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Clogging nets-*Didemnum vexillum* (Tunicata: Ascidiacea) is in action in the eastern Mediterranean

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Research Article

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Abstract

Didemnum vexillum is an aggressive, rapidly growing colonial ascidian and regarded as a global alien invasive species in temperate waters. It has recently become established in the western Mediterranean and the vectors of its introduction were assumed to be shipping or oyster trade. A dense settlement of it was encountered on nets of the bluefin tuna (*Thunnus thynnus*) cages placed at 60–65 m depths off the İzmir Peninsula (eastern Aegean Sea, eastern Mediterranean) in December 2022. It had considerably clogged net's eye openings, hindering water circulations inside cages. It had a vertical distributional pattern on 35 m long-nets, occurring solely on depths from surface down to 15 m, around where a summer thermocline develops. It has entirely replaced the native black mussel *Mytilus galloprovincialis* on nets. This colonial ascidian changed the routine cleaning procedure of nets in the farming. Three possible ways of its introduction to the eastern Mediterranean were proposed, but the most reasonable one is its secondary transfer via nets or ships from Malta. Mechanisms of its invasion biology and behaviour should be studied and monitored in the region.

Introduction

The Mediterranean Sea is a hotspot area for introduction of species and nearly 1000 species have been reported from the region (Zenetos *et al.*, 2017; Galanidi *et al.*, 2023). They have created unpredictable, irreversible changes in the Mediterranean ecosystems, outcompeting native species, changing food webs, and destroying biogenic habitats (Çinar *et al.*, 2011, 2021). Some sessile invasive alien species densely attach to artificial substrata such as hulls of ships, pipes, and nets, and significantly reduce their usage efficiency (Çinar, 2006; Ferrario *et al.*, 2017; Tempesti *et al.*, 2022). The main fouling invasive alien species are algae, sponges, serpulid polychaetes, and ascidians (Koçak *et al.*, 1999; Çinar, 2006; Mineur *et al.*, 2007; Çinar *et al.*, 2008; Zenetos *et al.*, 2017; Lezzi & Giangrande, 2018; Evcen & Çinar, 2020). The newly described sponge species, namely *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004, densely invaded harbour environments and mussel farming ropes in the Mediterranean Sea, creating dense mats, resulting in dead of species underneath (Longo *et al.*, 2007; Evcen & Çinar, 2020). Serpulid polychaetes such as *Hydroides* and *Ficopomatus* species densely occur on artificial substrata in polluted and brackish waters (Çinar *et al.*, 2008; Çinar, 2013). Ascidians are known to be pioneer colonizers in benthic communities, and also good competitors and efficient space monopolizers (Vieira *et al.*, 2021).

A total of 28 alien ascidian species have been reported from the Mediterranean Sea (Izquierdo-Muñoz *et al.*, 2009; Galanidi *et al.*, 2023). They were introduced to the region via shipping or natural dispersal via the Suez Canal (Zenetos *et al.*, 2010; Ulman *et al.*, 2019; Çinar *et al.*, 2021). Some of them are highly invasive, covering large areas and outcompeting native species (Ordóñez *et al.*, 2015; Stabili *et al.*, 2015; Çinar, 2016; Montesanto *et al.*, 2021a). They are frequently found on submerged ropes or tyres in harbour environments, but also occur in natural substrata like rocks (Çinar *et al.*, 2006; Ramos-Esplá *et al.*, 2020; Montesanto *et al.*, 2021b). Along the coasts of Turkey, 14 alien species have been reported up to date, with eight species being found in the Levantine Sea, and 11 species in the Aegean Sea (Çinar *et al.*, 2021). Among the species, only one didemnid species (*Didemnum ahu* Monniot and Monniot, 1987) has been reported on a bivalve shell in İzmir Bay (Aegean Sea) (Aydın-Önen, 2020).

