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ORIGINAL PAPER

First record of the sea anemone *Diadumene lineata* (Verrill 1871) associated to *Spartina alterniflora* roots and stems, in marshes at the Bahia Blanca estuary, Argentina

Lucas M. Molina · Macarena S. Valiñas · Paula D. Pratolongo · Rodolfo Elias · Gerardo M. E. Perillo

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Abstract We report the occurrence of the orange-striped green anemone *Diadumene lineata* (Verrill 1871) (=*Haliplanella lineata*) in salt marshes at the Bahía Blanca Estuary for the first time in August 2005. We also found this species attached to roots and stems of *Spartina alterniflora*, an association that has never been registered before. After their determination, sampling was performed during a year to evaluate seasonal abundance of this sea anemone. Results showed that *D. lineata* was present through the whole year, indicating the existence of a stable population. All individuals sampled were found attached to roots or stems of *S. alterniflora*, with the higher abundances detected in summer. Further studies are necessary to precise the potential effects of this exotic sea anemone on salt marsh communities.

Keywords *Diadumene lineata* · *Haliplanella* · Sea anemone · *Spartina alterniflora* · Salt marshes · Bahía Blanca estuary

L. M. Molina (✉) · P. D. Pratolongo · G. M. E. Perillo
Instituto Argentino de Oceanografía, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CC 804, B8000FWB, Bahia Blanca, Argentina
e-mail: lmmolina@criba.edu.ar

M. S. Valiñas
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

M. S. Valiñas
Laboratorio de Ecología, Depto. de Biología (FCEyN), Universidad Nacional de Mar del Plata, CC 573 Correo Central, B7600WAG, Mar del Plata, Argentina

P. D. Pratolongo
Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, 8000 Bahia Blanca, Argentina

R. Elias
Departamento de Ciencias Marinas, Universidad Nacional de Mar del Plata, Deán Funes 3350, B 76002 AYL, Mar del Plata, Argentina

G. M. E. Perillo
Departamento de Geología, Universidad Nacional del Sur, San Juan 670, 8000 Bahia Blanca, Argentina

Introduction

The orange-striped green anemone, *Diadumene lineata* (Verrill 1871) (=*Haliplanella lineata*) has been reported as indigenous to the coasts of Japan (Mc Murrich 1921; Stephenson 1935; Fuller 2007). However, the occurrence of this species has also been registered worldwide. Some of the records include coastal Indonesia and New Zealand (Dunn 1982; Fuller 2007), the Pacific coast of North America, from southern California to British Columbia (Stephenson 1935; Carlton 1979 in Ting and Geller 2000; Cohen and Carlton 1995; Ruiz et al. 2000; Fuller 2007), the Atlantic coast of North America, from Florida to the Bay of Fundy (Linkletter 1977; Ting and Geller 2000), Hawaii (Coles et al. 1999; Zabin et al. 2004), the Mediterranean (Birkemeyer 1996; Cohen 2005), and North-western Europe (Walton 1908; Gollasch and Riemann-Zürneck 1996; Faasse 1996, 1997; Wolff 2005). In South America, *D. lineata* has only been reported in Brazil (Da Costa-Belem and da Cruz Monteiro 1977), and a few individuals were found in the San Matias Gulf, Argentina (Excoffon et al. 2004), about 500 km south of Bahia Blanca Estuary.

Within its native range, populations of *D. lineata* consist of colorful, relatively large, and sexually active individuals (Riemann-Züerneck 1998). However, in most exotic populations the commonly found specimens are smaller, and sexual reproduction has not been observed. In this later case, longitudinal fission and occasionally pedal laceration appear to be the sole means of propagation, resulting in a large number of tiny anemones within a relatively short time (Davis 1919; Johnson and Shick 1977; Schick and Lamb 1977; Minasian 1979; Ting and Geller 2000).

