of the two groups with a thorough description of the larvae of all homoscleromorph species. In his work, three families were recognized within Homosclerophorida, the Oscarellidae, Plakinidae, and Corticiidae, but the order was maintained within the sub-class Tetractinomorpha together with the Tetractinellida, Hadromerida, and Axinellida. It was only in 1973 that Lévi elevated

the Homosclerophorida to the rank of sub-class (p. 591), outside of Tetractinomorpha. *Corticium* was allocated for the first time to the Plakinidae, and the sub-class was divided into two families, one with spicules, Plakinidae, and another one without spicules, Oscarellidae. Thus, the organization of this group of sponges appeared rather clear, and Berquist (1978) followed Lévi’s view by giving the name Homoscleromorpha to this sub-class.

The description of a new genus, *Pseudocorticium* (Boury-Esnault et al. 1995), re-introduced some uncertainty into the adopted classification (Fig. 1G). Indeed, this new taxon had a morphology and an anatomy close to that of *Corticium* but it had no spicules, so Boury-Esnault et al. (1995) merged the two families Oscarellidae and Plakinidae in a single one, Plakinidae, represented by sponges both with and without spicules.

In *Systema Porifera*, Muricy and Diaz (2002) followed the latter classification, although they emphasized that this group remained problematic and that more anatomical, cytological, molecular, and chemical work was needed to better understand the internal relationships of the genera of this sub-class, as well as to elucidate the relationships of this sub-class with Demospongiae, Calcispongiae, and Hexactinellida.

The role of integrative taxonomy in resolving the *O. lobularis* species complex

The genus *Oscarella* is defined as “Plakinidae without spicules, with thin encrusting to lobate shape. Thin ectosome (<100 µm) is often limited to the pinacoderm. The aquiferous system has a syllebeid-like organization, with spherical eurypylous chambers uniform arranged around large, regular exhalant canals, and a large exhalant cavity” (from Muricy and Diaz 2002). The type-species *O. lobularis* was considered as a cosmopolitan species due to the paucity of characters that could discriminate among the species of *Oscarella*. Furthermore, many species described by earlier authors (e.g., Schmidt 1862, 1868) were synonymized and the high diversity was reinterpreted as intraspecific phenotypic plasticity (e.g., Vosmaer 1935). That approach was challenged by allozyme studies in the 1990s, which revealed substantial genetic differentiation among supposedly conspecific morphotypes (Boury-Esnault et al. 1992). Other datasets like metabolic fingerprint, mtDNA, and rDNA sequences have contributed more recently to a better evaluation of species diversity within the species complex *O. lobularis*.

Morphology, anatomy cytology, symbionts, and enzyme electrophoresis

In his work of 1895, Topsent pointed out the intraspecific phenotypic plasticity of *O. lobularis*. He noticed the different colors from yellow to violet, green, red, or blue, and noted that while most of the specimens were semi-cartilaginous, some others were soft (p. 561). Seventy years later, after an extensive sampling started in 1966, Jean Vacelet observed a similar phenotypic plasticity. Moreover, he noted that cartilaginous specimens possessed abundant collagen and abundant turgescient vacuolar cells, whereas soft specimens had a reduced content of collagen and few vacuolar cells, but abundant bacteria (J. Vacelet, personal communication). A genetic work using allozyme electrophoresis found a high level of genetic differentiation between the soft-textured violet morph of *O. lobularis* and three other color morphs that were all cartilaginous (Table 1). A cytological work conducted on the same specimens showed a congruent pattern of cytological differentiation (Boury-Esnault et al. 1992) (Fig. 2A–D). Collectively, these results supported allocating the two morphs to different species. The soft, violet specimens were allocated to *O. lobularis*, and the cartilaginous specimens to *O. tuberculata*, based on examination of Schmidt's (1868) type-specimen (Boury-Esnault et al. 1992).

