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To cite this article: R. Manconi, F. D. Ledda, A. Serusi, G. Corso & G. A. Stocchino (2009) Sponges of marine caves: Notes on the status of the Mediterranean palaeoendemic *Petrobiona massiliana* (Porifera: Calcarea: Lithonida) with new records from Sardinia, Italian Journal of Zoology, 76:3, 306-315, DOI: [10.1080/11250000802629471](https://doi.org/10.1080/11250000802629471)

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Published online: 06 Aug 2009.



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## Sponges of marine caves: Notes on the status of the Mediterranean palaeoendemic *Petrobiona massiliana* (Porifera: Calcarea: Lithonida) with new records from Sardinia

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(Received and accepted 28 October 2008)

### Abstract

The discovery of conspicuous populations of the rare species *Petrobiona massiliana* is reported from shallow water karstic caves of Sardinia. The morphological comparative analysis versus previous data from the entire geographic range highlights that spicular traits seem to be highly conservative in contrast with a notable plasticity of growth form. Lifestyle and adaptive strategies that favoured the successful spread of *P. massiliana* in karstic caves and its persistence as extremely isolated populations in a fragmented habitat are discussed together with potential environmental constraints. The data indicate that conservation of this Mediterranean palaeoendemic species with a spot-like distribution may be a challenge for the western Mediterranean Marine Protected Areas to confer protection to numerous co-occurring cave-dwelling species.

**Keywords:** Biodiscovery, cryptic habitat, biogeography, life strategies, rare species conservation

### Introduction

Mediterranean caves harbour extremely rich sponge assemblages characterized by both true cave-dwellers and/or resembling bathyal fauna together with a peculiar relict sponge fauna survived to the geological/climatic vicissitudes of the Tethys Sea (Sarà 1958, 1961; Vacelet 1964, 1976, 1994; Riedl 1966; Rützler 1966; Pouliquen 1969, 1972; Harmelin et al. 1985, 2003; Vacelet et al. 1994; Vacelet & Boury-Esnault 1996; Pansini & Pesce 1998; Corriero et al. 2000; Perez et al. 2004; Bussotti et al. 2006; Manconi et al. 2006; Bakran-Petricioli et al. 2007; Novosel et al. 2007; Oertel & Patzner 2007).

In the 1950s, the discovery in the northwestern Mediterranean of peculiar calcareous sponges resulted in the description of a new monotypic genus *Petrobiona* apparently related to the Mesozoic fossil order Pharetronida (Vacelet & Lévi 1958) and to a few recent Minchinellidae Dendy and Row, 1913 reported in the Mediterranean Sea exclusively from caves and/or bathyal zones (Pouliquen &

Vacelet 1970; Vacelet et al. 2002). The type species of the genus, *Petrobiona massiliana* Vacelet and Lévi, 1958 was found in some caves of Marseille (Vacelet & Lévi 1958). A second species, *P. incrustans* Sarà, 1963, described from a semi-submerged cave of the Apulian coast, is at present a junior synonym to *P. massiliana* (Vacelet et al. 2002). The most ancient fossil of this species from the Pleistocene cliffs of southern Italy is 30,000 years old, while more recent subfossils (1500 years) are known from a terrestrial cave in the Crete Island (Vacelet 1980, 1991; Vacelet et al. 2002). Very few records of *P. massiliana* occurred until now for about 50 years (Vacelet & Lévi 1958; Sarà 1963; Rützler 1966; Pouliquen 1972; Pansini & Pronzato 1982; Bouduresque et al. 1986; Corriero et al. 1997, 1999; Vacelet et al. 2002; Harmelin et al. 2003), notwithstanding the extensive surveys in the Mediterranean caves.

Although the karstic area along northwestern Sardinia is characterized by a high density of marine, anchialine and terrestrial caves (Mucedda et al.

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2002; Mulas et al. 2006), only few data exist on their fauna, namely sponges, flatworms, molluscs and arthropods (Segre 1956; Grafitti 1990; Jaume & Boxshall 1995, 1996a, 1996b; Manconi et al. 2006; Curini-Galletti, personal communication).

In this paper we report the discovery of the Mediterranean palaeoendemic *P. massiliana* from northwestern Sardinian shallow water caves.

### Materials and methods

Sponge surveys were carried out in the Marine Protected Area (MPA) of Capo Caccia-Isola Piana (Sardinia, NW-Mediterranean) in eight karstic caves, namely Cabirol 2, Cervi, Fantasmi, Galatea, Laghi, Madonnina, Nereo, and Terrazze (Figure 1). The dark zone of these submerged shallow water caves was surveyed in June 2002–July 2007 by SCUBA visual census to collect conspicuous

sponges. The rarity of the species, its scarcity in some sites and the status of the area as a MPA suggested the collection of only a few specimens from each cave. Water temperature and depth were measured at the time of sponge collection. Data reported on caves are from Mucedda et al. (1997, 2002), and Cicogna et al. (2003).

Collected sponges were preserved in alcohol 70% or dry. Morphological macro-traits such as growth form, size and colour were evaluated by a stereo-microscope, and representative body fragments were dissected to characterize surface morphology and distribution of inhalant and exhalant apertures. Calcareous skeleton fragments and spicules were prepared by digestion in sodium hypochlorite at ambient temperature for light microscopy and scanning electron microscopy (SEM). Procedures for SEM follow Manconi and Pronzato (2000). Skeleton micrometries were performed on 10 spicules for each spicular type for 14 specimens.