Gollasch and Riemann-Zürneck (1996) found that *D. lineata* can propagate along shipping lanes since as adults they are able to travel in fouling assemblages attached to the hulls of ships. There may be also some transport of adults on drifting fragments of eelgrass or seaweed (Cohen 2005). However, since this species rarely reproduces sexually outside its native range, the carrying of adults in hull fouling and asexual reproduction may be major mechanisms for dispersion, while larval transport in currents or in ballast water is unlikely to occur (Cohen 2005). These later observations suggest two different stages in the life cycle of the anemone, with the sexually active phase restricted to the relative narrow range of conditions of its native range (Fukui 1991), but a migrating stage with wide tolerance across environmental gradients (Riemann-Zürneck 1998).

Individuals of *D. lineata* are commonly associated to firm surfaces in shallow subtidal waters, including shallowly-buried rocks in mudflats (Cohen 2005). Although associations with mussels, oysters and debris are often observed on mudflats and marsh channels (Fautin and Hand 2007), to the best of our knowledge the association of this anemone to marsh macrophytes has never been reported before. In the present study we document the presence of *D. lineata* in salt marshes at the Bahia Blanca Estuary, Argentina, and its association to stems and roots of the native marsh macrophyte *Spartina alterniflora*. In addition, we provide the first estimates of density through a complete annual cycle, as an approach to assess the prevalence of this non-native species in the study area.

Materials and methods

Study area

The Bahia Blanca Estuary is a wide coastal wetland complex in temperate South America, comprising a total surface of 2,300 km^2 which included about 410 km^2 of marshes and more than 1,150 km^2 of mudflats (Perillo et al. 2000; Isacch et al. 2006). Mean tidal range varies from 2 m at the mouth to 3.8 m at the middle and upper reaches, and salinity typically increases from the mouth to the head, where the restricted circulation and the high evaporation may produce concentrations larger than 38 psu (Piccolo and Perillo 1990).

Spartina alterniflora is a widespread marsh macrophyte, commonly dominating intertidal wetlands on temperate Atlantic coasts of the American continents. In North America, wide pure stands of *S. alterniflora* can be found from the Gulf of Saint Lawrence to the Gulf of Mexico. In South America, the natural range of the species extends between 10° and 40° S (Mobberley 1956), with the greatest expression along the humid temperate coasts of northern Argentina. At the Bahía Blanca Estuary, located in the northern limit of the Patagonian desert, vegetation in the intertidal zone is scarce and, unlike the better known counterparts of Eastern North America, *S. alterniflora* marshes only occur in discontinuous patches at the mouth of the estuary. Under the seasonally hypersaline conditions in the inner estuary, vegetation is virtually absent in the intertidal zone except for the circular mounds of *Sarcocornia perennis,* colonizing the upper marshes (Perillo and Iribarne 2003).

Within the study area, major transformations relate to the presence of the largest deepwater harbor system in Argentina, that comprises Ingeniero White Port and a series of subsidiary harbors related to a petrochemical industrial park, and a naval base (Perillo and Sequeira 1989). The specific site where *D. lineata* was found corresponds to a *S. alterniflora* marsh of about 30 ha located 15 km southeast of Ingeniero White Port, and just 4 km northwest of the Puerto Belgrano Naval Base (Fig. 1). Substrate is composed of a mixture of sand and mud, with extensive bare flats occupying most of the intertidal fringe. Vegetation is restricted to the upper intertidal zone, with plant densities varying from 100 to more than 300 ramets m^{-2}, according to a strong seasonal pattern (Molina pers. obs.).

Field sampling and data analysis

In August 2005 we found a few isolated sea anemones in sediment samples, during a field survey intended to determine root biomass of *S. alterniflora* in the upper intertidal zone of the salt marsh. To identify and perform a detailed description of this animal, 30 individuals were collected from the study area and carried to the laboratory. The collected sea anemones were maintained in aquarium and fed with newly hatched *Artemia persimilis* nauplii. Taxonomic determination at the species level was made following Fautin and Hand (2007, pp. 134–145). Individuals were observed under a Nikon SM-Z1500 binocular microscope (2×) and live sea anemones were photographed using a Nikon DXM1200F digital videocamera attached to the microscope.