Ecological and reproductive traits

The two Mediterranean sibling species, *O. lobularis* and *O. tuberculata*, have distinct ecological habits. *O. tuberculata* tends to occupy a wide range of depths and is found in a large variety of habitats, whereas *O. lobularis* is generally restricted to more shallow water (5–35 m) and to limited habitats. The two species also differ in regard to their reproductive cycles as revealed by their pluri-annual *in situ* monitoring (Ereskovsky et al. 2013). First, *O. lobularis* has shorter periods of gametogenesis and embryogenesis than does *O. tuberculata* (Fig. 2E). Second, greater variability in water temperature and the warming of seawater have a positive influence on the reproductive

effort of both sexes in *O. lobularis*. By contrast, there is a slight negative correlation between an increase in annual mean temperature and the reproductive effort in *O. tuberculata*.

Metabolomic fingerprinting

The use of chemical markers has a long tradition in sponge systematics and its advantages and limitations have been extensively discussed (for a review see Erpenbeck and van Soest 2007). The choice of the molecules, the question of the homology of the compounds, and the natural variability of their expression related to phenotypical plasticity were identified as some of the most serious issues regarding the application of individual chemical characters in sponge taxonomy. A novel approach called metabolomic fingerprinting allows circumventing some of the problems and represents a rapid, untargeted, and high throughput method that can be used for a large number of small samples and that gives access to the whole metabolome (Fiehn 2002; Wolfender et al. 2009). Ivanišević et al. (2011) were first to apply this approach to Porifera and demonstrated that the chemical diversity sponges may be useful for understanding fundamental issues in homoscleromorphan systematics and evolutionary biology. A first validation of the metabolomics approach was the measurement of intraspecific and interspecific variability in chemical diversity within the two sister species *O. lobularis*/*O. tuberculata* which showed that the intraspecific variability was significantly lower than interspecific variability (Ivanišević et al. 2011). At the same time, this analysis has demonstrated that the divergence between these species was actually much more subtle than indicated by the chemistry of natural products. Indeed, while Loukaci et al. (2004) only reported a marked difference indicated by the major compounds of the two sister species, the metabolomic approach indicated that about 95% of both metabolomes were actually identical (Ivanišević et al. 2011) (Fig. 2F).

mtDNA sequences

To discriminate rapidly between sister-species of animals, the Folmer fragment of the gene for cytochrome oxidase subunit 1 (*cox1*) is commonly used. The Erpenbeck fragment (I3-M11 fragment), especially designed for sponges and often more informative (Erpenbeck et al. 2002), was used to discriminate between the species of *Oscarella*. However, in the case of the *O. lobularis*/*O. tuberculata* sister-species, these sequences were nearly identical (only one difference at position 249) (Ivanišević et al. 2011). Thus

Table 1 Estimates of Nei's (1978) genetic identity (above the diagonal, in *italics*) and genetic distance (below the diagonal, in **bold**) between four color morphs of *Oscarella*

	Blue	Green	Yellow	Violet
Blue	—	<i>0.96</i>	<i>0.97</i>	<i>0.26</i>
Green	0.05	—	<i>1</i>	<i>0.25</i>
Yellow	0.03	0	—	<i>0.25</i>
Violet	1.36	1.41	1.4	—

Modified from Boury-Esnault et al. (1992).

