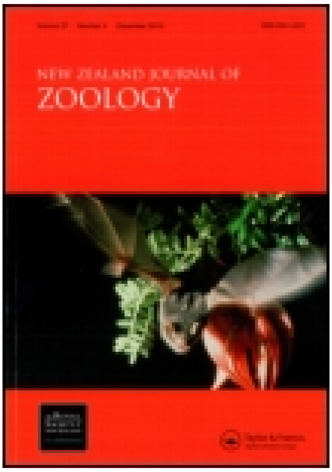

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Outbreak of a neurotoxic side-gilled sea slug (Pleurobranchaea sp.) in Argentinian coasts

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SHORT COMMUNICATION

Outbreak of a neurotoxic side-gilled sea slug (*Pleurobranchaea* sp.) in Argentinian coasts

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Since 2009 we have been registering the outbreak of an unknown side-gilled sea slug of the genus *Pleurobranchaea* in coastal waters of Argentina, southwestern Atlantic Ocean. Examination of both external macroscopic features and internal structures via electron microscopy did not allow us to conclusively identify the species. Nevertheless, since Argentinian specimens closely resemble *Pleurobranchaea maculata* (a species native from Australia and New Zealand known to contain a potent neurotoxin) we performed a bioassay in our specimens, which was positive for neurotoxins. The presence of this pleurobranch in Argentinian waters is of concern beyond the ecological effects of the outbreak of a marine predator and requires urgent attention. This work provides a guide to distinguish between this unidentified species and the sympatric cryptogenic *Pleurobranchaea inconspicua*, based on features that are readily observable in fresh and preserved specimens. We discuss the potential effects of this outbreak on local communities.

Keywords: Argentina; Buenos Aires; gastropoda; marine neurotoxins; population outbreak; southwestern Atlantic

Introduction

Interspecific differentiation among side-gilled sea slugs of genus *Pleurobranchaea* is problematic because of their high morphological similarity. All members of *Pleurobranchaea* are medium to large sea slugs, pale grey to brownish in colour. The dorsum has a reticulate pattern. All are active hunters that prey on a wide variety of animals, though in some cases they have been described as having a preference for sea anemones (Ottaway 1977; Willan 1984; Cattaneo-Vietti et al. 1993). Recently, high levels of tetrodotoxin (TTX) were found in adults, eggs and early larval stages of *Pleurobranchaea maculata* (McNabb et al. 2010), though concentration varies greatly across individuals, seasons and populations (Wood et al. 2012). TTX is an extremely potent neurotoxin that inhibits the propagation of action potentials in muscle and nerve cells; the ingestion of just 1–2 mg of TTX can cause death in adult humans (Noguchi & Arakawa 2008).

Although the genus has a worldwide distribution in coastal waters, until now the only species recorded on the Atlantic coast of South America is *Pleurobranchaea inconspicua* (Muniain et al. 2006). This species is reported to have an amphi-Atlantic distribution, but in the western Atlantic has been recorded from Cape Hatteras, North Carolina 35°N, 80°W to Golfo San José 42°S, 64°W (Argentina).

We have been routinely sampling the benthic fauna within the port of Mar del Plata, Argentina

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(38° 04 S; 57° 53 W), for over a decade and found, in spring 2009, an unknown large side-gilled sea slug (Farias 2009). After careful examination we rejected *P. inconspicua* as the species identity and tried to determine the actual identity by means of classic taxonomy using external and internal anatomic features. Since the first finding in 2009, we registered an outbreak of this species in a wide geographical range, which continues to date. In the context of a broader study, here we advance the presence of a stable population of this new side-gilled sea slug in Argentinian waters, its potential identity based on morphological features, and the finding of potent neurotoxins contained in its tissues.

Materials and methods

Collection of specimens

Specimens were collected by hand during routine SCUBA surveys from 2009 to date, and during the cruise 'Litoral Bonaerense 2014' of the R/V *Puerto Deseado* using a bottom trawl net. Animals were photographed and transported alive to the laboratory, where they were cryo-anaesthetised and then processed according to the requirements of the different analyses. For further genetic analysis, some individuals were fixed directly in 96% ethanol and stored. For observations of the external morphology, individuals were fixed in formalin for 48 h and then transferred to 70% ethanol for long-term storage in the collection of the Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina, catalogue number MACN-In: 39571. For the examination of internal anatomy, some individuals were dissected and the reproductive system, jaws and radulae were removed. All parts were dehydrated through an alcohol series and then dried in hexamethyldisilazane, mounted on aluminium discs, and coated with gold–palladium for scanning with an electron microscope JEOL, JSM-6460 LV. Species identification was attempted first by comparing our specimens with the detailed descriptions of the external and internal anatomy of *P. inconspicua* in Muniain et al. (2006), and then following Marcus & Gosliner (1984).

