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A New Species of *Oscarella* (Demospongiae: Plakinidae) from California

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We describe a new species of *Oscarella* Vosmaer, 1877 (Porifera: Homosclerophorida) from a rocky coast and marine aquariums in central California and analyze its spatial distribution and temporal fluctuations to determine if it is a non-indigenous species. We also compare the efficiency of two different fixatives, Bouin's solution and glutaraldehyde in seawater, and found that glutaraldehyde is better for comparisons of anatomical and cytological characters that are important for species identification.

This is the first record of the genus *Oscarella* from the Eastern Pacific. The new species is characterized by its light brown to rusty orange color, undulated appearance, bumpy, microlobate surface, soft, slimy consistency, two particular kinds of cells with inclusions, and occurrence in the intertidal high zone. It sometimes thrives in laboratory conditions, indicating that predators may limit its vertical distribution on the shore. The new species of *Oscarella* is clearly different in external morphology and cell content from all previously described species of *Oscarella*, thus giving low support to the hypothesis that it is an invasive species. Instead, it appears to have been previously overlooked because it is inconspicuous and relatively uncommon in the field, and because it could not be distinguished from *Halisarca sacra* de Laubenfels, 1930 or *Halisarca* sp. in keys to the West Coast sponge fauna. We present how it can be distinguished from *Halisarca*, and discuss the present knowledge of that genus in the northeast Pacific in comparison to *Oscarella*.

KEY WORDS: California, *Halisarca*, Homosclerophorida, intertidal, *Oscarella*, Porifera, sponge.

Marine invasive species often appear first in aquariums, to be later established in the field. A classic marine example is the alga *Caulerpa taxifolia* in the Mediterranean, but there are many others (e.g., Ribera and Boudouresque 1995). In the mid 1980s, an unidentified slimy sponge without skeleton (either spongin or spicules) appeared growing on submerged rocks and the sides of aquariums and sea tables at the Joseph M. Long Marine Laboratory of the University of California, Santa Cruz, on the northern edge of Monterey Bay. At first, it was taken to be a species of *Halisarca* Johnston, 1842, the only similar organism in the main key for intertidal sponges of central California (Hartman 1975). However, in 1990, M. Cristina Diaz examined a piece of the sponge with scanning electron microscopy and identified it as a member of the genus *Oscarella* Vosmaer, 1887 (Demospongiae: Plakinidae) (Diaz and van Soest 1994), a genus mainly known in the Mediterranean and previously unknown from the eastern Pacific. The sponge was noticed in the

late 1980s in several of the exhibit tanks of the Monterey Bay Aquarium, which is located on the southern side of Monterey Bay, and at Long Marine Laboratory, where it continues to be present (JSP, pers. obs.). It also became established in the exhibit tanks and teaching labs of the Seymour Marine Discovery Center, adjacent to Long Marine Laboratory, shortly after it opened in 2000. After seeing the sponge at these facilitates in early 2004, Scott Nichols (pers. commun.) found the same sponge in an aquarium at the Bodega Marine Laboratory of the University of California, Davis.

In addition to the question of its identity, it was puzzling that the sponge had not been seen in the field, especially since field biologists have been working in the rocky intertidal of the Monterey Bay area of central California throughout the 20th century. Moreover, de Laubenfels (1932) had extensively monographed the sponges of California. Finally, in September 2002, one of us (JSP) found the sponge growing on the sides and undersurfaces of intertidal rocks on the northwest corner of Carmel Point, in Carmel Bay, central California (Figs. 1, 2A).

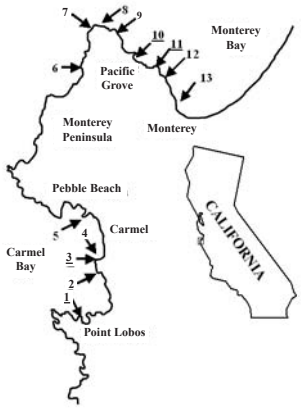

FIGURE 1. Map of Carmel Bay and the Monterey Peninsula showing the location of 13 intertidal sites examined between September 2002 and May 2004; underlined numbers indicate sites where specimens of *O. carmela* sp. nov. were found. 1. Whaler's Cove, Point Lobos State Park; 2. Carmel Point, southwest corner; 3. Carmel Point, northwest corner; 4. Carmel Point, north side, between site 3 and Carmel Beach; 5. Arrowhead Point on east side of Stillwater Cove; 6. Asilomar State Beach State Park, south end next to Spanish Bay Beach; 7. The Great Tide Pool, south of Point Pinos; 8. Point Pinos; 9. Lucas Point, between Coral Street and Esplande Park; 10. Lovers Point, east side, both sides of Lovers Point Beach; 11. Hopkins Marine Station (Cabrillo Point); 12. Monterey Bay Aquarium (Point Alones), underneath building; 13. Monterey Harbor, between the Coast Guard Pier and Fisherman's Wharf. Square on inserted map of California outlines the Monterey Peninsula and Carmel Bay.

Most species of *Oscarella* have been described from the Mediterranean, but there are also two Indo-Pacific species and a few doubtful Australian and South African records (e.g., Boury-Esnault et al. 1992; Muricy et al. 1996; Muricy and Diaz 2002; Bergquist and Kelly 2004). Moreover, many species of *Oscarella* occur in Brazil and the Caribbean (GM, pers. obs.), but to a large extent they are uncommon and most have yet to be described. The same might be true for California, and this is likely an undescribed species indigenous to this area. However, with no previous records of the genus *Oscarella* along the Pacific coast of North America, the question arises whether it could have been introduced from elsewhere, possibly via disposal of aquarium water. If it is non-indigenous, it is not a very attractive addition to the Californian fauna: it is a tan, bumpy, slime-like sponge that overgrows other organisms. If it is conspecific with any of the Mediterranean species of *Oscarella*, that would support the invasion hypothesis, and there is a risk that it could disrupt natural biotas.

Our goals were to determine whether this skeleton-less sponge belongs to *Oscarella* or *Halisarca*, and whether it is a new species, either indigenous but unnoticed before or introduced but undescribed, or a known species introduced from somewhere else. We also analyzed the spatio-temporal distribution of the species in the Monterey Bay region, and compared the efficiency of two different fixatives, Bouin's solution and glutaraldehyde in seawater, for the histology and taxonomy of these skeleton-less sponges.