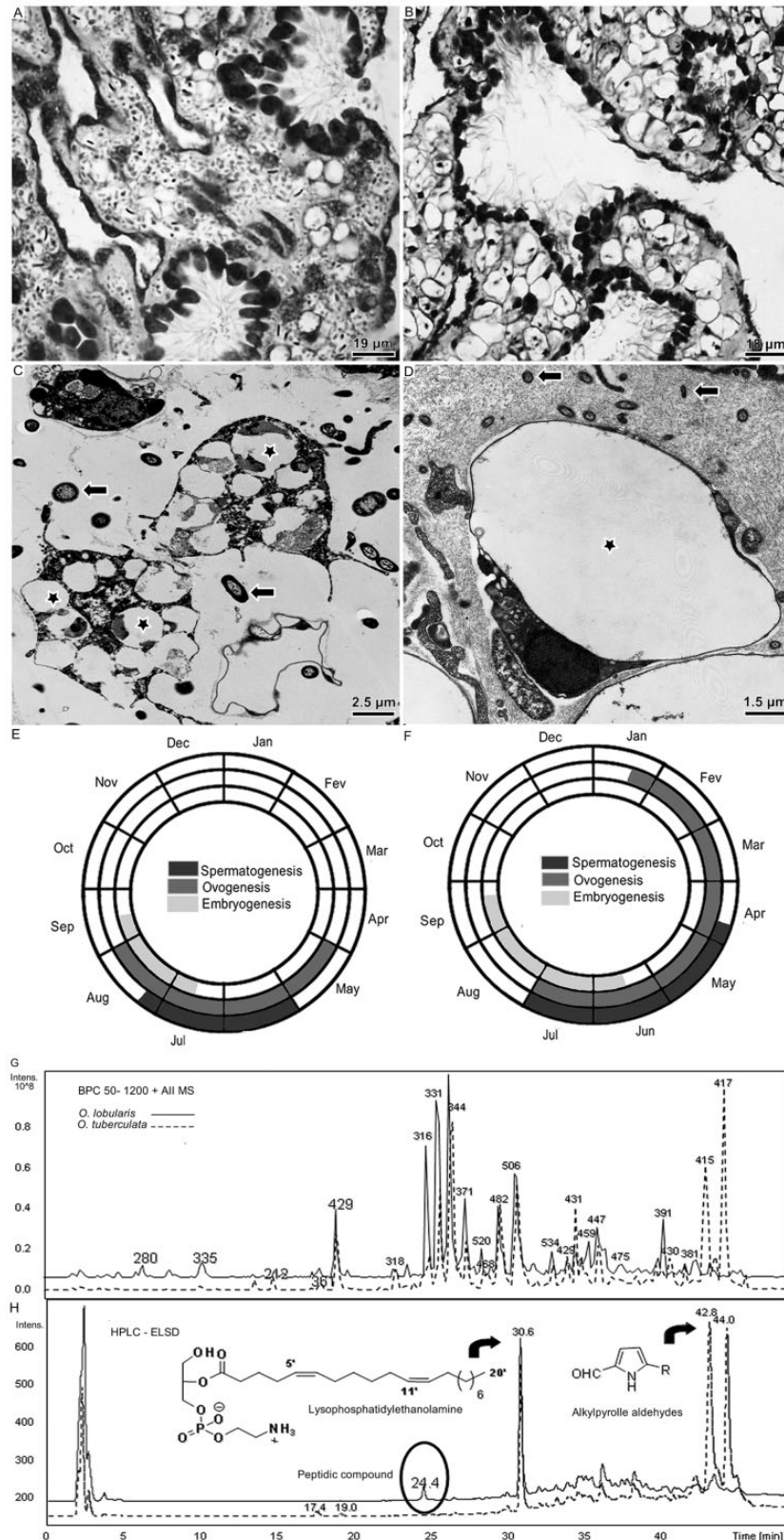


Fig. 2 (A) Semi-thin section through the body of *Oscarella lobularis* showing the choanocyte chambers, surface and canals lined by pinacocytes, the mesohyl with vacuolar cells, numerous bacteria (black dots), and loose fibrils of collagen. (B) Semi-thin section through the body of *Oscarella tuberculata* showing the choanocyte chambers, surface and canals lined by pinacocytes, and the mesohyl with numerous vacuolar cells and a dense matrix of collagen fibrils. (C) TEM micrograph of the mesohyl of *O. lobularis* containing vacuolar cells, numerous bacteria (arrows), and loose collagen fibrils. (D) TEM micrograph of the mesohyl of *O. tuberculata* with turgescent