Toxicity testing

To test whether individuals from Mar del Plata contain neurotoxic compounds, we performed a mouse bioassay following Anon. (2005). Nine individuals were processed, obtaining 40 g of homogenised tissue. The homogenate was mixed with 40 mL 0.1 M hydrochloric acid, the pH was adjusted to < 4.0 and the mixture was boiled gently for 5 min before cooling, reaching a final pH of 3.4. The mixture was centrifuged and 1 mL of the supernatant (acidic extract) was injected intraperitoneally into albino CF1 strain mice, weight range 19–21 g, in triplicate. To determine a relative value of toxicity we calculated the respective Mouse Units (MU)/100 g of tissue and converted it to µg saxitoxin eq./g, using the Sommer's table with a conversion factor of 0.19.

Results

Collection of specimens

Specimens were first found within the Mar del Plata Port (38°04S, 57°53W), but recently NEF, on board of the R/V *Puerto Deseado*, has added new records that extend their distribution up to 60 m depth within the Buenos Aires coastal area. In addition we have recently confirmed records as far south as Puerto Madryn, Chubut (42°50′S, 65° 05′W) approximately 1200 km along the shoreline from the first finding.

Species identification

A first anatomical comparison eliminated *P. inconspicua* (Muniain et al. 2006). Externally, *P. inconspicua* has a brown reticulate colour pattern with white spots scattered all over the mantle, but more conspicuous on the dorsal foot surface, while our specimens had a mottled pattern of various shades of brown and cream without clearly distinguishable white spots (see Fig. 1). On the posterior dorsal part of the foot *P. inconspicua* has a brown median line and a tiny dark hooked spur, called a caudal spur, both of which were lacking in our specimens. The absence of a penial stylet also contributed to excluding *P. inconspicua*.

Further examination of the external body features, buccal pieces and reproductive system (the