Didemnum vexillum was originally described from New Zealand (Kott, 2002) and regarded as a global alien invader in temperate waters (Turon *et al.*, 2020). It has a high growth rate that enables it covering large areas quickly, overgrowing almost every other sessile species (Lambert, 2009). This colonial ascidian is a relatively recent invader in the Mediterranean Sea, first encountered on oyster crops in aquaculture facilities in the Ebro Delta (Spain) (Ordóñez *et al.*, 2015), and on biota (mussels, sessile ascidians, and other fouling organisms) and maritime structures in the Venice Lagoon, based on material collected in 2012 (Tagliapietra *et al.*, 2012). However, in the Ebro Delta, its first observation goes back to 2002, but becomes more apparent in the 2010s (Ordóñez *et al.*, 2015). It was also the case

in the Venice Lagoon where it was first observed in the late 2007 (Tagliapietra *et al.*, 2012). This species was considered as invasive alien species in the western Mediterranean and Adriatic Sea.

In the present study, the occurrence of the highly invasive alien species *D. vexillum* was reported in the eastern Mediterranean (Ildır Bay, Aegean Sea, Türkiye) and information regarding its morphological and ecological characteristics was documented.

Material and methods

The colonies of *D. vexillum* were collected on 13 December 2022 on nets of bluefin tuna cages (38°29′39″N-26°23′24″E), which were located at 60–65 m depths off the coast of Izmir Peninsula in the eastern Aegean Sea (Figure 1). The bluefin tuna farm system was composed of eight cages, each of 200–250 ton fish, and the breeding period for fish is 6 months, usually between June and January. Nets extend to a depth of 35 m.

The surface water temperature in the sampling time was 15 °C. Its colonies on the net were photographed by a compact Olympus underwater camera (Tough TG-6). The material taken on nets was first fixed with 4% formaldehyde and transferred to the laboratory. The colony was first rinsed under tap water and then placed in jars containing 70% ethanol. It was cut into small pieces to observe zooids, spicules, and larvae. The measurements of the zooids and spicules were made an ocular micrometre. The colony was deposited in ESFM (Ege University Faculty of Fisheries Museum).

Results and discussion

Taxonomy

Phylum: Chordata Haeckel, 1874
Subphylum: Tunicata Lamarck, 1816
Classis: Ascidiacea Blainville, 1824
Order: Aplousobranchia Lahille, 1886
Family: Didemnidae Giard, 1872
Didemnum vexillum Kott, 2002
Didemnum vexillum Kott, 2002, 625–628, fig. 1; Lambert, 2009: 5–28, figs 1–5.

Short description

It is a colonial species, creamy whitish in colour (Figure 2C–D, 3A). It was found growing on net surfaces of bluefin tuna cages, developing short and long lobes with a cloacal opening at tip of each lobe (Figure 2A–D). The colony has a characteristic reticulate pattern. The colony thickness is around 2–3 mm (Figure 3C, D). Spicules embedded into the surface layer of tunic, 10–50 μm ($N = 20$) in diameter (average: 31.3 μm ± 2.47 SE), with sharply tipped short rays, about 10–20 in visible field (Figure 3B). The thorax and abdomen almost 1 mm long (Figure 4A–C); thorax with six small pointed lobes in the oral syphon and wide atrial aperture on branchial sac; with four rows of stigmata, first row contains eight or nine stigmata per side; thoracic organ could not be observed (Figure 4B). The abdomen with a straight digestive system; containing a spherical-shaped testis with a spiralled sperm duct forming 6–8

Fig. 1. Map of the investigated area with the location of sampling sites (red dot).