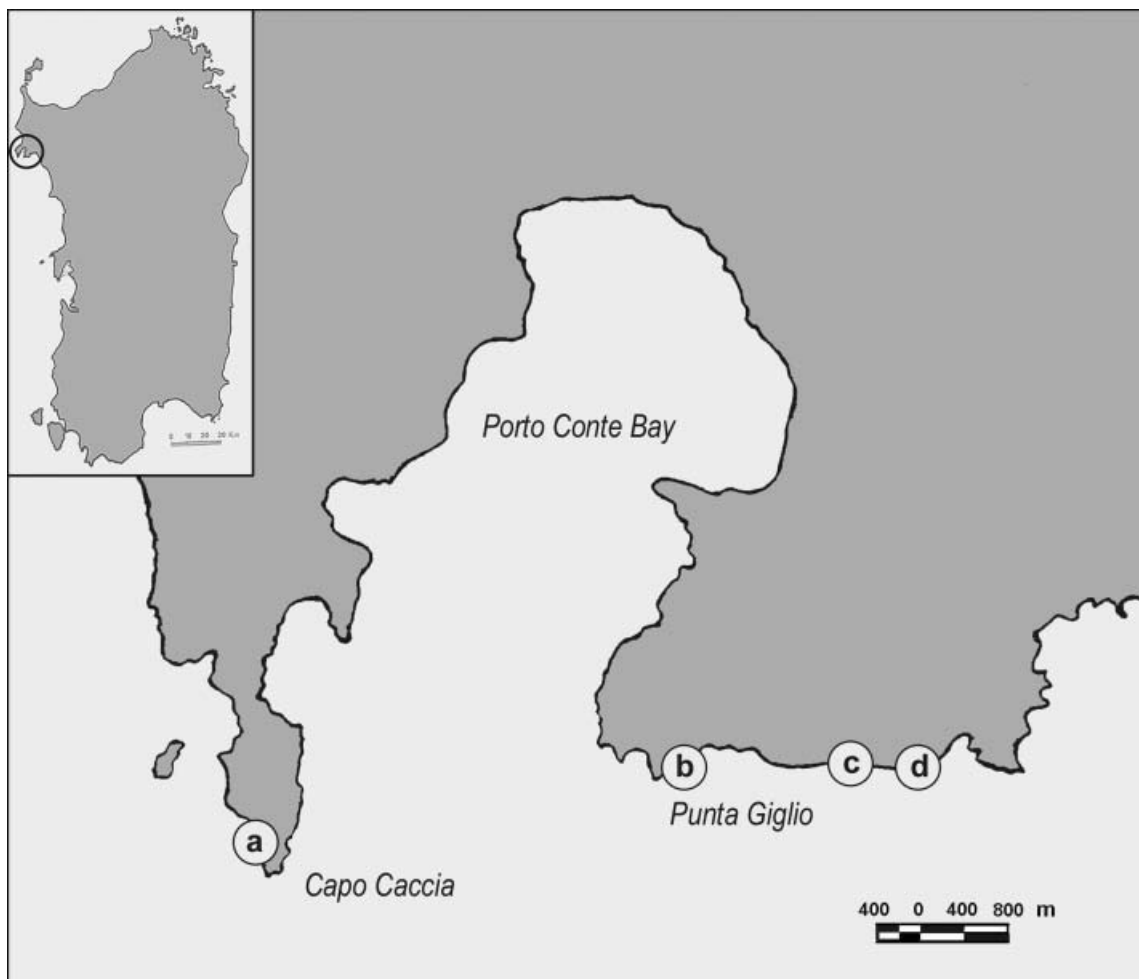


Figure 1. Study sites of *Petrobiona massiliana* in four caves along the karstic promontories of Capo Caccia and Punta Giglio (NW Sardinia). a, Nereo; b, Fantasmi; c, Terrazze; d, Galatea.

### Studied materials

FAN 3, FAN 4, FAN 5, Punta Giglio Promontory, Grotta dei Fantasmi n. 2708 (40°34'12"N, 8°12'24"E), 6 m of depth, 19.vi.2002, R. Manconi leg.; TER 1, TER 2, Punta Giglio Promontory, Grotta delle Terrazze n. 2740 SA/SS (40°34'16"N, 08°13'43"E) Catasto Speleologico Regionale, 4 m of depth, 10.iv.2004, E. Carzedda leg.; GAL 6, Punta Giglio Promontory, Grotta della Galatea n. 2747 SA/SS (40°34'05"N, 8°13'52"E) Catasto Speleologico Regionale, 5.5 m of depth, 10.iv.2004, R. Barbieri leg.; NER 7, NER 8, NER 9, NER 10, 15 m of depth, NER 11, NER 12, NER 13, NER 15, 28 m of depth, Capo Caccia Promontory, Grotta di Nereo n. 930 SA/SS (40°33'46"N, 8°09'44"E) Catasto Speleologico Regionale, 09.ix.2004, R. Manconi leg. All specimens, fragments, slides and SEM stubs are deposited in the collection of R. Manconi at the Dipartimento di Zoologia e Genetica Evoluzionistica, Università di Sassari.