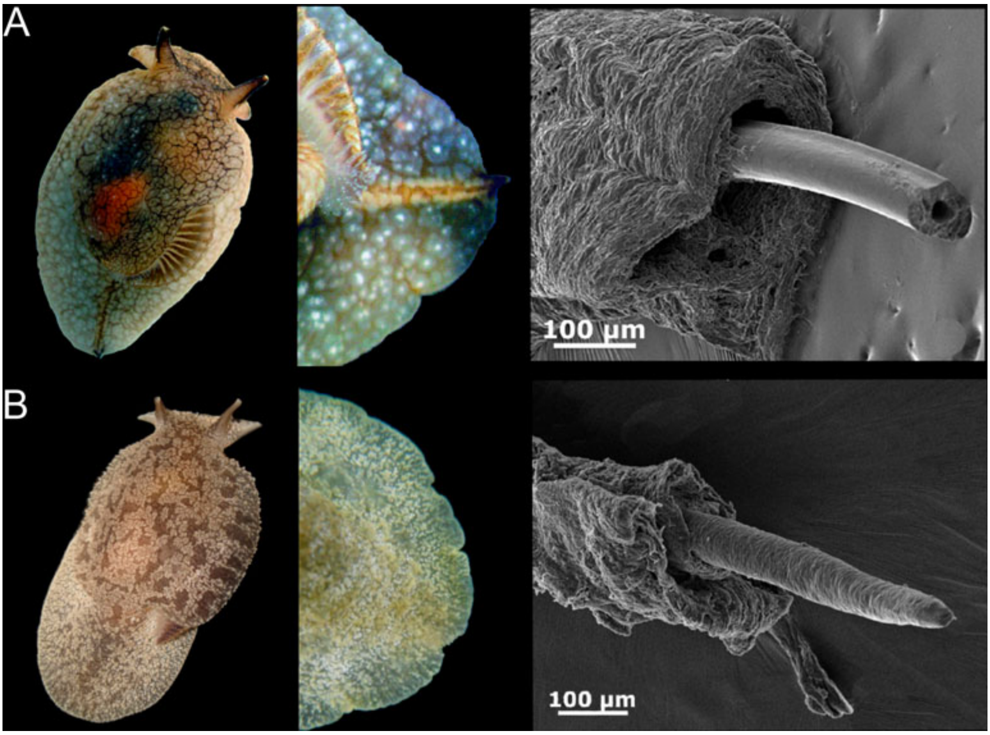

Figure 1 Morphological differences used to distinguish between **A,** *Pleurobranchaea inconspicua* and **B,** *Pleurobranchaea* sp.. Images of *P. inconspicua* are from Muniain et al. (2006). Images of *Pleurobranchaea* sp. are from the first individual found in Mar del Plata; total length: 93 mm. From left to right: full individual, dorsal view; detail of the caudal portion of the foot (note the absence of caudal spur in *Pleurobranchaea* sp., which is clearly present in *P. inconspicua*); electron microscopy of a cross-section of the penis showing the chitinous stylet present in *P. incospicua* but absent in *Pleurobranchaea* sp.

last two using electron microscopy) showed that the species closely resembles *P. maculata*. We reached this conclusion in two steps, using the key provided in Marcus & Gosliner (1984). First, given that the radular teeth are bicuspid, with the secondary cusp of each tooth well developed (Fig. 2) we confirmed that the species is a *Pleurobranchaea* other than *Pleurobranchaea californica*. Second, we verified the presence of a soft penis with no cuticle structures inside (i.e. no penial stylet, see Fig. 1), a 'short' vagina, and a penial sac developed but narrow.

Toxicity testing

After intraperitoneal injection with 1 mL of the acidic extract, the mice died rapidly (in less than 10 min), showing neurological symptoms compatible with paralytic shellfish poisoning, and so confirming the presence of neurotoxins in our samples. The calculated toxicity was equivalent to 1.12 µg saxitoxin eq./g of tissue.

Discussion

Since the first finding in September 2009, adult individuals and their ovipositions (loosely coiled mucous egg strings) have become increasingly abundant in the area and therefore this pleurobranch should be already considered a resident species of Mar del Plata, adding to the growing list of marine species reported for the first time in Argentinian waters during the last two decades (Orensanz et al. 2002). As is probably the case here, most of these

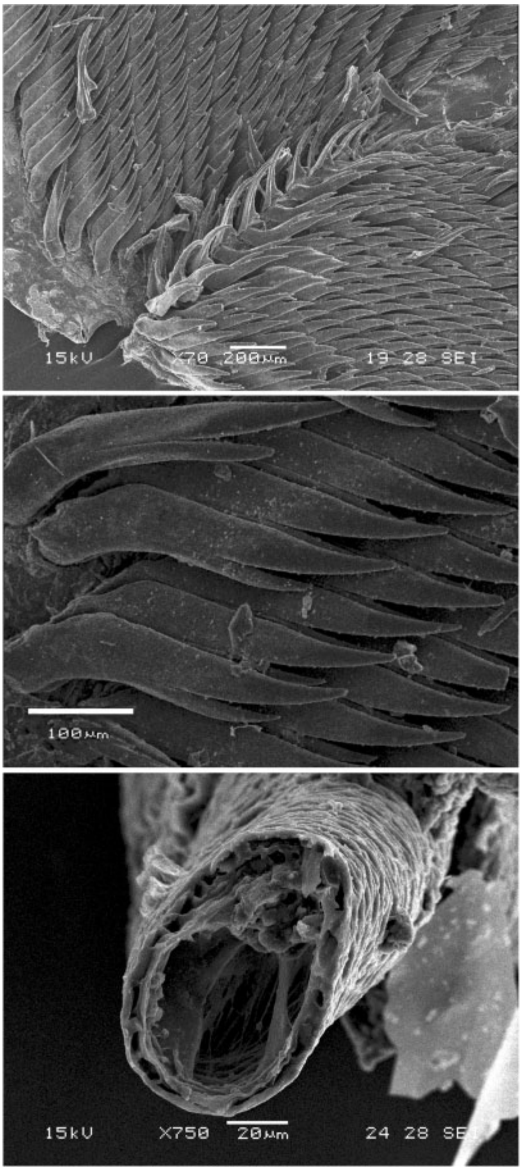

Figure 2 Electron microscopy of diagnostic features used to identify specimens found in Mar del Plata, Argentina, as *Pleurobranchaea* sp. **A,** Radula showing the medial line of bicuspid teeth; **B,** close-up of teeth showing the well-developed secondary teeth in more detail; **C,** close-up of the cross-section of the penis tip showing the lack of chitinous structures.