(continued)

cox1 sequences would not help to discriminate these two species if this character were used alone. By contrast, complete mtDNA sequences from *O. lobularis*/*O. tuberculata* were found to be different at 48/20,243 or 0.24% of sites (Gazave et al. 2010), which is about twice as many as complete mtDNA sequences from two color morphs of *O. tuberculata* (29 or 0.14% of sites) (Gazave et al. 2013). Furthermore, *atp6* and *tatC* regions of mitochondrial genomes provide some support for the monophyly of both *O. lobularis* and *O. tuberculata* when multiple individuals from each species were sampled (Gazave et al. 2013). Outside of the *O. lobularis*/*O. tuberculata* species group, partial *cox1* sequences (Fig. 3), partial *atp6* or *tatC* sequences, and complete mtDNA sequences can easily discriminate between known *Oscarella* species.

Conclusion

O. lobularis and *O. tuberculata* can be distinguished by their consistency due to collagen content, the cytology, their symbiont community, electrophoresis of their enzymes, life cycle, and metabolomic fingerprint. On other hand, the sequences of *cox1* are very close and alone would not allow discrimination between the two species. *O. lobularis* and *O. tuberculata* are clearly two species; six independent datasets of the seven used were able to differentiate between them (DeSalle et al. 2005). In the Mediterranean area, before 1992, all works confounded both species. All records, outside the Mediterranean and the adjacent Northeast Atlantic area, have to be checked carefully because *O. lobularis* is not a cosmopolitan species (e.g., Red Sea, Lévi 1958; Antarctic, Koltun

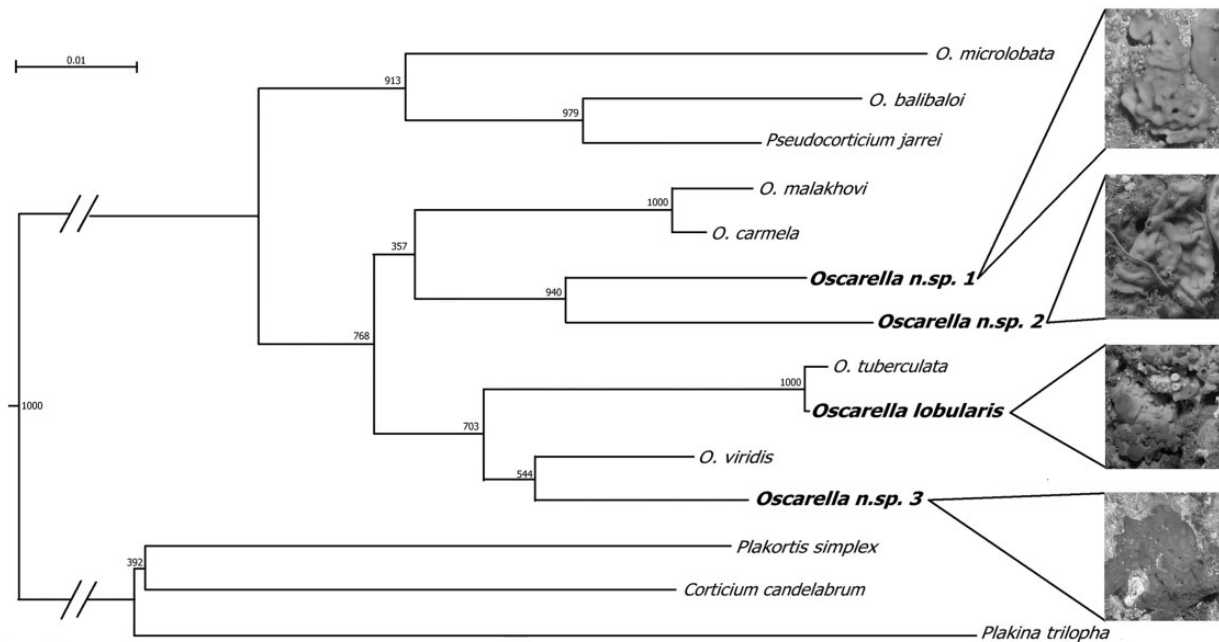


Fig. 3 Phylogenetic relationships among species of the family Oscarellidae, using the Erpenbeck fragment of *cox1* from mitochondrial DNA. The three species from Martinique (in bold) were presumed *Oscarella lobularis*. The results, however, show that the Caribbean specimens cannot be *O. lobularis* and we proposed these Caribbean sponges as new species. *Oscarella viridis* HQ269358.1, *Plakortis simplex* HQ269362.1, *Corticium candelabrum* HQ269363.1, *Plakortis trilopha* NC_014852.1.