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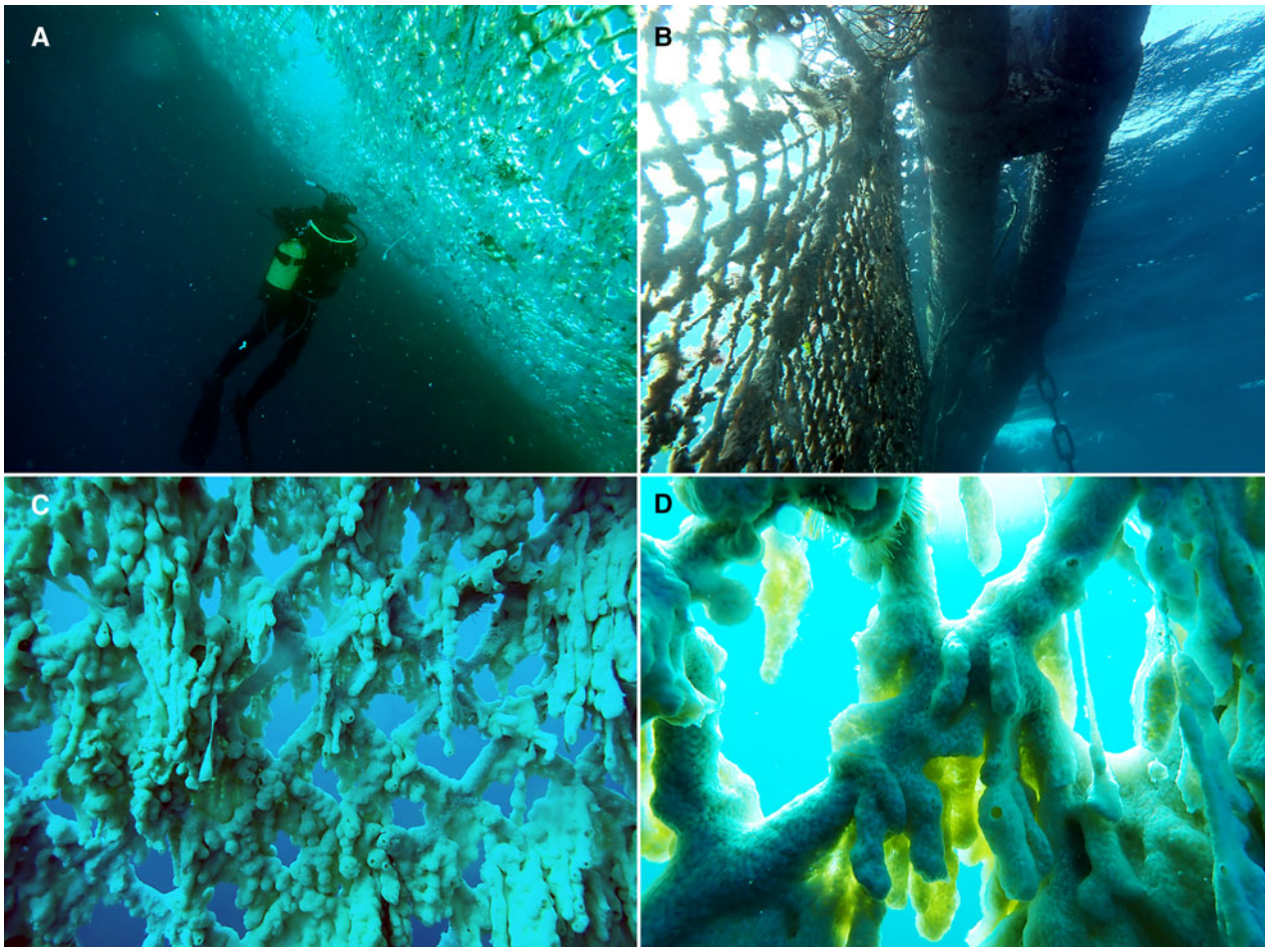

Fig. 2. A–B. Nets of bluefin tuna cages, C–D. *Didemnum vexillum* colonies on nets.

turns before passing straight towards atrial aperture (Figure 4C). It has also incubating oocytes with one large and two small eggs (Figure 4D). Larvae placed within the tunic beneath zooids; with six pairs of lateral ampullae and three adhesive papillae (Figure 4E).

Associated fauna

Nets surrounding the bluefin tuna cages were densely covered with the invasive colonial ascidian *D. vexillum*. This species almost clogged nets opening in some parts, hindering water circulations inside cages. A few species (some algae and actinarians) were observed to be associated with it, but no sessile encrusting invertebrates were observed on nets.