MATERIALS AND METHODS

Specimens were photographed with an Olympus Camedia 3040 digital camera and collected in November 2002 and May 2003 from rocks in a high intertidal pool at Carmel Point in Carmel

Bay, central California (Figs. 1, 2A). Additional specimens were collected from tanks in Long Marine Laboratory and the Seymour Marine Discovery Center of the University of California, Santa Cruz (UCSC). Small pieces of the sponges were collected with a pocketknife and immediately fixed in Bouin's fixative (75 ml saturated aqueous solution of picric acid: 25 ml 37–40% formalin (formaldehyde): 5 ml glacial acetic acid) or freshly prepared 2.5% glutaraldehyde in seawater (25% glutaraldehyde diluted and buffered with seawater). Bouin's-fixed samples were embedded in paraffin for thick sections and epon resin for semithin sections. Glutaraldehyde-fixed samples were post-fixed in 1% osmium tetroxide and embedded in epon resin for semithin and ultrathin sections. Thick and semithin sections were stained with toluidin blue or acid fucsin, observed in light microscopy (LM), and photographed with a Nikon Coolpix 4500 digital camera. Ultrathin sections were stained with uranyl acetate, contrasted with lead citrate and observed in a Zeiss M900 transmission electron microscope (TEM).

Systematics

Phylum Porifera Grant, 1836
Class Demospongiae Sollas, 1885
Subclass Homoscleromorpha Lévi, 1973
Order Homosclerophorida Dendy, 1905
Family Plakinidae Schulze, 1880

Genus *Oscarella* Vosmaer, 1887

Type species: *Halisarca lobularis* Schmidt, 1862 (by monotypy).

[*Oscaria*] Vosmaer, 1881: 163 (preocc. by Oscaria Gray, 1873 – Reptilia);
Oscarella Vosmaer, 1884: pl. 8 (explanation); 1887: 326 (nom. nov. for *Oscaria* Vosmaer).
Octavella Tuzet and Paris, 1964: 88.

Diagnosis (Muricy and Diaz 2002).— Plakinidae without skeleton, with thinly encrusting to lobate shape. Thin ectosome (< 100 μm), often limited to pinacoderm; true cortex absent. Mesohyl poorly developed, with a proportion of mesohyl to chambers varying from 0.5:1 to 1.2:1. The aquiferous system has a sylleibid organization, with spherical, eurypylous choanocyte chambers uniformly arranged around large, regular exhalant canals, and a large basal cavity.

***Oscarella carmela* Muricy and Pearse, sp. nov.**
(Figs. 2–4)

Diagnosis.— Intertidal or aquarium-dwelling *Oscarella,* light brown to tan or rusty orange in color, with bumpy, microlobate surface, wavy appearance, soft, slimy consistency, and two particular kinds of cells with inclusions (types 1 and 2).

Material examined.— (12, all from central California, USA) (MNRJ, Museu Nacional, Universidade Federal do Rio de Janeiro; CASIZ, California Academy of Sciences; SBMNH, Santa Barbara Museum of Natural History)

Holotype: MNRJ 8033, Carmel Point, Carmel Bay, intertidal, J. Pearse coll., 08 May 2003. Paratypes: MNRJ 8032, 07 November 2002, CASIZ 168925, 08 May 2003, Carmel Point, Carmel Bay, central California, intertidal, J. Pearse coll.; MNRJ 8034, 8035, CASIZ 168924, 06 November 2002, SBMNH 354351 (three specimens) 29 July 2004, tanks in Long Marine Laboratory, UCSC, Santa Cruz, J. Pearse coll.; MNRJ 8031 (three specimens), 06 November 2002, teaching lab of the Seymour Marine Discovery Center, UCSC, Santa Cruz, J. Pearse coll.

Description.— Thinly encrusting, irregular sponges, generally light brown, up to 20–30 cm

in diameter, with extremely soft, slimy consistency. Thickness, color, and surface irregularities variable. Most specimens tan in color, 2–3 mm thick, and lumpy or undulated with conspicuous channels and oscula (Fig. 2B–F). Some specimens gray-tan and smoother, or pale brown to rusty orange, with very bumpy surfaces and orange blotches. These different morphologies could be ecophenotypes or different stages of growth. In some cases, one morphology graded into another in what appeared to be the same individual. On the glass walls of aquariums, the sponge is nearly transparent and clusters of choanocyte chambers and spherical embryos can be seen inside. The sponge is easy to peel off smooth surfaces. Under low magnification (60×) the colored spherical choanocyte chambers can be seen in living material, and often, the colorless spherical hollow embryos.

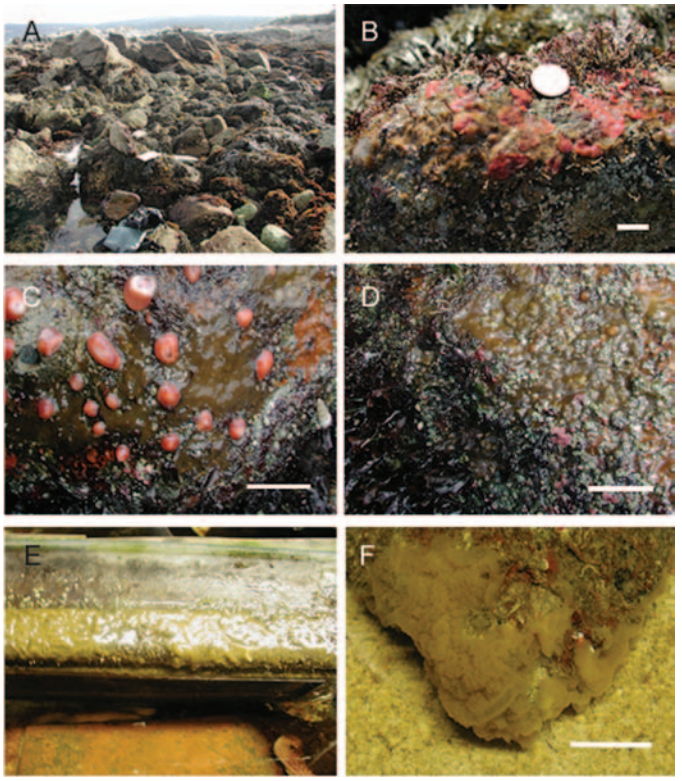

FIGURE 2. *Oscarella carmela* sp. nov. A. Habitat at the northwest corner of Carmel Point, with boulders in the intertidal zone. B-D. Three colonies on the upturned sides of boulders exposed during low tide; colonies of the bryozoan *Eurystomela bilabiata* partially overgrown in B, polyps of the corallimorpharian *Corynactis californica* partially surrounded in C (scale bar = 2 cm; coin in B is a U.S. quarter). E. Colony growing up to the water line on the side of a 8 cm high plastic box in an aquarium in Long Marine Lab. F. Colony growing on a rock in an aquarium in the Seymour Marine Discovery Center; the sponge is covered with seawater (scale bar = 2 cm).