Fig. 2 Continued

vacuolar cells, some bacteria (arrow), and a dense matrix of collagen fibrils. (E) Life cycle of *O. lobularis*. The reproductive period lasts 4.5 months; ovogenesis occurred from May until the end of August, spermatogenesis from June to mid-August, and embryogenesis from mid-July to mid-September. (F) Life cycle of *O. tuberculata*. The reproductive period lasts about 8 months. Ovogenesis starts at the end of January and continues until the end of July, spermatogenesis is from late April until the end of July, and embryogenesis from mid-June to late September (modified from Ereskovsky et al. 2013). (G) Interspecific variability illustrated by HPLC–ESI(+)MS (BPC) (top) and HPLC–ELSD (below) chromatograms of the metabolome of two sister species *Oscarella lobularis* (top black line) and *Oscarella tuberculata* (lower dashed line). Major *m/z* (above) and retention times in minutes (below) are indicated above peaks. The major metabolite present in both species (black arrow) is a lysophosphatidylethanolamine. Alkylpyrrole aldehydes (grey arrow) have been detected in *O. tuberculata* and a peptidic compound in *O. lobularis*. Modified from Ivanišević et al. (2011).

1964; Far Eastern Arctic, Koltun 1966; New Zealand, Gordon 2009; Atlantic Islands, Azores, Canarias, Madeira, Cruz 2002; Tropical East Atlantic, Lévi 1952).

The contribution of integrative phylogeny to an understanding of the internal relationships among homoscleromorph taxa

As seen in the first part of this article, the internal relationships of Homoscleromorpha have been controversial and the genera had been allocated either to one or to three families, depending on the author. However, several recent studies supported the presence of two major lineages within the group, corresponding to families Oscarellidae and Plakinidae in the traditional (Linnean) classification. Below we briefly review the support (or lack of it) for these major clades in several datasets used in sponge phylogenetics.

Morphology and cytology

Aquiferous system in Oscarellidae and Plakinidae can be sylleibid (*Oscarella* spp. and *Plakina* spp.) or leuconoid (*Corticium* spp., *Plakortis* spp., *Plakinastrella* spp., and *Pseudocorticium jarrei*). The cortex is thin in *Oscarella* spp. and *Plakina* spp. but thick in *C. candelabrum* and *P. jarrei*. There is a regular increase in the ratio of the volume of mesohyl/volume of choanocyte chambers from *Oscarella* spp. and *Plakina* spp. (0.7:1) to *C. candelabrum* (1:1) and *P. jarrei* (2.5:1). The choanocyte chambers are eurypylous in *Oscarella* spp. and *Plakina* spp., aphodal in *C. candelabrum*, and diplodal in *P. jarrei* (Boury-Esnault et al. 1995; Muricy et al. 1996, 1999). In summary, the anatomy of chaosome sylleibid versus leuconoid and diplodal versus eurypylous choanocyte chambers and the proportion of the mesohyl relative to choanocyte chambers are not useful for discriminating between the two clades.

Plakina spp. and *C. candelabrum* have a simple cellular organization (Muricy 1999; Muricy et al. 1999). Archeocytes and sclerocytes are the only amoeboid cells of the mesohyl although some vacuolar cells are present in one species: *P. jani*. In *O. lobularis* and *O. tuberculata*, the more abundant cells of the mesohyl are vacuolar cells (see above). In contrast, some oscarellid species, *P. jarrei*, *O. microlobata*, *O. imperialis*, *O. viridis*, and *O. balibaloï*, have several types of cell with inclusions that are sometimes paracrystalline (Muricy et al. 1996; Pérez et al. 2011). This last character could be informative about the relationships among some taxa. Unfortunately, cytological descriptions are not yet used routinely for description of homoscleromorph species.