## Results

### Taxonomic account and morphology

*Petrobiona massiliana* Vacelet and Lévi, 1958 of the monotypic family Petrobionidae Borojevic, 1979 (Calcarea Bowerbank, 1864; Calcaronea Bidder,

1898; Lithonida Vacelet, 1981) was found in four caves out of the eight surveyed, namely Terrazze, Galatea and Fantasmi caves along the southwestern cliff of Punta Giglio promontory, and in Nereo cave on the northwestern cliff of the Capo Caccia promontory (Figure 2).

The sponges from the Terrazze cave show a notably plated encrusting growth form with oscules, ca. 0.5 mm in diameter, irregularly to regularly scattered in the middle of the apical surface or along its margins (Figure 3a–c, Table I).

The single sponge collected from the Galatea cave bears two large oscules in the centre of the apical area and shows an encrusting growth form strictly adhering to the substrate by a wide base of attachment (Table I).

The sponges from the Fantasmi cave are from globose to subspherical to fan-shaped with multiple lobes and a stout well developed brown stalk (Figure 3d–e, Table I). Small oscules ca. 0.4 mm in diameter are scattered irregularly on the upper surface.

The sponges from the Nereo cave were constantly massive–elongated to finger-like shaped, with small lobes in the apical region and a single, conspicuous, apical oscule in the middle of the upper region receiving wide subdermal canals (Figure 3f–h, Table I).

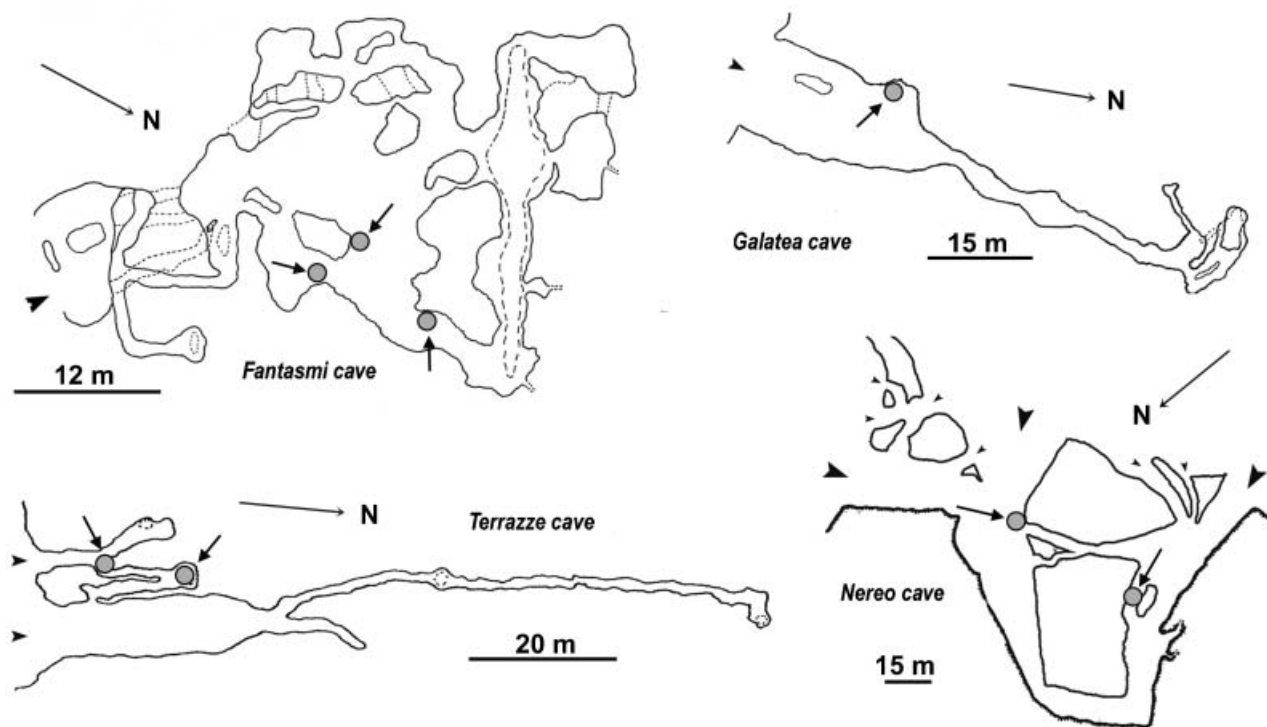


Figure 2. Maps of four northwestern Sardinian caves with topographic distribution (circles–arrows) of *Petrobiona massiliana*.