ANATOMY.— Because glutaraldehyde provided better fixation for fine structure than Bouin's solution (see below), the description of anatomy and cytology is based on glutaraldehyde-fixed material. Choanocyte chambers ovoid to spherical eurypilous, 25–65 μm in diameter, organized around large exhalant canals (Fig. 3A–G). Mesohyl between the chambers very thin (5–10 μm thick). Proportion of mesohyl to chamber volume less than 0.5:1.0. Ectosome thin, 5–10 μm thick. Basal cavities large, separated by septa without choanocyte chambers, 10 μm thick. Cinctoblastula larvae characteristic of the family Plakinidae, 50–300 μm in diameter (Fig. 3H).

CYTOLOGY.— Choanocytes ovoid to pyramidal, irregular, 2–5 μm in diameter by 3–5 μm high (Fig. 4A–C). Nucleus basal or apical, 1.5–2.2 μm in diameter. Cytoplasm with up to eight phagosomes, 0.5–1.5 μm wide. Choanocyte collars with 25–33 microvilli. Apopylar cells not observed. Pinacocytes flat or elongated to ovoid, 5–10 μm long by 0.5–2 μm wide, flagellated, anchored in the mesohyl by short pseudopodia (Fig. 4C–D). Nucleus ovoid, 1–2 μm in diameter, nucleolated. Cyto-plasm with numerous small phagosomes, 0.3–0.7 μm, and often also one larger vacuole with clear contents. Choanoderm and pinacoderm lined by a basement membrane-like structure, which is a continuous, 5–10 nm thick layer of condensed collagen fibrils in the mesohyl closely adjacent

to the base of the cells. Collagen deposition in the mesohyl heterogeneous (Fig. 4D). As seen in both LM and TEM, ameboid cells of the mesohyl include archaeocytes and two types of cells with inclusions. Archaeocytes roughly ovoid, irregular, 5.0 × 3.5 μm, with abundant pseudopodia (Fig. 4E, F). Cytoplasm filled with phagosomes 0.3–1.5 μm wide containing food particles, including bacteria, in various stages of digestion. Nucleus ovoid or irregular, 1.0–2.5 μm, nucleolated. Type 1 cells with inclusions ovoid, 5.0 × 3.0 μm, irregular, with short pseudopodia (Fig. 4E). Nucleus 2 μm in diameter, ovoid or compressed by the abundant cytoplasmic inclusions. Cytoplasm filled with inclusions of two kinds, 0.2–1.0 μm wide, one kind with relatively dark and homogeneous contents and the other with clear, heterogeneous, filamentous contents. Type 2 cells with inclusions have irregular shape, 5.0×3.0 μm, rarely with short pseudopodia (Fig. 4F, G). Cytoplasm with 5–15, 0.5–2.0 μm wide inclusions with homogeneous contents, and 3–8 larger vacuoles, 1–4 μm in diameter, with clear and filamentous contents. Nucleus 1.2–2.5 μm in diameter, ovoid or compressed by abundant cytoplasmic inclusions. This cell type appears to be involved in the secretion of ground substances of the intercellular matrix, because it is often seen liberating the contents of its larger, clear vacuoles in the mesohyl (Fig. 4G). Endobiont bacteria of two kinds (Fig. 4H): type B1 most common, ovoid, 1.0–1.5 by 0.6–1.0 μm, with a wrinkled cell wall, a clear and filamentous nuclear region and a darker cytoplasmic region; type B2 rod-like, 1.0 × 0.2 μm, with darker and rather homogeneous contents, and without a clear distinction between nuclear and cytoplasmic regions.

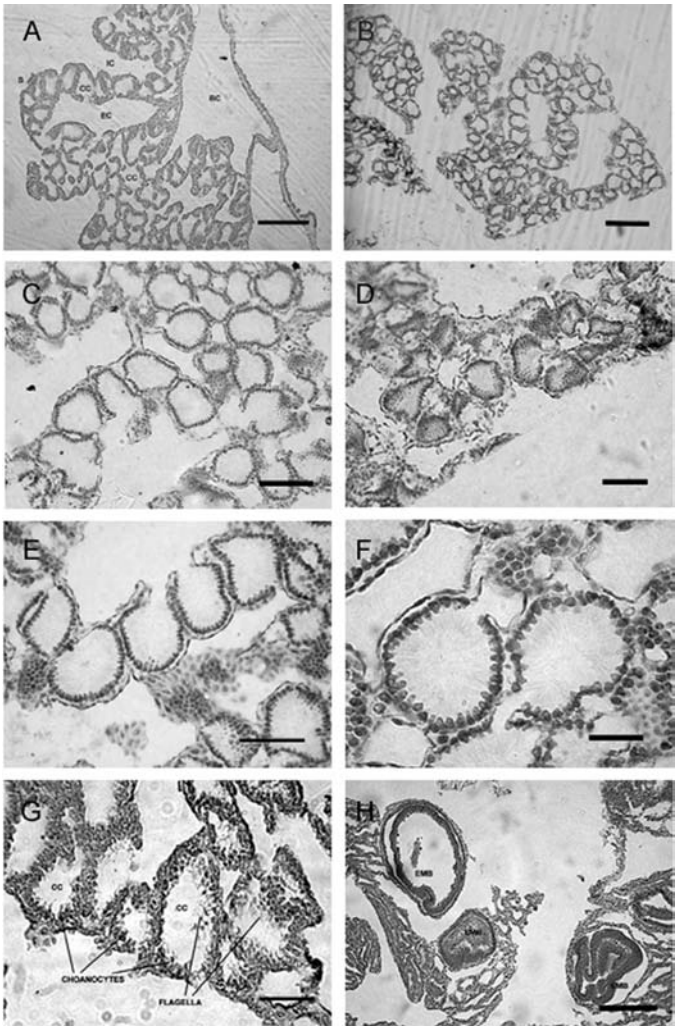

FIGURE 3. Histology of *Oscarella carmela* sp. nov. (LM). (A–B) transverse section showing the arrangement of choanocyte chambers around exhalant canals and the large basal cavities (scale bar = 100 μm); (C–E) choanosome (scale bars = 50 μm); (F–G) choanocyte chambers (scale bars = 25 μm); (H) embryos (scale bar = 100 μm). Fixation: A, G and H, Bouin's fixative; B–F, glutaraldehyde / osmium tetroxide.