After identification of the specimens and given the unusual association between anemones and *S. alterniflora* roots and stems in the upper intertidal zone, we carried out a systematic seasonal sampling, through a complete year (from spring 2005, to winter 2006), to test the permanence of this species, and seasonal patterns of abundance. At each season six PVC cores of sediment (10 cm in diameter and 10 cm in depth) were taken from the root zone of the salt marsh. Sediment and plants were washed and sieved through a 500 μm mesh screen. Specimens of sea anemones retained were preserved in 5% neutralized formaldehyde, and were counted. Differences in

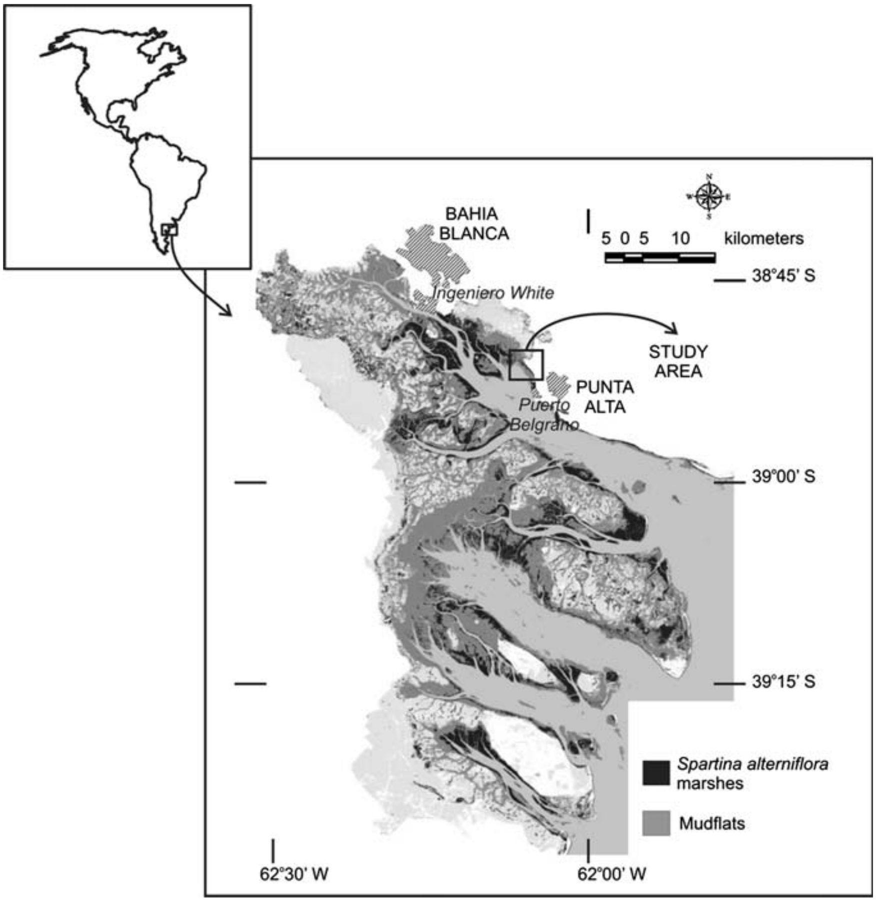

Fig. 1 Location of the study area in the Bahia Blanca Estuary. Main harbors are shown, as well as the area covered by *Spartina alterniflora* marshes and mudflats

seasonal abundances were compared with one-way ANOVA (Zar 1999). When ANOVA assumptions were not met, transformations following Underwood (1997) were applied. When differences in abundance were found, multiple comparison Tukey tests were used (Zar 1999). Additional information on the air and water temperatures, precipitation, and air humidity over the sampling period was obtained from the weather station *Puerto Rosales*, located about 8 km south from the study area.

Results and discussion

Given the diagnostic characters stated by Stephenson (1935, pp. 197–207), and further descriptions by Hand (1989, pp. 85–94), the sea anemone found associated at roots and stems of *S. alterniflora* in Bahía Blanca Estuary, could be identified as the orange striped green anemone, *Diadumene lineata* (Verrill 1871) (Figs. 2, 3), according to Fautin and Hand (2007). The observed individuals presented a column clearly divided into scapus and capitulum, with a permanent parapet and fosse, and generally showed between 50 and 100 slender, often very long tentacles. The typical color of the scapus was olive green or brownish, with vertical orange stripes, occasionally yellow or white. The capitulum, disc and tentacles were translucent grey or greenish, occasionally dotted in white (Fig. 3a). In live sea anemones, we observed the presence of acontia, readily emitted through mouth and cinclides (Fig. 3b), when the animal is disturbed. Measurements performed on the pedal disc of anemones maintained in aquarium gave a diameter of 6 ± 2 mm (mean ± standard deviation, $N = 30$).