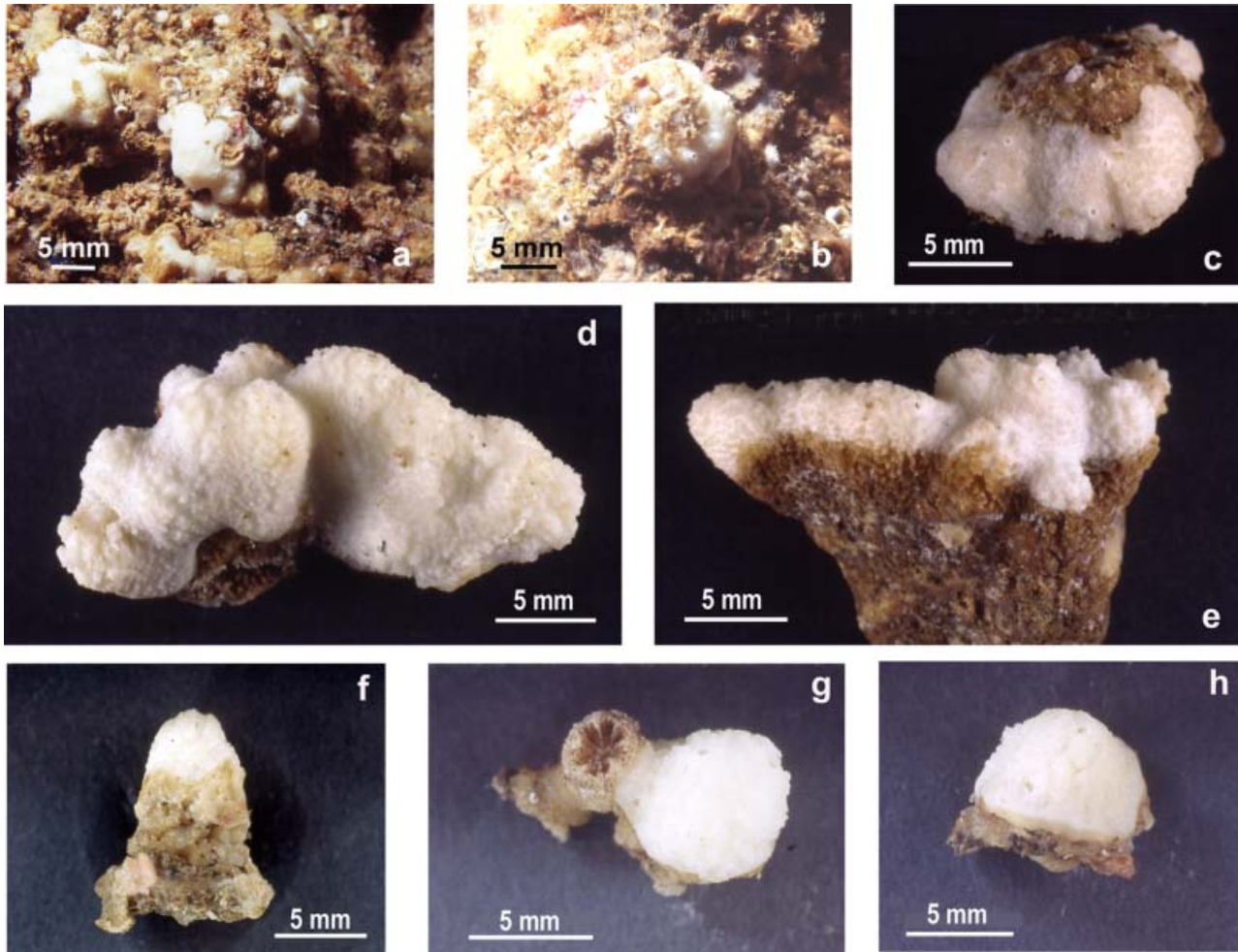


Figure 3. Specimens of *Petrobiona massiliana* from karstic caves of northwestern Sardinia. **a–c**, Terrazze cave in situ (**a,b**) and preserved (**c**); **d–e**, Fantasmi cave in top (left) and lateral (right) views; **f–h**, Nereo cave. The colour version of this figure is available online.

The calcareous spicules (Figure 4) of the specimens from the four caves were homogeneous in shape with slight differences in size (Table II). Microdiactines ranged from tubercled to spined. Malformed spicules, mainly diapason triactines showing asymmetries in lateral actins, were also found.

The release of abundant free-swimming larvae by the Nereo cave specimens from the deepest site was observed in September at the lab just after sampling. Unfortunately, larvae degenerated due to an accident during fixation, and it was not possible to obtain more data.

#### Habitat

The surveyed caves are almost horizontal, but the Nereo cave shows an extremely complex topography. Freshwater infiltrations were apparently absent in the four caves despite the presence of numerous cracks on the walls and the roof.

In the Galatea cave, *P. massiliana* was found exclusively on the walls at a depth of 5.5 m. Water temperature in April was 13°C. This population appeared to be restricted to a zone ca. 30 m from the entrance (Figure 2, Table I). The presence of wide ripple marks on the sandy bottom suggested high water movement levels in the outermost zone. The presence of iron and manganese oxides was scanty.

In the Terrazze cave, *P. massiliana* was at a depth of 4 m on the walls of a dark S-shaped tunnel (ca. 20 m in length) on the left of the entrance (Figure 2). The water temperature in April was 14°C (Table I). The absence of sand deposits and the presence of abundant large boulders on the rocky bottom suggest the cave may be subjected to a higher water movement.

Large facies of *P. massiliana* were found in the Fantasmi cave at 6 m of depth exclusively in the central dark chamber at ca. 30 m from the entrance, both on walls and on large scattered rocky blocks

Table I. Habitat and habitus of *Petrobiona massiliana* from NW-Sardinian caves compared to other Mediterranean records. Not recorded (-).