ECOLOGY.— Specimens of *Oscarella carmela* occur as thin sheets on the sides of granite outcrops and on the edges and undersurface of boulders in high-zone tide pools of Carmel Bay and the

Monterey Peninsula (Fig. 1, Fig 2A). These sponges apparently need to remain continually immersed, but do well in the variable environment of high-tide pools. The tops of the boulders in this habitat supported typical high-zone algae (*Silvetia compressa, Fucus gardneri, Endocladia muricata, Mazzaella affinis, Mastocarpus* spp.) whereas the sides of boulders immersed in the tide pools often were covered with the red alga, *Prionitis lanceolata,* and encrusting and upright coralline algae. The undersurfaces of the boulders supported a rich variety of sessile and encrusting animals, e.g., red poecilosclerid sponges, cnidarians (*Corynactis californica* and *Plumularia* sp.), bryozoans (*Eurystomella bilabiata* and others), polychaetes (*Spirorbis* sp.), gastropods (*Petaloconchus montereyensis*), and ascidians (*Aplidium californicum* and *Distalpia occidentalis*). Often individuals of *O. carmela* were overgrowing other encrusting organisms, especially the bryozoan *E. bilabiata* (Fig. 2 B). Surprisingly, *O. carmela* has not been found in mid or low zones, although thorough searches down to the low zone were made at all the sites where it was found in the high zone.

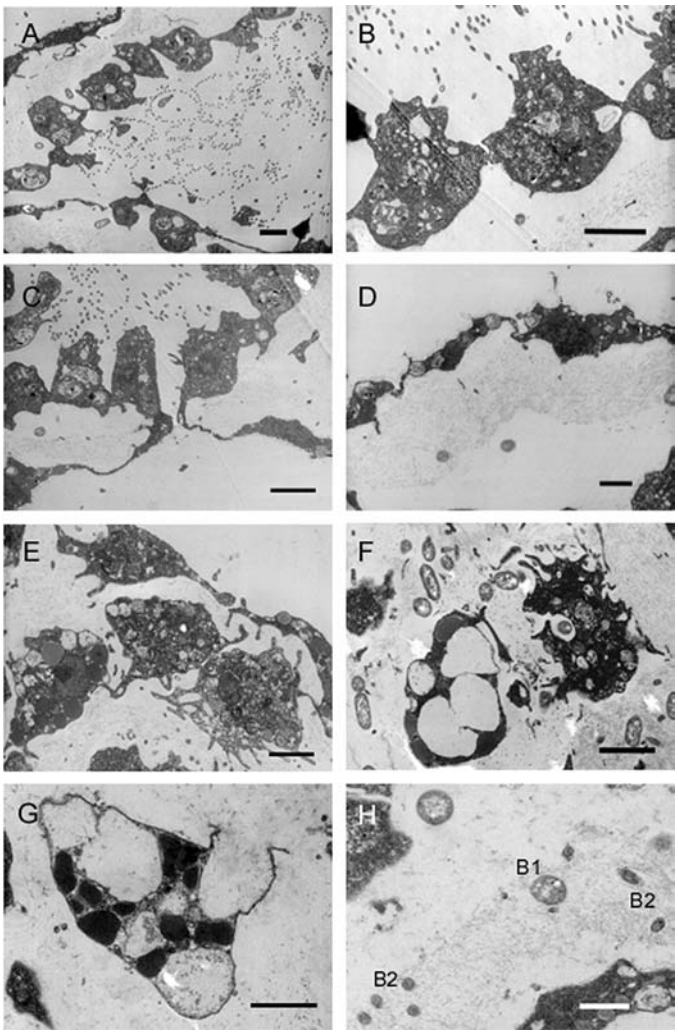

FIGURE 4. Cytology of *Oscarella carmela* sp. nov. (TEM). (A) section through a choanocyte chamber; (B) choanocytes; (C) prosopyle of a choanocyte chamber showing choanocytes (top) and endopinacocytes (bottom); (D) pinacocytes; (E) type 1 cell with inclusions (left), two archaeocytes (middle and right), and the loose junction between two pinacocytes (top right); F, type 2 cell with inclusions ("spherulous-vacuolar cell", left) and archaeocyte phagocytizing a bacterium (right); (G) type 2 cell with inclusions ("spherulous-vacuolar cell"); (H) endobiont bacteria types 1 (B1) and 2 (B2). Scale bars: A–G = 2 μm; H = 1 μm.

Specimens of *O. carmela* often grew profusely in aquariums and sea tables of the Long Marine Laboratory–Seymour Marine Discovery Center complex of the University of California, Santa Cruz, covering submerged rocks and plastic trays right up to the edge of the water line (Fig. 2E). In quiet aquarium water, the sponges developed conspicuous folds with bulging excurrent canals converging on large oscula (Fig. 2F). Asteroids (*Asterina miniata, Dermasterias imbricata, Pisaster ochraceus*) and abalones *(Haliotis rufescens*) in some of the sea tables and aquariums did not appear to disturb the sponges.

Specimens of *O. carmela* have been noticed in several of the exhibits of the Monterey Bay

Aquarium for many years, especially in the "Crevice Dwellers" tank, where they are particularly abundant during the spring (Veronica Franklin, pers. commun.). They grow on the artificial rock backing of the tank and on the shells of rock scallops (*Crassodoma gigantea*). The specimens have only been seen in tanks containing local biota and not in tanks with exotic species (e.g., the tank with Mediterranean cuttlefish); all seawater is filtered both before and after it leaves tanks with exotic species.

Distribution.— *Oscarella carmela* occurs in aquariums and sea tables of the Long Marine Laboratory–Seymour Marine Discovery Center complex, the Monterey Bay Aquarium, and in an aquarium of the Bodega Marine Laboratory of the University of California, Davis, approximately 150 km north of Monterey Bay (Scott Nichols, pers. commun.). However, specimens of *O. carmela* are not ubiquitous in marine laboratories and public aquariums along the California coast. For instance, it was not found in any of the aquarium tanks in the Steinhart Aquarium of the California Academy of Sciences in San Francisco nor in aquariums at the Long Beach Aquarium in southern California. Moreover, it has not been found in any of the tanks with running seawater at Stanford University's Hopkins Marine Station, which is adjacent to and receives all its seawater from the Monterey Bay Aquarium.