Molecular markers: nuclear markers 18S and 28S rDNA and mitochondrial DNA

A molecular study using 18S and 28S rDNA sequences on 12 species of Homoscleromorpha has shown that two clades are well supported: one with species with a skeleton (*Plakina* spp., *Corticium* spp., *Plakortis* spp., and *Plakinastrella* spp.) and the other with species without a skeleton (*Oscarella* spp. and *P. jarrei*) (Gazave et al. 2010).

To date, complete mitochondrial genome sequences have been determined for 16 species of homoscleromorphs representing six genera (Wang and Lavrov 2008; Gazave et al. 2010; Gazave et al. 2013). Recently, the complete mitochondrial genome has been used to design primers for the most phylogenetically informative regions within this molecule (*atp6* and *tatC*) in order to study phylogeny within the family Oscarellidae (Gazave et al. 2013). These mitochondrial genomes can be subdivided into two groups on the basis of the organization of the mitochondrial genome: one group corresponding to *Oscarella* and *Pseudocorticium* and the second to *Plakina*, *Plakinastrella*, *Plakortis*, and *Corticium* (Fig. 4A). The genomes in the first group (family Oscarellidae) are characterized by several features, including the retention of *tatC*, a gene for subunit C of the twin arginine translocase not found anywhere else in the animal kingdom, presence of two duplicated tRNA genes, and a characteristic arrangement of genes in which genes in one part of the genome have a transcriptional polarity opposite to that of the genes in the other part (Gazave et al. 2010). The genomes in the second group (family Plakinidae) are characterized by the loss of 20 of the 25 tRNA genes required for mitochondrial translation, necessitating the import of corresponding tRNAs from the cytosol. In addition, one or two introns are present in *cox1* of *Plakinastrella* sp., *Plakina crypta*, and *P. trilopha* but absent elsewhere (Gazave et al. 2010). Analysis of mitochondrial coding sequences provides strong support for the presence of two main clades within Homoscleromorpha: family Plakinidae consisting primarily of spiculate species and family Oscarellidae consisting of aspiculate species.

Metabolomic fingerprints

The alignment of homoscleromorph metabolic fingerprints resulted in a classification congruent with the phylogenetic trees (Ivanišević et al. 2011). The clade Oscarellidae is composed of *Oscarella* spp. and *P. jarrei* and is aspiculate; the clade Plakinidae is composed of *Corticium* spp., *Plakina* spp., *Plakinastrella* spp., and *Plakortis* spp. and is spiculate (Fig. 4B).