Individuals of *D. lineata* were always present since 2005, when the species was first registered in the study area. The sea anemone was observed at every visit to the field, and seasonal sampling showed significantly higher abundances in summer and lower in winter and autumn. There were no differences in abundance between autumn and spring ($df = 3$, $F = 6.13$, $P < 0.01$, Fig. 4).

Because of their settlement in the upper intertidal zone, anemones are subjected to periods of air exposure larger than 6 h twice a day. Given the very high evaporation rates on typically hot and dry summer days in Bahía Blanca (Fig. 5), the larger abundances suggest that some mechanism to avoid desiccation may be acting. Although at lower densities, individuals of this species were also found in winter samples, collected under extremely cold conditions, with air temperature records as low as −5°C.

Former records of this species indicate that *D. lineata* is an extremely euryhaline and eurythermal species (Shick and Lamb 1977; Ting and Geller 2000), commonly found in fouling communities, attached to hard substrates like pilings or floats, shells, or rocks and debris on the mud surface of

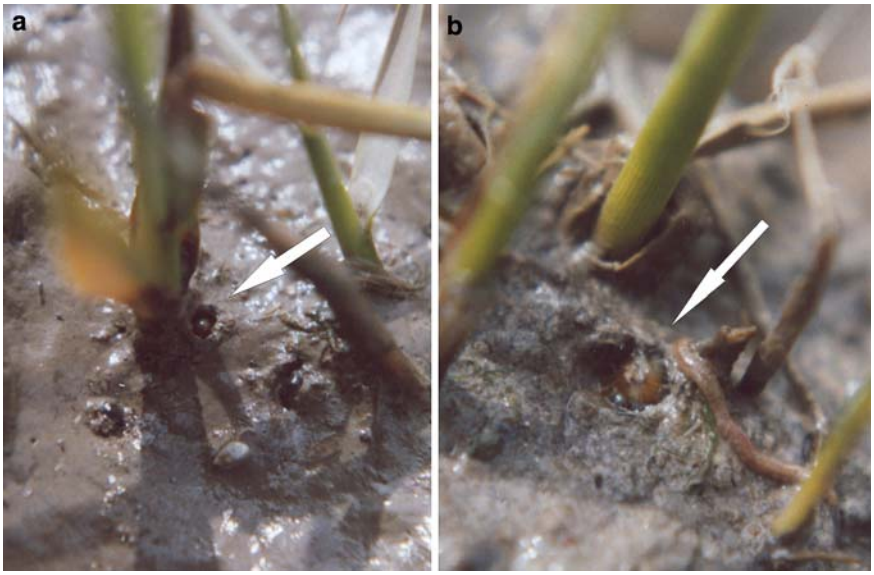

Fig. 2 (**a**) Individuals of the orange striped green anemone (*Diadumene lineata*) found in Bahia Blanca as observed in the field, attached to *Spartina alterniflora* roots. (**b**) Detail