Specimens of *O. carmela* were first found in the field by JSP at Carmel Point on September 2002 and sampled on November 2002. The sponges were on boulders in high intertidal pools directly west of the intersection of Scenic Road and Ocean View Avenue, and to the north of the "Sunburst" house on the ocean side of Scenic Road (see: *http://limpets.noaa.gov/monitoring/rockyIntertidal/sites/mbnms/carmelpoint.html*). The site at Carmel Point was visited again on January 2003, several weeks after unusually heavy storms. Many of the boulders in the high tide pools had been disturbed and rearranged, but thin colonies of *O. carmela* were found on the undersurfaces of some of them. On April 2003, a thorough search of the boulders was made throughout the area from the high zone, through the mid zone dominated by the alga *Mazzaella flaccida,* to well into the low zone dominated by surfgrass (*Phyllospadix* spp.). Three out of about 20 boulders at the original high-zone site had specimens of *O. carmela* growing on their undersurfaces. Only one small colony of *O. carmela* was found elsewhere, on the underside of a boulder in a high-zone shallow tide pool west of the "Sunburst" house, approximately 30 m southwest of the area where the first individuals were seen.

The site at the NW corner of Carmel Point was examined six other times: May, August, and December 2003, and January, February, and April 2004. Specimens were found under boulders in the original pools on all these dates, and when looked for again on August 2003 and January 2004, small individuals at the site west of the "Sunburst" house were also found.

Between April 2003 and May 2004, thorough searches were made of 12 additional intertidal sites during low tides around Carmel Bay and the Monterey Peninsula (Fig. 1). One or more specimens were found at three of these sites: (1) Whaler's Cove at Point Lobos, rip-rap boulders adjacent to the boat launch, February and April 2004, three colonies total; (2) Lovers Point Beach, Pacific Grove, east of the beach at base of cliff below the intersection of Grand Avenue and Ocean View Boulevard, 6 February, 15 February, and 7 March 2004 (three colonies total), but not found on 21 February 2004, nor in boulder field just SE of Lovers Point on 7 March 2004; (3) Hopkins Marine Station, directly north of shop building, March, May, and July 2004, four colonies; but none found during thorough searches on November 2002 and May 2003. At all of these sites, specimens of *O. carmela* were found on the undersurfaces of partially submerged boulders in high-zone pools, similar to where they were found at Carmel Point. Similar habitats occurred at the other eight sites examined, but no individuals of *O. carmela* were found. Particular attention was given to the area under and around the Monterey Bay Aquarium, including the outflow channels from the facility,

which would likely carry propagules from the exhibit tanks.

In addition to the Monterey Peninsula area, six sites with boulders in the high zone between Monterey and San Francisco to the north were examined without finding specimens of *O. carmela.* These sites were: (1) James V. Fitzgerald Marine Reserve, Moss Beach; (2) Pigeon Point, south side; (3) Point Año Nuevo, south side; (4) Terrace Point adjacent to the outflow from the Long Marine Laboratory–Seymour Marine Discovery Center facilities; (5) Point Santa Cruz (Lighthouse Point), east side; (6) Elkhorn Slough, northwest of Highway 1 bridge. Although the type locality of another slime sponge, *Halisarca sacra* de Laubenfels, 1930, was in Elkhorn Slough close to this site, neither it nor *O. carmela* was found there.

On the other hand, after seeing specimens of *O. carmela* in aquariums at Long Marine Laboratory, J.H.R. Goddard (pers. commun.) told us he had seen it at Cape Arago, Oregon, where it was distinctly different from specimens of *Halisarca* found there (see below). A photograph he shared with us, taken at North Cove, Cape Arago on 22 May 1985, shows a thin, orange-tan specimen, which appears to be identical with specimens of *O. carmela* around the Monterey Peninsula. In addition, W.C. Austin informed us that he found a slime sponge with spherical choanocyte chambers characteristic of *Oscarella*, and distinct from *Halisarca* sp., in the Vancouver Aquarium in British Columbia (pers. commun., 1 June 2004).

Etymology.— The name carmela derives from the site where this species was first found in the field, Carmel Bay in Central California.

Discussion

Comparison of fixation methods.— Proper fixation is important for the identification of *Oscarella* species. These sponges are very sensitive, and a delay of only a few minutes before placing them in fixative may make them stop filtering, start contracting, and their tissues and cells loose their normal aspect. Bouin's solution, 4% formalin or 70% ethanol preserve canals and choanocyte chambers well enough to allow their recognition as belonging to *Oscarella* and not to, for instance, *Halisarca* (see below). However, these solutions rarely preserve the natural shape of chambers and cells or allow observation of cell contents, which are important taxonomic characters at the species level. We suggest that the best way to fix these sponges for both semithin (LM) and ultrathin sections (TEM) is to put small pieces of the sponge into freshly prepared 2.5% glutaraldehyde in seawater immediately after collection, preferably while still underwater. In the laboratory, the fixative should be changed to ensure it has the desired concentration. After 24 hrs, the pieces should be post-fixed with osmium tetroxide and then dehydrated until the ethanol concentration is 70%–100%. Following this treatment, they can be stored in ethanol for long periods before making sections for observation in LM or TEM. Boury-Esnault et al. (1984) compared the results of fixation in four different fixatives for TEM and SEM (scanning electron microscopy) in *Oscarella lobularis* and *Corticium candelabrum*, with varying osmolarity (1100 –1440 mosm) and different concentrations of glutaraldehyde (0–3.0 %), seawater, cacodylate buffer (0–0.4 M), and NaCl (0–7%). They concluded that their fixative C (2.5% glutaraldehyde in 0.4 M cacodylate buffer, pH 7.4, and seawater 4 vol.: 5 vol., 1120 mosm; rinse in seawater 1100 mosm) was the best fixative for homosclerophorid sponges.

