Mediterranean Marine Science

Vol 13, No 2 (2012)

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doi: 10.12681/mms.307

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net

New records of recently described chemosymbiotic bivalves for mud volcanoes within the European waters (Gulf of Cádiz)

J. L. RUEDA[1], J. URRA[2], S. GOFAS[2], N. LÓPEZ-GONZÁLEZ[1], L.M. FERNÁNDEZ-SALAS[1] and V. DÍAZ-DEL-RÍO[1]

[1] Centro Oceanográfico de Málaga, Instituto Español de Oceanografía, Puerto pesquero s/n, Fuengirola 29640, Málaga, Spain
[2] Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, Campus de Teatino s/n, Málaga 29671, Spain

Corresponding author: jose.rueda@ma.ieo.es

Received: 26 March 2012; Accepted: 19 July 2012; Published on line: 14 September 2012

Abstract

Chemosymbiotic bivalves are important members of cold seep communities and information on their distribution in the European waters is still quite scarce. This study reports the presence of living populations and shell remains of some recently described bivalves such as *Lucinoma asapheus, Solemya elarraichensis* and *Acharax gadirae* as well as *Bathymodiolus* sp. in the mud volcanoes of the Spanish Atlantic waters. Living populations of these species were thus far only found in Anastasya, Aveiro and Almazán mud volcanoes, together with other chemosymbiotic metazoa (*Siboglinum* spp.), suggesting the presence of moderate seepage activity. In other mud volcanoes (Albolote, Gazul), the benthic communities are dominated by sessile filter feeders on authigenic carbonates (chimneys, slabs) and only the shell remains of some chemosymbiotic bivalves were found, indicating earlier or very low seepage conditions. The present study elaborates on the known distribution of *L. asapheus* and *S. elarraichensis* to the European waters of the Gulf of Cádiz.

Keywords: Cold seep, Mollusca, Solemyidae, Lucinidae, Gulf of Cádiz, mud volcano.

Introduction

Cold seeps and mud volcanoes are seafloor manifestations of vertically migrating subsurface fluids, representing heterogeneous environments that are widely distributed in different continental margins worldwide (Milkov, 2000). In the European continental margin, geological and biological aspects of cold seeps and mud volcanoes have been studied over the last decades in both the Mediterranean Sea (e.g. Anaximander Field) and the Atlantic Ocean (e.g. Tasyo Field) (Olu-Le Roy *et al.,* 2004; Vanreusel *et al.*, 2009). The Gulf of Cádiz represents an area of extensive seepage activity in the eastern Atlantic Ocean, with the presence of ca. 50 mud volcanoes located in the Spanish, Portuguese and Moroccan waters (200 to 4,000 m depth) (Medialdea *et al.*, 2009; León *et al.*, 2012). Recent studies have revealed the presence of endosymbiont-bearing fauna, mainly frenulate polychaetes (e.g. *Siboglinum, Lamellibrachia*) and bivalves (e.g. *Thyasira, Acharax*) in the mud volcanoes of the Moroccan (e.g. El Arraiche Field, western Moroccan Field) and Portuguese margins (e.g. Captain Arutyunov, Carlos Ribeiro, Bonjardim and Porto) (Vanreusel *et al*., 2009; Oliver *et al*., 2011). Nevertheless, information on the presence of some of these chemosymbiotic species and communities is absent for those mud volcanoes located in the Spanish waters of the Gulf of Cádiz. This is particularly of interest because of the high spatial and temporal heterogeneity of the cold seeps and mud volcanoes, which results in strong differences in their habitats and associated communities, such as summit vs flanks within a mud volcano, or anoxic sediments vs cold water corals substrates (Campbell, 2006; Cordes *et al*., 2010; Fernández-Zambrano *et al.*, 2012). Information on the distribution of cold seep communities and species is also of importance for improving the knowledge on the distribution of these uncommon species and the conservation strategies of their populations within the European waters.