Locality/cave	Depth (m)	Brightness	Water movement	Water temp. (°C)	Habitus (Ø cm)	References
Marseille Gulf (Type locality)	10	Dark	–	–	Massive lobate 3.0 × 1.0	Vacelet & Lévi 1958
Apulia (Teatrino Cave)	0.5–1	Dark, semi-dark	High	–	Encrusting 2.0–3.0 in facies	Sarà 1963
Sorrento Peninsula (Tuffo Tuffo Cave)	0.8	Dark	High	–	Massive, lobate 0.5–2.5 (height) in facies	Rützler 1966
Marseille Gulf (Endoume Cave)	4	Dark	High	–	Massive	Pouliquen 1972
Marseille Gulf (Figuier Cave)	7	Dark	Medium	–	Encrusting in facies	Pouliquen 1972
Marseille Gulf (Trèmies Cave)	5.5	Semi-dark	Low	–	Encrusting in facies	Pouliquen 1972
Sorrento Peninsula (Mitigliano Cave)	12	Dark	Medium	–	Encrusting	Pansini & Pronzato 1982
Ustica (Verde Cave)	7	Dark	Medium-high	–	–	Corriero et al. 1997 1999
Zembra, Tunisia (Entorche Cave)	10	–	–	–	–	Boudouresque et al. 1986
Port Cros islands (Bagaud Cave)	8	Dark, semi-dark	Medium	–	–	Harmelin et al. 2003
NW Sardinia (Terrazze Cave)	4	Dark	High	14	Encrusting 1.3–1.5 × 1.6–2.1 0.7–0.8 (height)	Present paper
NW Sardinia (Galatea Cave)	5.5	Dark	High	13	Encrusting 1.3 × 2.0 0.7 (height)	Present paper
NW Sardinia (Nereo Cave)	15 28	Dark	Medium	24	Massive lobate Subspherical/fan-shaped 0.7–1 × 0.8–1.9 0.75–1.3 (height) in facies	Present paper
NW Sardinia (Fantasmi Cave)	6	Dark	Low	18	Massive, lobate, subspherical/flabellate 0.8 × 2.2 0.7–1.9 (height) in facies	Present paper

covered by iron and manganese oxides. Sponges were absent on the roof of the chamber and in the tunnel of white rock leading to the entrance (Figure 2, Table I). The water temperature in June was 18°C. Water movement is low, as suggested by the presence of a muddy bottom. Two large facies of *P. massiliana* at 15 and 28 m of depth were found in the large tunnels of the Nereo cave, with specimens densely and regularly distributed among other sponge species, bryozoans, cnidarians, and serpulids (Figure 2, Table I). The water temperature in September was 23°C. The cave morphology with large tunnels and 10 entrances at different depths (10–31 m) facing SE indicates a notable water movement.

On most *P. massiliana* specimens associated organisms such as sponges, bryozoans, foraminifers (*Miniacina miniacina*), serpulids and rare antozoans were found.

## Discussion

According to the literature, the growth form of *P. massiliana* is widely variable, also in specimens

collected from neighbouring caves. The range of morphs, from thin crusts (Figure 3c) (Terrazze and Galatea caves), slender finger-like shape (Figure 3f) (Nereo cave) to relatively large fan-shaped erect sponges (Figure 3d,e) (Fantasmi cave), indicating a high growth form plasticity, under the control of hydrological and biotic factors.

It may be that the relatively small body size and the erect columnar habit (Figure 3f) in the Nereo cave together with the aggregate distributional pattern (facies) could be related to the spatial competition with the other components of the benthic community (mainly sponges, bryozoans, madreporarians and serpulids). On the other hand, the species was not found in the same microhabitats colonized by lithistids (Manconi et al. 2006), as previously suggested by Pouliquen (1972). In any case, such shape variability supports the opinion of Vacelet et al. (2002) on the status of *P. incrustans* as a junior synonym of *P. massiliana*.

In contrast to the variability of the growth form, the spicular complement appears to be very constant in the different specimens. Although the spicular