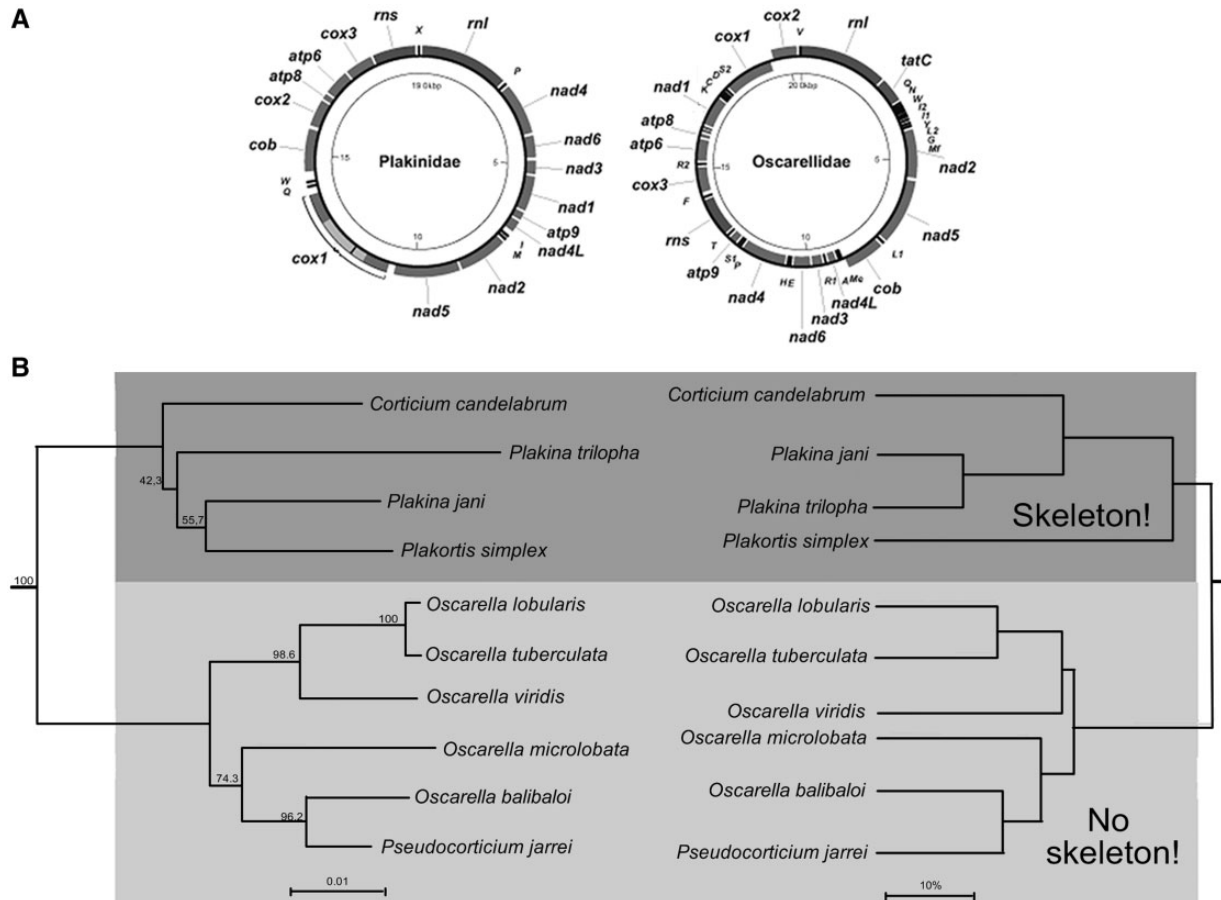


Fig. 4 (A) Comparison between the complete mitochondrial DNA of Oscarellidae (right) subdivided into two parts, with opposite transcription, and characterized by the retention of *tatC* gene and 27 tRNAs genes, and complete mitochondrial DNA of Plakinidae (left); all genes are transcribed in one direction, and there are only 6 tRNAs (modified from Gazave et al. 2010). (B) Classification of homoscleromorph sponge species based on the Erpenbeck fragment of the *cox1* mitochondrial gene (left tree) compared with the classification based on metabolomic fingerprints (right tree). The *cox1* molecular phylogenetic reconstruction using the Neighbour Joining method is compared with the metabolic fingerprint classification established on the basis of Euclidean linkage distance. Values on nodes indicate the percentage of bootstrap replicates (over 10,000 replicates). Dark gray = species with skeletons (Plakinidae); light gray = species without skeletons (Oscarellidae). Note that in both cases the topology of the trees is similar. Modified from Ivanišević et al. (2011).

An integrative phylogeny is needed to resolve the internal relationships within Oscarellidae and Plakinidae

Internal relationships within Oscarellidae and Plakinidae remain poorly understood and need to be investigated by an integrative approach. As one of the first steps in this direction, the phylogeny of Oscarellidae has recently been studied by combining molecular (nuclear and mitochondrial) as well as morphological and cytological data (Gazave et al. 2013). Two clades previously obtained within Oscarellidae with the I3-M11 portion of the *cox1* mitochondrial gene and the metabolomic fingerprint (Ivanišević et al. 2011) received strong support from this study (Gazave et al. 2013).