Bivalves are generally important members of cold seep communities, with 6 species occurring in cold seeps and mud volcanoes of the eastern Mediterranean Sea (Olu *et al.,* 2004; Taviani, 2011; Rodrigues *et al.,* 2011, with references therein) and more than 10 species in those of the Gulf of Cádiz (Taylor & Glover, 2010; Oliver *et al*., 2011). Recently, four new species have been

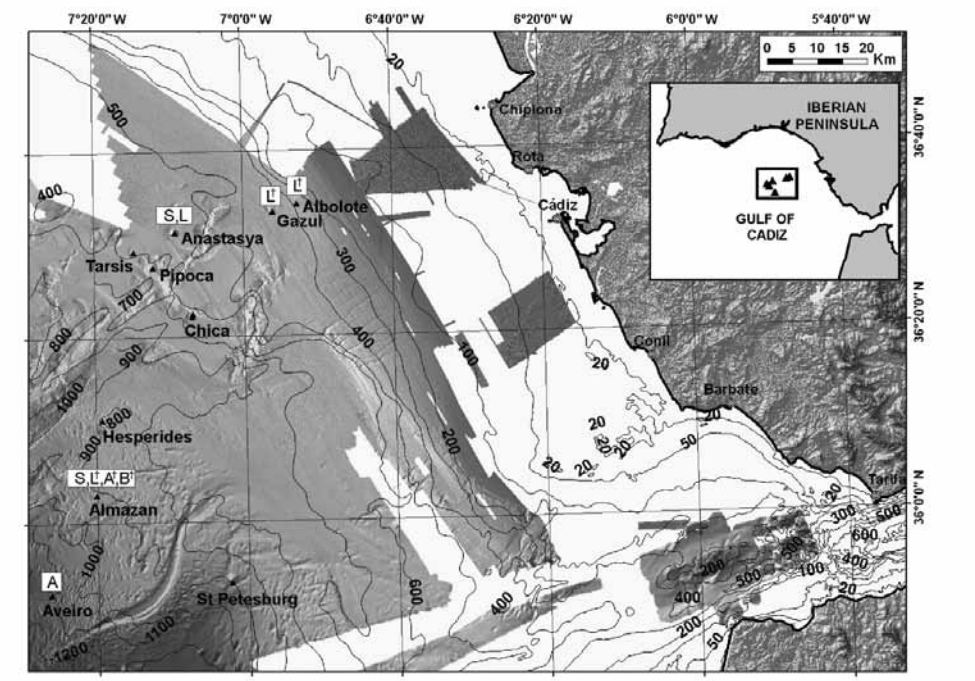

Fig. 1: Location of mud volcanoes in the Spanish waters of the Gulf of Cadiz, indicating the presence of living individuals of chemosymbiotic bivalves and of shell remains in the samples collected in this study. A: *Acharax gadirae*; B: *Bathymodiolus* sp.; L: *Lucinoma asapheus*; S: *Solemya elarraichensis*. † : Only shell remains collected.

described from material collected in the mud volcanoes of the Moroccan margin, including the lucinid *Lucinoma asapheus* Oliver, Rodrigues & Cunha, 2011, and the solemyids *Solemya (Petrasma) elarraichensis* Oliver, Rodrigues & Cunha, 2011 and *Acharax gadirae* Oliver, Rodrigues & Cunha, 2011, the latter was also found in the mud volcanoes of the deep water field within the Portuguese Margin. This study adds new records of some of these recently described bivalves for the mud volcanoes located within the Spanish waters of the Gulf of Cádiz. Information on the shell remains of these and other chemosymbiotic bivalves is also recorded for these mud volcanoes. These remains may represent indicators of past seepage activity in certain mud volcanoes or of potential living populations not found yet, as observed in those of the Moroccan Margin.

Materials and Methods

Samples were collected in different areas (summit, flanks, depression, adjacent bottoms) of nine mud volcanoes located within the Spanish waters (Albolote, Gazul, Anastasya, Tarsis, Pipoca, Chica, Hespérides, Almazán and Aveiro), from 300 to 1,100 m water depth, during the INDEMARES/CHICA 0610 and 0211 surveys (Fig. 1). This mud volcano field is exposed to the highly saline (36.1-36.9‰) and warm (ca. 13ºC) Mediterranean Outflow Water (MOW) that forms a strong bottom current flowing towards the W and NW above the less saline (34.9-35.2‰) and cold (3-8ºC) North Atlantic Deep Water (NADW) (Nelson *et al.,* 1999). Sampling was performed using different methods such as the box-corer (BC: ca. 0.09 m^2 of the sampling area on each occasion, n=48), benthic dredge (DA: ca. 300 m^2 of the sampling area, n=56) and beam-trawl (BT: ca. 2,000 m^2 of the sampling area, n=40) on board the R/V Emma Bardan and Cornide de Saavedra. Sediments collected with the box-corer (generally down to 15-20 cm depth) were sectioned at 5 cm intervals and sieved (0.5 mm) in order to study the vertical distribution of some of these chemosymbiotic species. Measurements of pH and Eh along the sediment column were performed on board, at every 5 cm by means of pHenomenal portable pH- and Eh-meter before sub-sampling. Grain size analysis was done by dry sieving (from > 2 to 0.063 mm) and Sedigraph III 5120 (< 0.063 mm) in the laboratory.