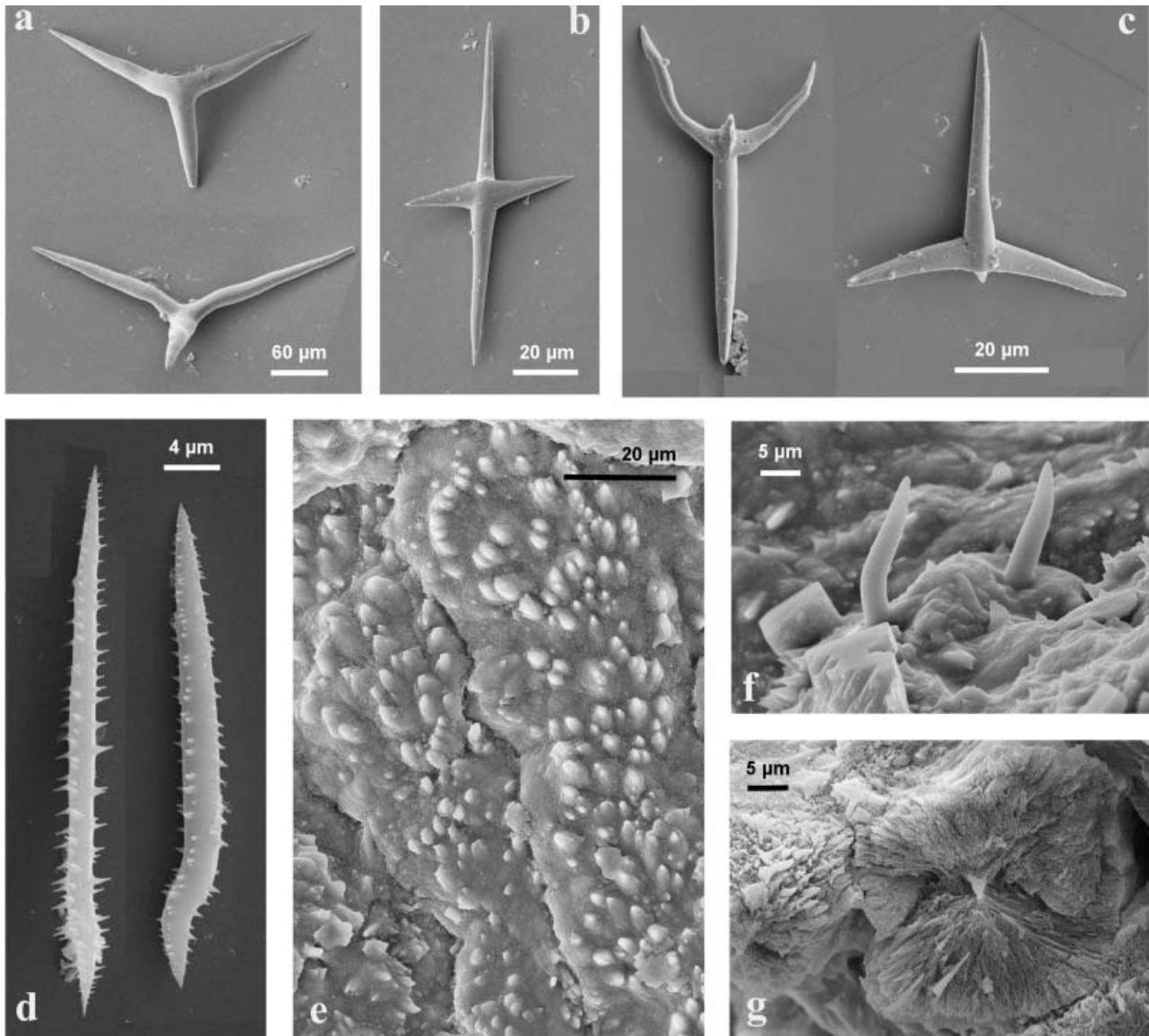


Figure 4. *Petrobiona massiliana* from the Fantasmi cave. Micrographs (SEM) of spicular complement **a**, triactines; **b**, tetractine; **c**, diapason tetractines; **d**, microdiactines with spines; **e**, surface of the compact basal skeleton; **f**, apices of a diapason tetractine partially embedded in the basal skeleton; **g**, cross-section of the basal skeleton.

morphometries from the different Mediterranean caves are not useful for a statistical analysis (the numbers of specimens and measured spicules are not reported in the literature), the available data show that this trait seems to be highly conservative across the entire geographic range (Table II). However, the spicular malformations recorded in the Terrazze and Galatea caves at the level of diapasons could be suggestive of some kind of disturbance.

In the caves of the Punta Giglio and Capo Caccia promontories, *Petrobiona massiliana* colonizes the rocky walls at a variable distance from the entrances at depths ranging from 6 to 28 m (Table I). The wide

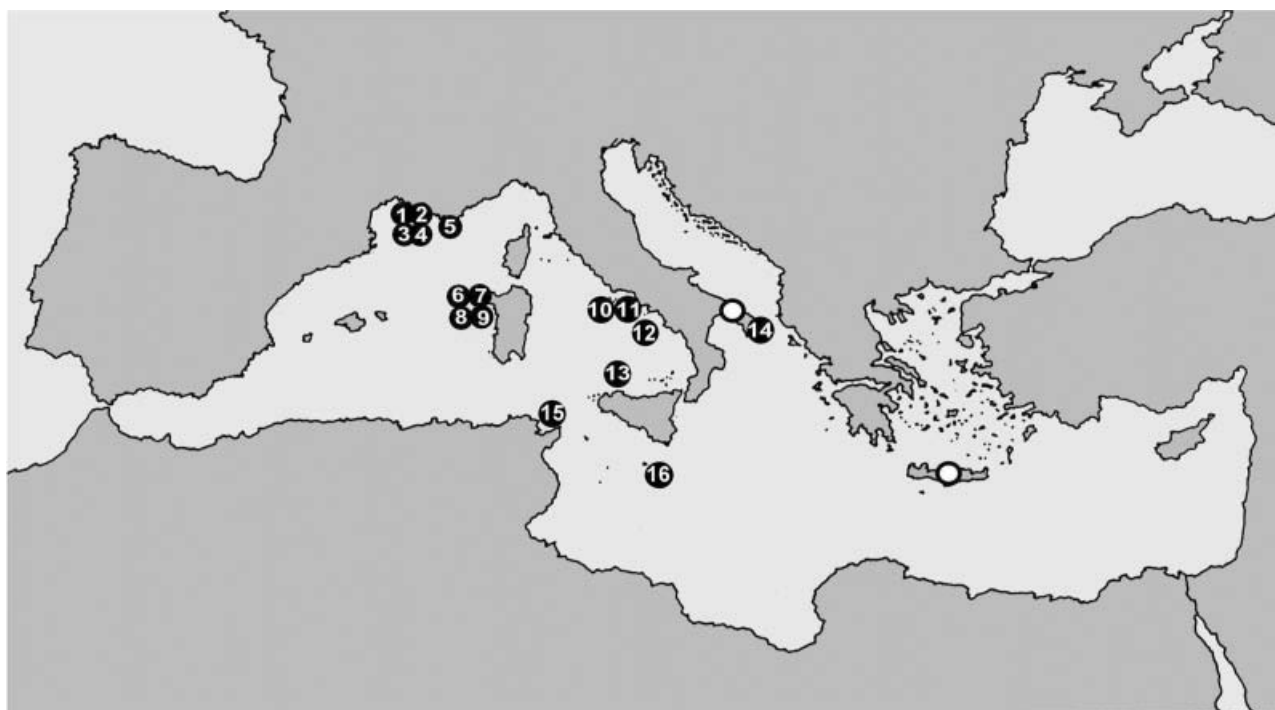
array of cave typologies and occupied habitats confirms the ability of this species to acclimatise to a large spectrum of environmental conditions in cryptic habitats (Vacelet & Lévi 1958; Sarà 1963; Rützler 1966; Pouliquen 1972; Pansini & Pronzato 1982; Corriero et al. 1997, 1999; Harmelin et al. 2003).