new species are invasive species, native from Indo-Pacific waters, such as the golden mussel *Limnoperna fortunei* (Darrigran & Pastorino 1995), the oyster *Ostrea japonica* (Orensanz et al. 2002), the spider crab *Pyromaia tuberculata* (Schejter et al. 2002), the kelp *Undaria pinnatifida* (Casas et al. 2004), and the Asian whelk *Rapana venosa* (Giberto et al. 2006).

The extent to which *Pleurobranchaea* sp. has spread along the coast is uncertain given the lack of systematic surveys in shallow waters off the coast of Argentina. To date we registered this species as far south as Puerto Madryn, Chubut (42°50′S, 65°05′W), and in the coasts of Buenos Aires up to 60 m depth. There are also findings in Golfo San Matias (40°44′S, 64°57′W) but this needs further confirmation.

The presence of this new species has many potential effects on local communities. Pleurobranchs are voracious predators (Willan 1984), so that outbreaks like that reported here often have a direct negative effect on local prey species. In New Zealand, these sea slugs restrict the vertical distribution and abundance of the anemone *Anthothoe albocincta* through predation (Ottaway 1977). Within the Mar del Plata port there is a stable population of a closely related anemone, *Anthothoe chilensis* (Excoffon et al. 1999), which represents a potential prey.

Competitive interactions with local predators may also be important. Possibly, *Pleurobranchaea* sp. is already competing with the cryptogenic *P. inconspicua*. However, we did not find any *P. inconspicua* in the area with which to perform the necessary experiments. Although the virtual absence of *P. inconspicua* might suggest competitive exclusion, specimens of *P. inconspicua* described from Argentina (Muniain et al. 2006) were collected more than 100 km from Mar del Plata and we do not know if there was a stable population of *P. inconspicua* in the port of Mar del Plata during the past years. However, potential competitive interactions between these two species of sea slugs deserve further study because of their similarity and the overlapping distribution ranges. The sea star *Asterina stellifera* is another species for special consideration as it also consumes anemones within the port (Farias et al. 2012). During the last decade this sea star has been declining steadily along the entire distribution, with the port as the only location where high densities are still reported. Therefore competitive interactions with *Pleurobranchaea* sp. may pose a threat to this species. This recent outbreak adds to recent invasions that already have a great impact in the coastal waters of Mar del Plata, such as the arrival of the invasive kelp *Undaria pinnatifida* (Meretta et al. 2012). It is difficult to predict if *Pleurobranchaea* sp. will develop into a major disturbance in an already highly modified ecosystem (Orensanz et al. 2002) but its recent outbreak underscores the need for monitoring the shallow water ecosystems along the coast of Buenos Aires province, where Argentina's principal ports are located.

Although we confirmed the presence of potent neurotoxins in specimens from Mar del Plata, we cannot say whether TTX is the actual cause of such toxicity as was reported for New Zealand populations of *P. maculata*. To confirm this, further analyses (such as high-performance liquid chromatography or liquid chromatography-mass spectrometry) must be performed.

It is clear that the species reported here is not *P. inconspicua*, and the descriptions and pictures of living animals that we provide permit a rapid distinction of the two sympatric pleurobranchs currently present in Argentinian waters by the simple observation of the external features. Unfortunately, species of *Pleurobranchaea* are not that easy to identify without detailed study of the internal anatomy, and even with such studies the identification can be difficult or even impossible, as exemplified by this very work. The morphological features studied here resemble those of *P. maculata*, but other congeners as *Pleurobranchaea tarda* or *Pleurobranchaea bubala* (distributed in the Atlantic coast of North America and the west coast of Africa, and in the southern tip of South Africa, respectively) cannot be ruled out and probably a definite identification will require the aid of DNA analysis.

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