Taxonomy of *Oscarella*.— The identification of *Oscarella* at the species level is difficult. The species are homogeneous in most of the few taxonomic characters available: encrusting shape, sylleibid aquiferous system organization, choanocyte chambers spherical-ovoid with 30–60 μm in diameter, cinctoblastula larvae, thin ectosome, etc. (Boury-Esnault et al. 1984, 1992; Muricy et al. 1996; Muricy and Diaz 2002). The differences among species are mostly in external traits: color,

consistency, and aspect of the surface. These characters, however, must be used with care, inasmuch as they can vary within a single species. Specimens of *Oscarella lobularis* (Schmidt, 1862) and *O. tuberculata* (Schmidt, 1868) from the Mediterranean, for example, may appear in several different colors: white, yellow, green, red, violet, and blue. However, each species is constant and unique in its cell contents (Boury-Esnault et al. 1992). Other species appear in only a single color such as *O. viridis* Muricy et al., 1996 (green) and *O. microlobata* Muricy et al., 1996 (brown). The consistency seems to be more constant within species, but it is also more subjective to describe. Finally, the aspect of the surface (smooth, microlobate) is also helpful, but one cannot separate all species on that basis alone. *Oscarella carmela* varies from light brown to tan or dull orange color, and from a smooth to microlobate surface; when out of the water, especially, the microlobes contract and the surface becomes smooth. The new species is similar in color and surface aspect only to *Oscarella microlobata*.

Because species of *Oscarella* have no skeleton, and histological characters are homogeneous among different species, less usual characters, such as the types of cells with inclusions present, are critical for species identification. Cells with inclusions are special sponge cells with different cytoplasmic inclusions, most of which have unknown functions (e.g., Simpson 1984; Muricy and Diaz 2002). They are abundant and diverse in species of *Oscarella*, and in the Mediterranean each species has a particular set of cells with inclusions that are useful characters for species identification (Muricy et al. 1996). It is, therefore, important that the specimens are fixed in glutaraldehyde, so they can be observed under TEM to identify cells with inclusions accurately. Cytological characters are also useful for the identification of other sponges without skeleton, such as *Halisarca* (*cf*. Vacelet and Donadey 1987). Molecular methods (e.g., allozyme electrophoresis, DNA sequencing) may also help in the definition of species boundaries in *Oscarella*.

The new species differs from *Oscarella microlobata* and all other Mediterranean *Oscarella* species in its cell contents, which are simple, with only two kinds of cells with inclusions: spherulous cells with two different inclusions, osmiophilic and filamentous, and vacuolar-spherulous cells (Fig. 4E-G). In contrast, *O. microlobata* has one type of vacuolar cells and three types of cells with inclusions, all different from those of *O. carmela*: spherulous cells with granular inclusions, spherulous cells with paracrystalline inclusions, and single-inclusion cells (Muricy et al. 1996). Besides being green, *O. viridis* differs from *O. carmela* by having two exclusive types of spherulous cells, called crescent-shaped cells and microgranular inclusions cells (Muricy et al. 1996). *Oscarella imperialis* is yellowish-white and has four types of cells with inclusions, two of which with different paracrystalline inclusions and two with large vacuoles with clear or osmiophiolic inclusions (Muricy et al. 1996); none of these cell types occur in *O. carmela*. *Oscarella tuberculata* is distinguished by the large groups of turgid vacuolar cells that fill most of the choanosome, and *O. lobularis* has two types of vacuolar cells, one of which is exclusive, and no spherulous cells (Boury-Esnault et al. 1992). The new species, therefore, has a set of cells with inclusions clearly distinct from those of all Mediterranean species of the genus, which are the only other species of *Oscarella* studied at TEM so far. *Oscarella carmela* is distinct also in its intertidal habitat: all known Mediterranean species of *Oscarella* occur in caves or in subtidal rocky shores (Boury-Esnault et al. 1992; Muricy et al. 1996).

Two other species of *Oscarella* were recently described from the Indo-Pacific (Bergquist and Kelly 2004). These specimens were fixed in 70% ethanol, and no attempt was made to characterize their cell composition. However, it is possible to distinguish both from the new species by their external characters: *Oscarella stillans* Bergquist and Kelly, 2004 forms a series of fused tubes up to 3.5 cm long, some with solid branches, and it is dark honey yellow in color. It also has a characteristically high collagen deposition in the mesohyl, giving it a collagenous consistency.

Oscarella nigraviolacea Bergquist and Kelly, 2004 differs from the new species by its dark violet, almost black color, and the oscules situated on top of papillae. According to Bergquist and Kelly (2004), other possibly valid species of *Oscarella* are *O. tenuis* Hentschel, 1909, *O. membranacea* Hentschel, 1909 (both from south Australia), and *O. ochreacea* Bergquist and Kelly, 2004 (*nomen novum* for *O. lobularis* sensu Vacelet et al. 1976) from Madagascar. However, these species must be better characterized before meaningful comparisons can be made.

Distinction from *Halisarca* and other sponges without skeletons.— With the finding of *Oscarella* along the California coast, there is now a need to distinguish *Halisarca* sp. from *O. carmela*, inasmuch as they belong to two superficially similar but unrelated sponge families. *Halisarca* sp. is the only tan colored sponge in the current standard keys for sponges in central California (Hartman 1975) and the northeast Pacific (Kozloff 1974) that has neither a collagen nor spicule skeleton. In these keys *Oscarella* would not be distinguished from *Halisarca*. The complete absence of a skeleton is also shared with *Chondrosia* Nardo, 1833, another common sponge genus, and three less common genera, *Hexadella* Topsent, 1896, *Bajalus* Lendenfeld, 1885, and *Pseudocorticium* Boury-Esnault et al., 1995. However, among these genera, only *Hexadella* is recorded for the West Coast of North America, and it is distinguished from *Halisarca sacra* and *O. carmela* by its bright yellow color when live (Kozloff 1996).

These genera can also be distinguished through examination of transverse sections to observe the shape and size of the canals, choanocyte chambers, and larvae. In *Oscarella*, the choanocyte chambers are ovoid to spherical, 30–60 μm in diameter, eurypilous, and arranged in a sylleibid organization (the chambers are regularly arranged around the exhalant canals); the larvae of *Oscarella* are incubated, hollow cinctoblastulae (Fig. 3H). Such larvae are typical of the family Plakinidae (including *Oscarella* and *Pseudocorticium*; Diaz and van Soest 1994; Muricy and Diaz 2002). In *Halisarca*, the choanocyte chambers are elongate, up to 280 μm long, tubular, ramified, with a large exhalant opening, and arranged in a leuconoid organization. The larvae are incubated, solid parenchymellae. In *Chondrosia*, the choanocyte chambers are spherical, 10–30 μm in diameter, diplodal, and arranged in a leuconoid organization. There is always a thick, easily recognizable collagenous cortex, absent in the other genera. *Hexadella* has a distinct collagenous ectosome, which provides some mechanical support in the absence of a skeleton. The choanosme is soft, with eurypilous, sac-shaped choanocyte chambers, approximately 60–80 μm in diameter. Its spherulous cells are typical of the Verongida (Bergquist and Cook 2002).