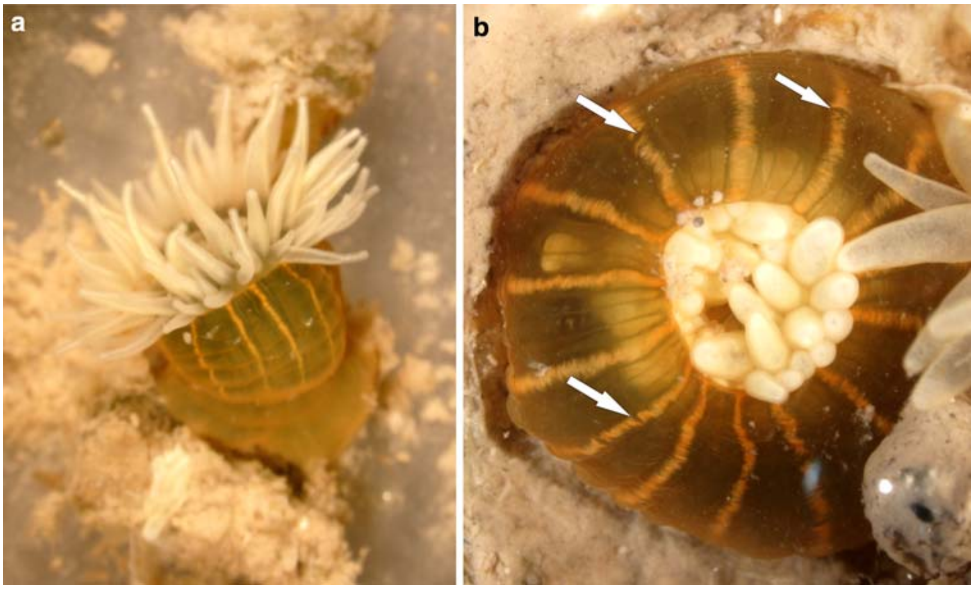

Fig. 3 (**a**) Live individuals of *Diadumene lineata* in aquarium observed under binocular microscope (2×). Note the white tentacles and orange stripes, (**b**) detail of the cinclides indicated by white arrows

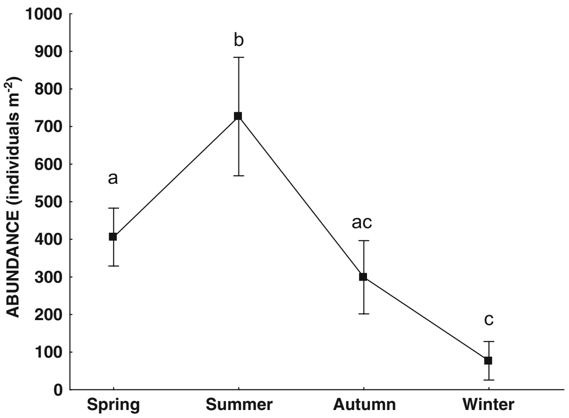

Fig. 4 Seasonal variation in abundance (individuals m^{-2}) of the sea anemone *Diadumene lineata* within the root zone of *Spartina alterniflora* salt marsh (mean ± standard error). Different letters indicate significant differences between seasons (Tukey post hoc comparison, $P < 0.01$)

mudflats and marsh channels (Morris et al. 1980; Cohen 2005; Fautin and Hand 2007). At some places, their abundance is thought to be limited by the availability of hard substrate (Cohen 2005). In this case, we found the sea anemone associated to marsh macrophytes, which not only provide hard substrate, but also are proven to greatly modify the physical and biological environment.

By shading the substrate, plant canopies can locally lower soil temperatures preventing desiccation. Stems and leaves attenuate currents and wave energy (Leonard and Luther 1995; Nepf et al. 1997; Nepf and Koch 1999), and physiological processes change geochemical balances, modifying the species composition of planktonic and benthic communities within the marsh (Declerck et al. 2007). There is a large amount of biological interactions in wetlands that support and shape food webs in coastal waters (Bertness and Leonard 1997). In the present study, the association found was as strong, that every observed sea anemone in the upper intertidal zone was found attached to the plant, and it is still not clear the effect that this alien sea anemone may have on the marsh functions.