Distribution

Didemnum vexillum is native to Japan (Lambert, 2009). It is spreading in cool temperate areas via human assistance (Kleeman, 2009). It has become introduced in several regions including New Zealand, the eastern Atlantic (Netherlands, France, Ireland, United Kingdom, Spain), western Mediterranean, and both the west and east coasts of the United States and Canada (Lambert, 2009; Stefaniak *et al.*, 2012; Tagliapietra *et al.*, 2012; Ordóñez *et al.*, 2015; McKenzie *et al.*, 2017). The present study expands its distributional range to the eastern Mediterranean (Aegean Sea).

Habitat

This species densely colonizes natural and artificial substrates at depths ranging from 1 to 81 m depth (Bullard *et al.*, 2007; Lambert, 2009; Carman & Grunden, 2010). It fouls heavily on aquaculture establishments such as mussel and oyster cages (Lambert, 2009). Its current distribution pattern indicates that it is a temperate species and can tolerate a temperature range between −2 and 28 °C (Bullard *et al.*, 2007; Valentine *et al.*, 2009; Ordóñez *et al.*, 2015). Water temperatures above 8–10 °C are required for colony growth (Kleeman, 2009). Its occurrence in the Mediterranean Sea suggests its capability to adapt to warmer waters. The maximum thermal limit of this species was known to be 24 °C (Bullard *et al.*, 2007), but Ordóñez *et al.* (2015) proved that it can sustain temperature 28 °C in the Mediterranean Sea. It prefers salinities above 26 psu (Bullard & Whitlatch, 2009).

In the study area, the surface temperature near the bluefin tuna cage system was 15 °C (December), but it increases to 26 °C in the summer period in the area (Eryilmaz & Yücesoy-Eryilmaz, 2016). It was only found on cage nets only, and there has been no observation at the moment that this species also colonizes natural substrata. It has a vertical distributional pattern on the cage nets which goes down to 35 m depth. It colonizes nets from surface down to 16 m, after that, nets were fouled only by a brown alga species. This vertical distribution might be associated with the thermocline that usually develops around 15–20 m depth in the region in the summer period (Eryilmaz & Yücesoy-Eryilmaz, 2016).

The vector for introduction

The sexual reproduction of the species generates short-lived lecithotrophic tadpole larvae that have a short distance dispersal

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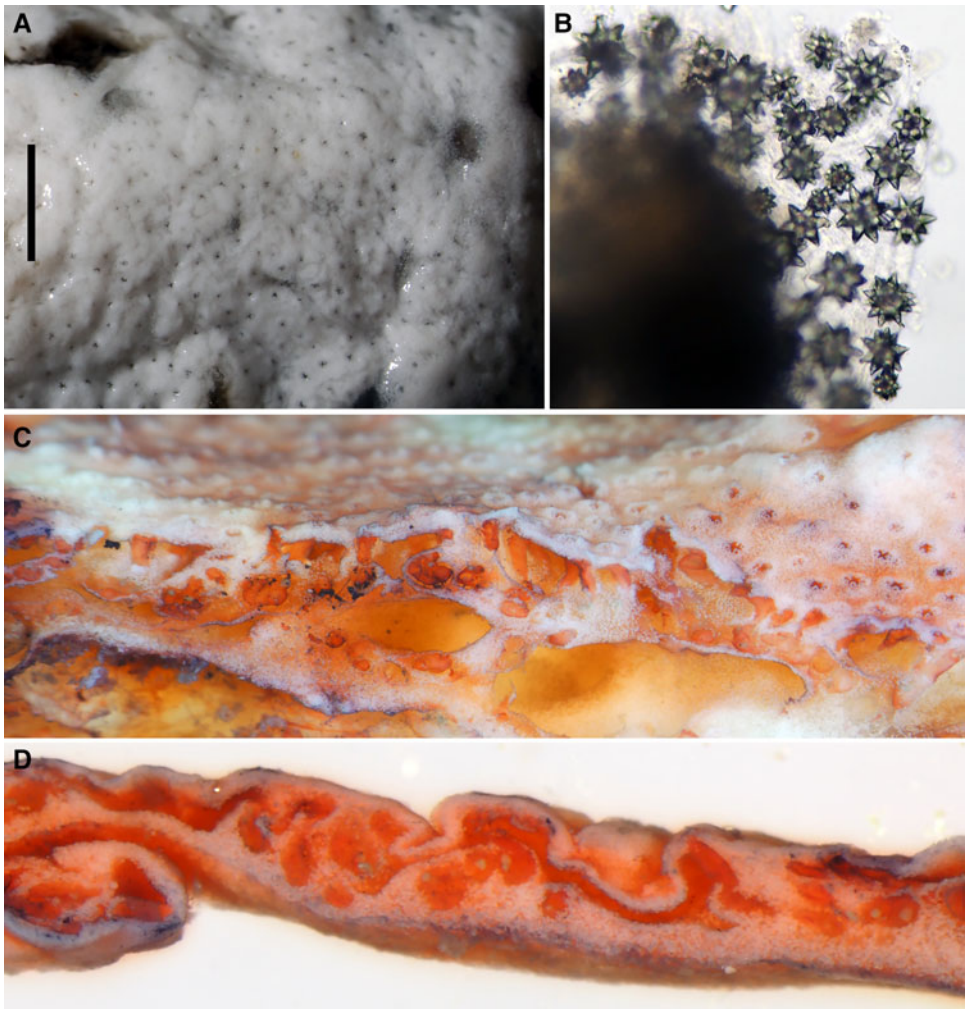