Material collected with the benthic dredge was sieved using a sieve column of 10, 5 and 1 mm mesh sizes. Large specimens were generally sorted just after sampling on board and the small size individuals were separated from the sediment in the laboratory using stereo microscopes. Individuals were preserved in 70% ethanol, 10% formaldehyde, 2.5% glutaraldehyde or dried in the case of shell remains. The material collected was deposited in the reference collection of Centro Oceanográfico de Málaga from Instituto Español de Oceanografía and also in the collection of Departamento de Biología Animal from the University of Málaga.

Table 1. Summary of examined material of chemosymbiotic bivalves from the mud volcanoes of the Gulf of Cadiz explored during INDEMARES-CHICA cruises. DA: Benthic dredge; BT: Beam-trawl; BC: Box-corer

Albolote mud volcano
Lucinoma asapheus
- 2 valves (DA01_0211, 334-371 m depth, flank of MV).
Gazul mud volcano
Lucinoma asapheus
- 2 valves (BC06.3_0610, 369 m depth, summit of MV)
Anastasya mud volcano
Lucinoma asapheus
- 2 live individuals (BC12_0211, 457 m depth, summit).
- 4 valves (BC24_0211, 458 m depth; BC25, 459 m depth, summit) [photo]
- 41 valves (BT08_0211, 469-562 m depth, summit and flanks)
Solemya elarraichensis
- 5 live individuals (BC12_0211, 457 m depth, summit) [photo]
- 1 live individual (BC25_0211, 459 m depth, summit)
- 7 valves (BC24_0211, 501 m depth, summit)
Tarsis mud volcano
No chemosymbiotic bivalves found in 6 BT, 6 DA & 5 BC samples collected
Pipoca mud volcano
No chemosymbiotic bivalves found in 5 BT, 7 DA & 7 BC samples collected
Chica mud volcano
No chemosymbiotic bivalves found in 3 BT, 7 DA & 4 BC samples collected
Hespérides mud volcano
No chemosymbiotic bivalves found in 4 BT, 9 DA & 5 BC samples collected
Almazán mud volcano
Lucinoma asapheus
- 1 valve (BT25_0211, 875-945 m depth, summit and flanks)
- 7 valves (BT26_0211, 930-940 m depth, seafloor depression)
- 4 valves (DA46_0211, 894-908 m depth, seafloor depression)
- 1 valve (BC35_0211, 829 m depth, summit)
Solemya elarraichensis
- 2 live individuals & 2 valves (DA42_0211, 927-915 m depth, flanks and summit) [photo]
Acharax gadirae
- 1 valve (BT26_0211, 930-940 m depth, seafloor depression)
- 3 valves (DA47_0211, 922-938 m depth, adjacent bottoms)
- 8 valves (BT25_0211, 875-945 m depth, flanks and summit)
- 3 valves (BC35_0211, 829 m depth, summit).
Bathymodiolus sp.
- 2 valves (BC35_0211, 829 m depth, summit)
Aveiro mud volcano
Acharax gadirae
- 3 live individuals (BT27_0211, 1115-1151 m depth, summit and flanks) [photo]

Results

Four species of chemosymbiotic bivalves have been found in the mud volcanoes (MV) of the Spanish waters, including 3 species that were collected alive, viz, *Lucinoma asapheus*, *Solemya (Petrasma) elarraichensis* and *Acharax gadirae*, and one only represented by shell remains (*Bathymodiolus* sp.) (Fig. 2; Table 1).