Conclusions

Several factors favor the presence of *D. lineata* in most locations, like their ability to reproduce asexually and survive a wide range of environmental conditions, and the absence of predators like intertidal nudibranchs or gastropods. Further work is required to determine whether the association to *S. alterniflora* plays a significant role in the permanence

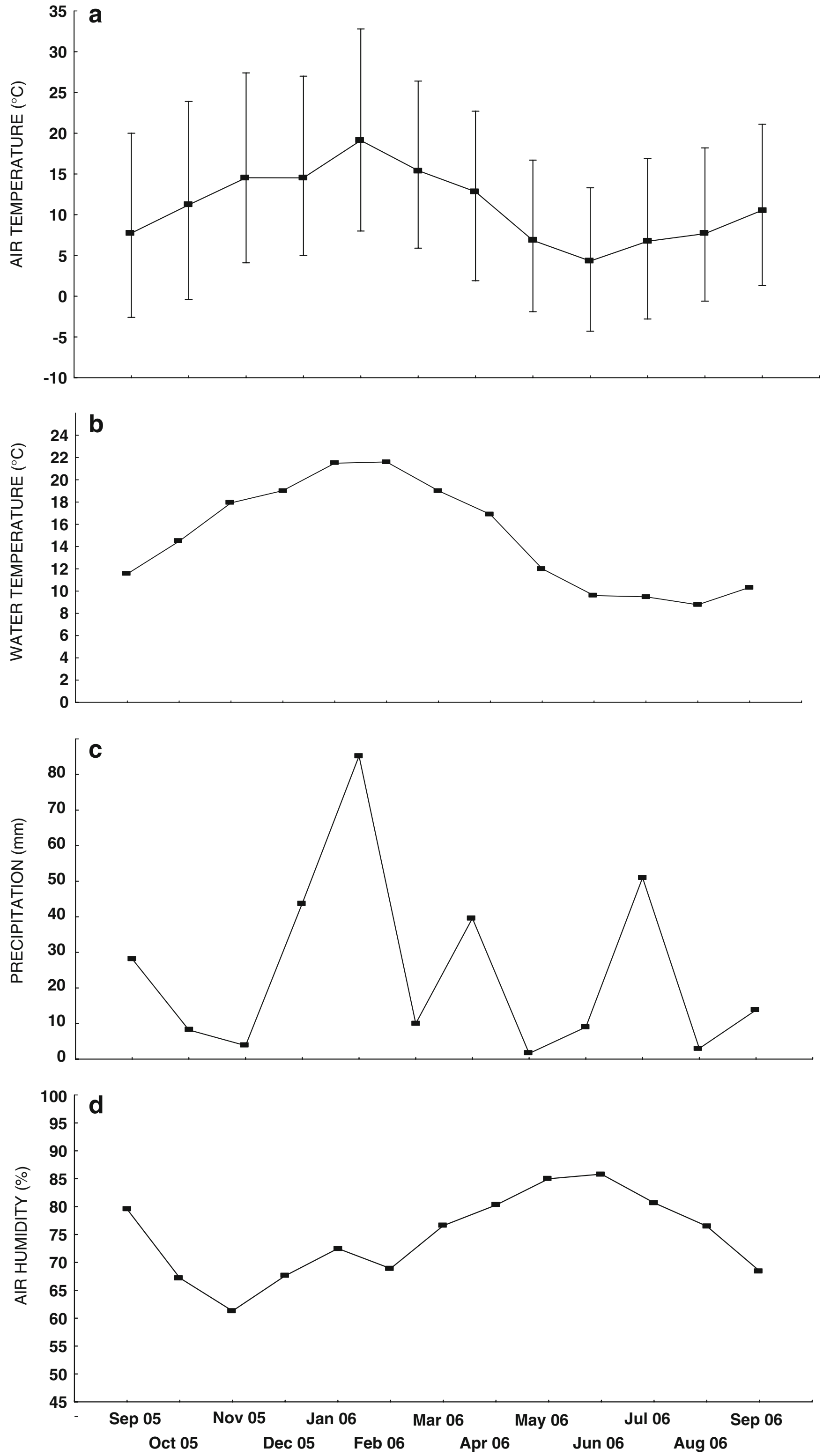

Fig. 5 Cimatological data from *Puerto Rosales* weather station, located 8 km south of the study area: (**a**) monthly mean air temperature, bars correspond to minimum and maximum records, (**b**) monthly mean water temperature, (**c**) total monthly precipitation, and (**d**) monthly mean relative humidity

and behavior of *D. lineata* in Bahía Blanca salt marshes. The invasive potential and ecological impact of *D. lineata* in the study area needs to be determined, especially its plausible predatory behavior, which may affect community structure.

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