Water movement seems to strongly affect the topographic distribution of this sponge in Sardinian caves: few scattered encrusting specimens or isolated specimens occur in high-water movement conditions (Terrazze and Galatea caves), whereas typical large facies of erected specimens prevail when water movement decreases (the wide tunnels of the



Table II. Spicular traits of *Petrobiona massiliana* from NW-Sardinian caves compared to other Mediterranean records. \*Original description; •Systema Porifera; total=total length; bas=basal; lat=lateral; ax=axial; thick=thickness.

Locality/cave/specimens	Triactines (µm)	Diapason triactines (µm)	Pugiole tetractines (µm)	Microdiactines (µm)	References
Marseille type locality	50–200 × 20–40*	70–100 total*	300 ax × 80 lat × 8–27 thick*	30–40 × 2*	Vacelet & Lévi 1958*
Holotype	25–200 × 6–40	30–70 × 5–8.5 bas 20–50 × 4–7 lat	40–130 × 22–28 lat• 16–40 × 5.5–8.5 lat• 8–100 × 10–28 ax• 30–70 × 5.5–8.5 ax•	30–60 × 2–3•	Vacelet et al. 2002•
Apulia Teatrino Cave	150 × 20–30 30–50 × 5–15	70–75 × 5 total	200 max ax × 100 max lat × 15 max thick	30–40 × 2–3	Sarà 1963
Sorrento Peninsula	45–300 × 15–48	75–130 × 6–8 total	130–150 ax × 80–135	30–65 × 2–4	Rützler 1966
Tuffo-Tuffo Cave			lat × 7.5–18 thick	23 × 7.5–10	
Punta Giglio Terrazze	45–170 × 10–25	35–60 × 3–8 bas 23–48 × 3–5 lat	100–160 ax × 60–105	25–40 × 3	Present paper
Cave TER 1-2			lat × 5–10 thick		
Punta Giglio Galatea	55–175 × 10–25	45–63 × 5–8 bas 20–55 × 4–5 lat	80–155 ax × 50–95	25–38 × 2–3	Present paper
Cave GAL 6			lat × 5–19 thick		
Punta Giglio Fantasmi	50–200 × 10–30	35–63 × 3–8 bas 20–55 × 3–8 lat	70–190 ax × 45–100	28–55 × 2–4	Present paper
Cave FAN 3-4-5			lat × 5–15 thick		
Capo Caccia Nereo	35–200 × 5–30	40–73 × 5–10 bas 20–50 × 4–8 lat	50–180 ax × 35–170	18–43 × 3	Present paper
Cave NER 7-8-9-10			lat × 4–20 thick		

Figure 5. Distribution of the Mediterranean palaeoendemic sponge *Petrobiona massiliana*. Marseille Gulf (1, type locality); Endoume (2); Figuer (3); Trèmies (4); Bagaud (5); Galatea (6); Terrazze (7); Fantasmi (8); Nereo (9); Mitigliano (10); Tuffo Tuffo (11); Ischia (12); Verde (13); Teatrino (14); Entorche (15); Malta (16). Numbers refer to records of living sponges. Fossil records from Apulia and Crete are indicated by white circles without numbers.



Nereo cave, and the innermost zone of the Fantasmic cave). The origin of the facies could be also explained by philopatry due to the low dispersal ability of sponge swimming larvae.

This distribution pattern confirms the preference of *P. massiliana* for low water movement conditions, as reported by Pouliquen (1969). On the other hand, Corriero et al. (2000) suggest that the absence of *P. massiliana* from an Adriatic semi-submerged cave (Tremi islands) could be due to high water turbulence, a hypothesis in agreement with previous observations referred to other Mediterranean sites (Vacelet 1976; Pansini et al. 1977).