Fig. 3. A. A *Didemnum vexillum* colony, general view, B. spicules of colonies, C–D. colony vertical section showing main canals. Scale bar: A = 2 mm, B = 55 μm, C = 2 mm, D = 1.7 mm.

ability (meters to kilometres) (Fletcher *et al.*, 2013). Translocation by human activities explains its sudden appearances in distant locations in the world's oceans (Lambert, 2009). Kleeman (2009) categorized its dispersal by larval release or fragmentation from adult colonies as natural processes (current and hitchhiking) and human vectors (hull fouling, ballast water/sea chests, fishing and dredging, and aquaculture). Its presence in the Gulf of Maine and France was explained by imports of large quantities of the Pacific oyster [*Magallana gigas* (Thunberg, 1793)] from Japan and other invaded areas (Dijkstra *et al.*, 2007). In the Ebro Delta, it might have been introduced to the area as epifauna with the oyster spat from the French Atlantic coast (Ordóñez *et al.*, 2015). Shipping was considered to be a possible vector for its introduction to the other invaded area in the Mediterranean Sea, the Lagoon of Venice (Tagliapietra *et al.*, 2012).

According to the interview by workers in bluefin tuna farming, this species first appeared in the area in nearly 5 years ago (2018), so its introduction to the area can be explained by three possible means. (1) Bluefin tuna trade is being held between Japan and Türkiye. Possibly due to the tax advantage, the Japanese merchant ship arrives in Malta and anchors there. A Maltese ship sails to Türkiye to convey farm-harvested fish to the Japanese ship. This invasive species may have primarily introduced to Malta by the Japanese ship and secondarily to Türkiye by the Maltese ship. (2) Bluefin tuna specimens breeding in the cage systems in Ildır Bay are fished off the coasts of Cyprus or Malta, and transferred to the area in nets slowly hauled by ships. Although this species has not yet been recorded in either Malta or Cyprus, the invaded areas in the western Mediterranean and Adriatic Sea by this species are close to Malta and colonies attached to nets or ships may have transferred this species to the region. (3) Another possibility is leisure yachting activity. The İzmir Peninsula is one of the favourite destination of yacht tourism. Many leisure yachts from the Mediterranean and other regions sail to Ildır Bay, nearby the bluefin tuna cages are located, and colonies as a part of foulers on hulls of ships might have been translocated to the area. However, workers in sea-bream and sea-bass cage farming in the area told us that this species do not exist on their nets, indicating its local colonization and its introduction via the bluefin tuna transportation. It is unknown at this stage if this species has invaded natural or other artificial substrata (docks, pier, pontoons, etc.) in the area.