There are also differences in external morphology among these genera, but such traits are less reliable for identification: *Chondrosia* has a firm, cartilaginous consistency, and a smooth surface. Both *Oscarella* and *Halisarca* have a soft and fragile consistency, but *Halisarca* has an even, smooth surface in which superficial canals can be seen sometimes. The lobulated surfaces of *Oscarella* and *Hexadella* do not have superficial canals, although prominent excurrent canals can often be seen below the surface (Fig. 2F). Species of these genera vary in thickness. Those of *Oscarella* and *Hexadella* are usually thicker (5–30 mm thick) than species of *Halisarca* (usually less than 5 mm). On the other hand, our specimens of *O. carmela* were usually only 1 mm thick or less, and rarely thicker than 5 mm even when growing in quiet water in the laboratory. In contrast, W. C. Austin (unpublished manuscript, 2004) reports that specimens of *Halisarca* growing in quiet waters in British Columbia can be up to 1 cm thick (see also Goddard [1984, 2001]) for photographs of thick specimens of *Halisarca* in Oregon).

Status of *Halisarca* on the West Coast of the United States and Canada.— The only described species of *Halisarca* in the northeastern Pacific, *H. sacra*, is based on specimens collected by Ed Ricketts in 1929 from rocks resting on a sheltered mud flat just within the former mouth of Elkhorn Slough, Monterey Bay. However, there has been some question about the validity of the

species (Ristau 1977); Hartman (1975) changed its designation from *H. sacra* de Laubenfels, 1930 in Hartman and Smith (1954) to *Halisarca* sp. without comment. Smecher (2004) includes *H. sacra* in his website but considers it to be synonymous with *Halisarca dujardini* Johnston, 1842. He also reports that specimens are in the British Columbia Provincial Museum (Smecher 2004), but upon inquiry he replied that the museum does not have the facilities to do proper sections (C. Smecher, pers. commun.). Similarly, Austin (1985) includes *Halisarca sacra* in his checklist, as does Austin et al. (1999–2001) on their website which includes a photo, but the latter with the caveat that it may be synonymous with *H. dujardini* Johnson 1842. Austin (unpublished manuscript, 2004) describes specimens of *Halisarca* collected from southeast Alaska, British Columbia, and northwest Washington and sectioned to reveal the tubular choanocyte chambers, and suggests that they are synonymous with *H. sacra* de Laubenfels, 1930. Goddard (1981) acknowledged P. Bergquist and W. Hartman for identification from prepared histological sections of *Halisarca* from Oregon, but they refrained from giving a specific name.

There are no confirmed records of *Halisarca* in California except for the original collections in Elkhorn Slough, the type locality of *H. sacra* de Laubenfels, 1930 (de Laubenfels, 1932) (types in the National Museum of Natural History, K. Rützler, pers. commun., 2004). MacGinitie (1935) mentions the species in his classic monograph on Elkhorn Slough, but apparently only on the basis of the original description; it is not mentioned at all in MacGinitie and MacGinitie (1949). In their survey of Elkhorn Slough, Nybakken et al. (1977) list *H. sacra* only because it was mentioned in MacGinitie (1935), and they did not find it themselves (J. Nybakken, pers. commun., 2004). Similarly, Ricketts and Calvin (1939) reported that *H. sacra* is known to occur in Elkhorn Slough, apparently on the basis of the original collection and description, and that report is retained unchanged in later editions of their book (Ricketts et al. 1985). The genus is not included in Bakus and Abbott's (1980) account of Californian intertidal sponges, Thompson et al.'s (1985) list of sponges from San Diego, California, or Bakus and Green's (1987) account of sponges in the Southern California Bight.

On the other hand, the nudibranch *Hallaxa chani*, which apparently feeds exclusively on *Halisarca* sp., (Goddard 1981, 1984, 2001), ranges from southeast Alaska to central California (Gosliner and Johnson 1994) and into the Channel Islands (Goddard, pers. commun., 2004). Consequently, *Halisarca* sp. probably also occurs in California but, if so, it would likely be seen rarely because it is so obscure and perhaps kept at low densities by predation by *H. chani* and a second nudibranch predator, *Cadlina modesta* (Goddard 1984, 2001). The Santa Barbara Museum of Natural History has two ethanol-preserved specimens collected from southern California that were identified by R. Given as *H. sacra* (SBMNH 353403, Moray Reef, Corona del Mar, Orange County, 50 ft, 26 March 1958; SBMNH 353402, Scorpion Anchorage, Santa Cruz Island, Santa Barbara County, 30–40 ft, 24 March 1963). These specimens were examined by one of us (JSP). Although histological sections were not made to reveal the choanocyte chambers, thick sections indicated that the specimens are definitely not *O. carmela,* based on thickness, surface texture, distinct stiff ectosome, thick collenchymatous choanosome, and large excurrent canals. They almost certainly are specimens of *Halisarca,* or perhaps of *Hexadella.*

Halisarca sp. is included in species lists prepared in the early 1970s during undergraduate student surveys at Point Año Nuevo and Pigeon Point, north of Monterey Bay (Pearse, 1980; unpublished). However, no vouchers were retained and because the keys used then (a draft of Hartman's 1975 keys) would not distinguish between *Halisarca* sp. and *O. carmela,* the identity of those records is uncertain. Similarly, using available keys, JSP identified skeleton-less sponges as *Halisarca* that were collected in the early 1970s at the same site at Carmel Point where *O. carmela* is now found (unpubl. obs.). It is likely that those specimens were *O. carmela,* which would sug-

gest that the species has been present at the type locality at least since the early 1970s. In support of this continuous presence, M. C. Diaz, who identified *O. carmela* growing at Long Marine Laboratory, noted it to be present at Carmel Point during a field trip in 1993 (K. Wasson, pers. commun., 2004). On the other hand, an undergraduate student survey of the sponges at Carmel Point in 1993 did not list *O. carmela* (or *Halisarca* sp.) (JSP, unpublished list), so it is rare and/or easily overlooked even at the type locality. Because of its slimy, non-sponge appearance, which could be confused with encrusting ascidians, and its unusual habitat in the high intertidal, it would not be difficult for students to miss.