Within Plakinidae, an earlier study revealed close relationships between *Plakinastrella* and *Plakortis*, as well as the non-monophyly of *Plakina* (Gazave et al. 2010). Clearly, more data are needed to confirm or reject these findings, and it is likely that recent discoveries of new plakinids, from New Caledonia and the Caribbean, which seem to have lost their skeletons, will generate new hypotheses about internal relationships within Oscarellidae and Plakinidae (work in progress by the present authors).

The role of integrative phylogeny at the level of the clade Homoscleromorpha

All Homoscleromorpha share morphological, anatomical, and reproductive characters such as

complete ciliated exopinacoderm (in *C. candelabrum* the ciliated exopinacocytes are restricted to the inhalant areas); a basement membrane lining the epithelium both of adults and larvae; a cinctoblastula larva; multipolar egression during development; and an epithelial invagination during metamorphosis (e.g., Cárdenas et al. 2012).

The molecular phylogenetic hypothesis that Homoscleromorpha are not Demospongiae, first proposed in 2004 (Borchiellini et al. 2004), was supported by several authors using different molecular markers (Philippe et al. 2009; Pick et al. 2010) and led to the elevation of Homoscleromorpha to the rank of class in the Linnean classification (Gazave et al. 2012). The relative alacrity (<10 years) of acceptance of this new classification has to be compared with the century needed to accept the classification of Bidder for the sub-classes Calcinea and Calcaronea in the Calcispongiae (Bidder 1898; Hartman 1958; Borojevic 1979; Manuel et al. 2003). This rapid acceptance is in part due to the focus on this taxonomic group since 1984–1992 by a team based in Marseille and the development of several datasets describing high biodiversity of homoscleromorph species from the Mediterranean Sea, including datasets based on molecular sequences (e.g., Boury-Esnault et al. 1984; Boury-Esnault et al. 1992; Muricy et al. 1996; Boury-Esnault et al. 2003; de Caralt et al. 2007; Maldonado and Riesgo 2008; Gazave et al. 2010; 2012; Ivanišević et al. 2011; Pérez et al. 2011). By contrast, the proposition by Bidder (1898) had to wait more than a century for the first molecular work on Calcispongiae (Manuel et al. 2003).

The relationships of Homoscleromorpha with the other three clades of Porifera are still in debate. A sister-relationship with Calcispongiae (Philippe et al. 2009; Pick et al. 2010; Nosenko et al. 2013) has been proposed. Homoscleromorpha and Calcispongiae share the presence of a cross-striated rootlet on the flagellated cells of the larva; however, this character is also present in Choanoflagellata and Eumetazoa and cannot be considered as a synapomorphy for the [Homoscleromorpha + Calcispongia] clade (Boury-Esnault et al. 2003; Cárdenas et al. 2012). Both clades also share the presence of collagen type IV, but this principal constituent of the basement membrane is also present in Demospongiae (Wörheide et al. 2012). For the time being, this sister-relationship is based only on molecular markers.

Conclusion

An integrative approach is crucial for α -taxonomy as well as for phylogeny. In fact, Schlick-Steiner et al.

(2009) recommended a minimum of three independent disciplines including morphology, genetics, and a third dataset to explore biodiversity. It is also important to convert our knowledge of phylogeny into a stable classification system. Such classification should be based on monophyletic genera, families, orders, and classes. The molecular phylogeny hypotheses also have to be reconciled with morphological data to be able to determine the synapomorphies of the clades, and when discrepancies between the molecular tree and the current classification emerge, each dataset has to be re-evaluated in order to detect homoplasies or loss of characters (Cárdenas et al. 2012). In addition, a concerted effort needs to be undertaken to develop new characters, such as metabolomic fingerprints, and organize a database to manage these new data, to supplement morphological and molecular data. We also need to convince other systematians to use an integrative approach, which may require collaborative projects such as the Porifera Tree of Life project.

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