A further potential environmental constraint for this species could be represented by freshwater infiltrations into marine caves. Although the north-western Sardinian karstic limestone is notably fissured, the superficial freshwater catchment basin is, at present, not conspicuous as rainfall is notably scarce, and freshwater input into the caves is very limited to absent (Mucedda et al. 1997). However, it is possible that in the long term, the degree of freshwater input and the related salinity variations may play a key role in the control of settlement and persistence of *P. massiliana* populations in submersed caves.

The almost exclusive habitat in caves and the success of *P. massiliana* in these extreme conditions could also be related to its cryptobiotic life strategy; namely, the ability to maintain aggregates of totipotent cells ("pseudogemmules made of archeocytic cells, rich in storage granules and food vacuoles") in narrow canals ("calicles") within the compact basal calcareous skeleton (Reitner 1989, 1991; Vacelet 1990; Vacelet et al. 2002). It could be argued that the ability of resistance and regeneration processes of these peculiar devices could be one of the key characters that favoured the survival of *P. massiliana* as a relict. Shallow-water caves may represent a refuge habitat for this species during unfavourable environmental/climatic conditions both in the short and long-term of the Mediterranean historical dynamics (salinity crises, freshwater/anchialine habitats in particularly humid climates, and cold/warm phases).

Since *P. massiliana* was never found in cold deep waters or cold caves, it could be a true cave-dwelling species (i.e. troglobious) as suggested by Corriero et al. (2000). The larval release by Sardinian specimens living in water at 24°C (28 m of depth) at the end of summer may indicate the thermophily of this species. Moreover, attempts are in progress to test *P. massiliana* as a bioindicator to provide environmental data relating to the past climate (Berry et al. 2005).

The present records confirm the restricted geographic range of *P. massiliana* in the western and central Mediterranean; the species occurs in a few scattered sites in the Adriatic, Ionian and Tyrrhenian seas, the Strait of Sicily, and the Lion Gulf (Figure 5) (Vacelet & Lévi 1958; Sarà 1963; Rützler 1966; Pouliquen 1972; Wendt 1979; Pansini & Pronzato 1982; Bouduresque et al. 1986; Reitner 1989; Corriero et al. 1997; Vacelet et al. 2002; Harmelin et al. 2003). The high frequency of finding and the notable abundance in northwestern Sardinia are, however, suggestive of a wider distribution of *P. massiliana* in caves of the entire western Mediterranean.

The presence of *P. massiliana* in distant karstic areas matches a biogeographic model of disjunct distribution with metapopulations isolated in cryptic habitats. The release of abundant free-swimming larvae by specimens from the Nereo cave in September fits well the reproduction occurring from May to October by lecithotrophic amphiblastula larvae as reported by Gallissian and Vacelet (1990). Dispersal and panmixis seem to be virtually possible by means of passive drifting by larvae from one cryptic habitat (caves, crevicular habitats) to another, in agreement with the model of stepping stones as suggested also for Sardinian cave-dwelling lithistids (Manconi et al. 2006). This hypothesis is also supported by the rare records of *P. massiliana* both in non strictly cryptic habitats, e.g. under boulders along rocky cliffs (Wendt 1979; Vacelet et al. 2002; Tunisia, 20 m of depth, J.G. Harmelin, personal communication), under large sponges (i.e. *Agelas oroides*, 20 m of depth, Malta, J. Vacelet, personal communication) and on beach rock surfaces in very shallow water (dead specimens only, Ischia, J. Reitner, personal communication). The topographic distribution of the adults frequently occurring in facies implies the low swimming and short dispersing capacity of larvae.

The status of *P. massiliana* as a rare Mediterranean palaeoendemic species required conservation measures since 1979; indeed, it is listed as a strictly protected species of the Mediterranean Sea (COE 1979) according with the Annex II of the Bern Convention and the Jakarta Mandate of the Convention on Biological Diversity (Costello 1996).

The taxonomic richness of Mediterranean Porifera (610 species) with a notable level of endemism (48%) (Vacelet 1980; Pulitzer-Finali 1983; Pansini 1995; Pansini & Longo 2003, 2006; Pronzato 2003) reaches one of its maxima in submarine caves that represent biodiversity hotspots. In the light of this, one of the major goals of the

Mediterranean MPAs encompassing karstic areas would be the assessment of biodiversity and measures for protecting endemic rare species in caves together with co-occurring species by a sustainable management of human activities (e.g. SCUBA diving). Appropriate conservation planning of *P. massiliana* represents one of the challenges of the western Mediterranean MPAs (e.g. Capo Caccia–Isola Piana, Ustica, Zembra–Zembretta, Punta Campanella, Ischia–Vivara–Procida and Port Cros).

## Acknowledgements

This paper is dedicated to the memory of Fabio Cicogna (CLEM) who supported the first investigations in caves by R. Manconi. We are grateful to the anonymous reviewers who helped improve the manuscript. We would also like to thank Drs R. Barbieri, E. Carzedda and G. Delitala for their kind cooperation in sampling and photography of specimens. This work was funded by the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MIUR-PRIN), the European Project INTERREG Sardinia–Corsica–Tuscany on Biodiversity, Fondazione Banco di Sardegna and Università di Sassari. The Ministero dell'Ambiente (Studio degli ambienti di grotte marine sommerse (Codice Habitat 8330) nelle Aree Marine Protette di Pelagie, Plemmirio e Capo Caccia) supported, in part, this research.

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