Distribution in the field and aquariums.— The spatial distribution of *O. carmela* is puzzling. Despite extensive searching in the vicinity of the Monterey Peninsula of central California, small, scattered individuals were found in only a few localities in the high intertidal zone. It was not abundant anywhere, and there was no evident pattern in the four sites where it was found in this study. On the other hand, it is difficult to find in the field, even when a search image is established. Thin sheets of encrusting ascidians (e.g., *Aplidium* spp., *Diplosoma listerianum*) and sponges (e.g., *Zygherpe hyaloderma, Astylinifer arndti*) were misidentified as *O. carmela* in the field by JSP; only inspection of specimens with a microscope revealed the error. Nonetheless, once noticed, it is quite conspicuous and hard to miss. Outstanding intertidal biologists (e.g., Ed Ricketts, Don Abbott) collected specimens in the intertidal of the Monterey Peninsula for most of the 20th century. Inasmuch as *O. carmela* is rather unattractive and usually occurs as small, slimy colonies adhering to large boulders, perhaps after de Laubenfels'1932 monograph was published, experienced intertidal biologists simply field identified it as *Halisarca sacra* and then went on to other things. Moreover, they could have been discouraged because of the difficulty in identifying slimy sponges. For example, in reference to *H. sacra*, Ricketts and Calvin (1939:176) wrote: "Since there are various additional species not mentioned, which, on the basis of field characters alone, could be confused with the above, and with previously treated species, the value of careful laboratory work should be kept in mind."

Another possibility is that *Oscarella carmela* normally occurs in deeper water, or in caves and hard-to-reach places, as do other species of *Oscarella* (Muricy and Diaz 2002). After all, it almost always occurs submerged in pools in the intertidal and does well in dimly lit aquariums. However, since being alerted about its appearance, two observant diving biologists (Anthony Draeger and Scott Nichols) have searched for it subtidally on the Monterey Peninsula without success.

Although rarely encountered in the field, and then only as small individuals, *Oscarella carmela* often thrives in aquariums, exhibit tanks, and sea tables in laboratories and public aquariums. This difference in abundance between the field and on-shore facilities indicates that there are factors operating to limit the sponge's establishment and growth in the field. Perhaps it is preyed upon heavily in the field by some unknown predator, which severely limits its growth subtidally and leads to it occurring mainly as small refuge populations under boulders in high intertidal pools. That appears to be the case for *Halisarca* sp. in the eastern Pacific (Goddard 1981, 1984, 2001), which is also rarely encountered, especially in California.

The source of the specimens of *Oscarella carmela* in aquariums continues to be unknown. It has been found in three on-shore facilities: (1) Long Marine Laboratory–Seymour Marine Discovery Center complex, (2) Monterey Bay Aquarium, and (3) Bodega Marine Laboratory. These are completely separate facilities, and it is unlikely that material was transferred among them. Moreover, Hopkins Marine Station receives all its running seawater from the Monterey Bay Aquarium, and *O. carmela* has not been found in any of the aquariums there. Furthermore, the sponge has not been found in the intertidal near two of the facilities when those habitats were searched. Consequently, the source is probably not via seawater being brought into the facilities.

Rather, small specimens probably are brought into the facilities on rocks and other material collected from the intertidal. There is also no evidence that the species has escaped from the facilities to colonize nearby intertidal areas.

Temporal dynamics.— It is not known whether the colonies of *Oscarella carmela* are new to Carmel Point and other sites around the Monterey Peninsula, signaling an environmental change, or were simply overlooked in the past. If specimens keyed as *Halisarca* from the same area at Carmel Point in the early 1970s by JSP were, in fact, *O. carmela*, then they have probably been there for a long time. They almost certainly were present since 1993, when M.C. Diaz saw them there (K. Wasson, pers. commun.).

After specimens of *Oscarella carmela* were recognized in high zone pools at Carmel Point in September 2002 by JSP, largely because they looked similar to specimens at Long Marine Laboratory, they were found there on each of nine subsequent visits, extending from November 2002 to April 2004. Specific individuals were not followed, mainly because the boulders were similar in appearance and appeared to be rearranged between visits by storms. In January 2003, after a major winter storm, the specimens were noticeably thin, and in the spring (April and May) they appeared to be particularly robust, as is the case for many intertidal organisms of the northeast Pacific (Foster et al. 1988). The abundance of *O. carmela* in the Monterey Bay Aquarium was somewhat seasonal too, with an increase in spring months and a die-off during winter (Veronica Franklin, pers. commun.). A similar pattern is found in calcareous sponges in Rio de Janeiro state, Brazil, belonging to the genera *Clathrina* and *Paraleucilla* (Michelle Klautau, pers. commun., 2003).

Indigenous or invasive?.— *Oscarella carmela* has been known for a long time in labs and local public aquariums before being noticed in the field, a pattern suggestive of an invasion. If this sponge were similar to any described species of *Oscarella* from other region, it could be concluded that it has invaded central California recently. Instead, the species has proved to be new, and it now seems likely that it is reasonably widespread at least between central California and British Columbia, thus suggesting that it is indigenous. This conclusion is consistent with the finding that nearly all introduced marine species in California are found in estuaries and harbors, and only a subset of these occur on the open coast (Wasson et. al., in press). Specimens of *O. carmela* have not been found in estuaries of central California that are populated by a high number of introduced species (San Francisco Bay, Andy Cohen and Jim Carlton, pers. commun.; Elkhorn Slough, Wasson et al., in press).

On the other hand, the taxonomy of the genus *Oscarella* is so poorly known worldwide that it is nearly impossible to know if it is really indigenous or introduced. Although we believe that there is a high probability that the new species is indigenous of this area, we cannot completely discard the possibility of an invasion from an unknown population of an undescribed species that originated elsewhere. There are no previous records of the genus *Oscarella* along the Pacific coast of North America, but this may be due to rarity and difficulty in identifying these species. The temporal dynamics of *O. carmela* shows high seasonal and pluri-annual fluctuations. Therefore, it is likely that, in this case, there has been an increase of abundance at a few localities of a previously rare, resident species, rather than an invasion.

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