Impacts

Didemnum vexillum is an aggressive and rapidly growing colonial ascidian (Bullard *et al.*, 2007). It forms dense encrusting mats with lobes in the introduced areas and is capable of changing or modifying habitats. It has a strong competitive ability, commonly overgrowing several sessile species including other ascidians, sponges, macroalgae, cnidarians, bryozoans, mussels, polychaetes, and crustaceans (Bullard *et al.*, 2007). It is capable of increasing their sizes 6–11 times in just 15 days (Valentine

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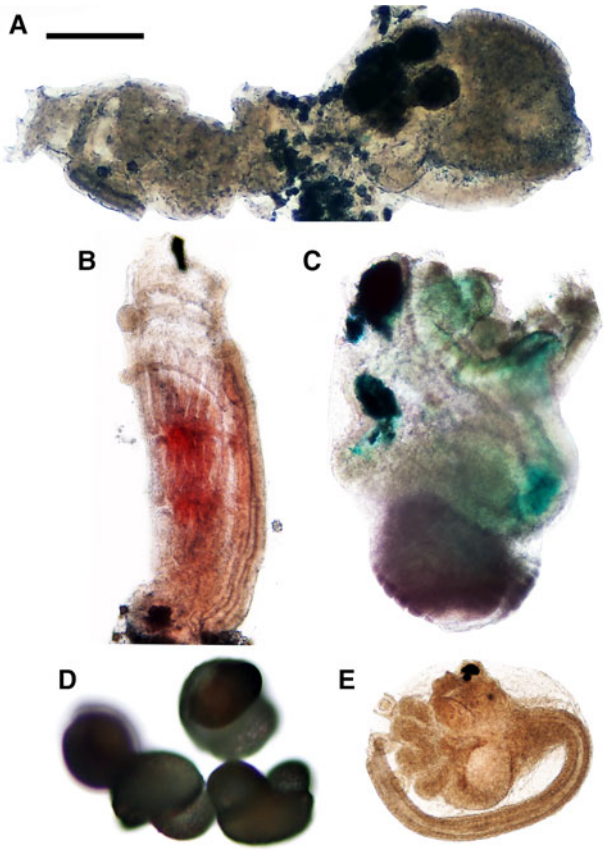

Fig. 4. A. Whole zooid, ventro-lateral view, B. thorax, C. abdomen, D. oocytes, E. larva. Scale bar: A = 0.17 mm, B = 120 μm, C = 90 μm, D = 55 μm, E = 215 μm.

et al., 2007a). On Georges Bank, off the coast of New England (USA), 50–90% surfaces of area within a 230 km^2 were covered by this species (Bullard *et al.*, 2007). The species smothered marked sized *Perna canaliculus* (Gmelin, 1791) mussels in New Zealand within 14 days (in summer) (Coutts & Sinner, 2004). It clogged syphons and caused condition loss or even death of mussels. *Didemnum vexillum* does not specifically attach itself to artificial substrata, but also colonizes natural habitats, having dramatic effects on native biota by altering habitat complexity and structure (Mercer *et al.*, 2009), and adversely affecting economically important activities such as fishing and aquaculture (Bullard *et al.*, 2007; Valentine *et al.*, 2007b). It poses a major economic threat to the mussel farms located along the northern Adriatic coasts and lagoons (Tagliapietra *et al.*, 2012).

In the eastern Mediterranean, this species solely occurred on nets of bluefin tuna cages and entirely outcompeted the native fouler, the mussel *Mytilus galloprovincialis* Lamarck, 1819 (interview with workers in bluefin tuna farming), which was regarded as a previous pest for the farm because of clogging mesh's eye openings. It seems that this species does not have a profound effect on the tuna fish breeding activity, as the breeding period is almost 6 months in the area (caging in June and harvesting in December or January). After harvesting tuna fish, nets are removed from the cage and underwent to cleaning. This species changed the cleaning procedure of nets in the farming. Normally, when mussels are attached, nets go to net washing device for cleaning. However, as the didemnid attaches nets firmly, nets cannot be adequately cleaned up by net washing machines, thus they are laid on the ground in the sun and retain there until they are completely dried out. It then detaches from nets easily and if required nets are subjected to antifouling paintings.

Data availability. The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Author's contributions. M.E. Çinar: Conceptualization, data curation, identification, writing, review, and editing. A. Özgül: conceptualization, data curation, sampling.

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Competing interest. None.

Ethical standards. Not applicable.

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