The lucinid *L. asapheus* was found alive at the summit of Anastasya MV at densities of 22.2 individuals m^{-2} (estimated from one BC sample) and at 0.05 and 0.15 meters below the seafloor (mbsf) (Table 1). The sediment was mud breccia grey in colour (GLEY2 4/5B; Munsell colour, 1998), with scattered organic matter spots and a strong sulphide smell. Values of pH decreased from 7.4 to 6.8 and a negative redox potential was observed at 0-0.05 mbsf level (Eh -140 mV), further decreasing from 0.1 to 0.2 mbsf level (Eh -388 mV). Several burrows and individuals of the thalassinid decapod *Calliax* sp. as well as frenulate polychaetes (*Siboglinum* sp.) were also found in the sediment, but typical species from the bathyal muddy bottoms were also found at the summit, including the pennatulaceans *Kophobelemnon stelliferum* (Muller, 1776) and *Funiculina quadrangularis* (Pallas, 1766) and

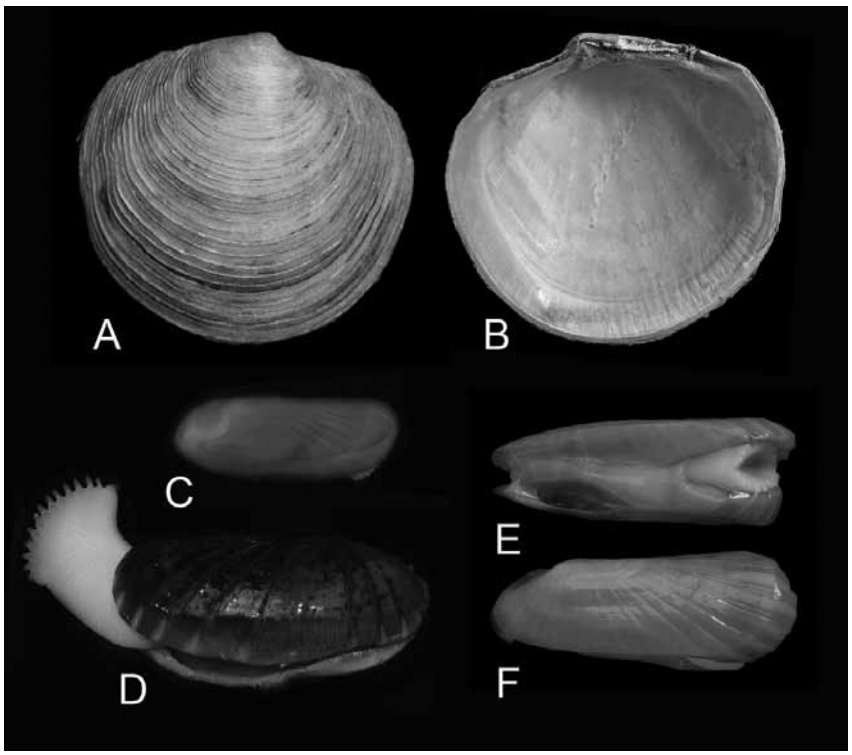

Fig. 2: Chemosymbiotic bivalve species found in the mud volcanoes (MV) within the Spanish waters. (A, B) External and internal views of the right valve of *Lucinoma asapheus* from Anastasya MV, shell length 23 mm; (C) External view of right valve of small size individual of *Solemya elarraichensis* from Anastasya MV, shell length 7.1 mm; (D) External view of left valve of *S. elarraichensis* from Almazán MV, shell length 31 mm; (E,F) Ventral and lateral view (right valve) of small specimen of *Acharax gadirae* from Aveiro MV, shell length 10.5 mm.

the bivalves *Abra longicallus* (Scacchi, 1835) and *Kelliella miliaris* (Philippi, 1844). Additionally, two more box-corer samples were collected at this summit but only the shell remains were found in one of them (Table 1). This seems to indicate a patchy distribution of this species within the summit of this mud volcano. Several shell remains were also collected at the eastern side of Albolote MV, summit of Gazul MV, Anastasya MV and Almazán MV, including its summit and seafloor depression.

Live individuals of the solemyid *S. elarraichensis* were also collected at the summit of Anastasya MV at densities of 66.7 individuals $\cdot m^{-2}$ (estimated from one BC sample) (Table 1). Small size individuals (< 10 mm shell length) were collected at the 0-0.05 mbsf level, whereas large individuals (20-30 mm) were collected between 0.05 and 0.2 mbsf. The sediment column had a strong sulphide smell and the same characteristics as that of *L. asapheus*, with a complex system of burrows that were occupied by *S. elarraichensis* and by *Calliax*. Other chemosymbiotic species occurring at the summit were frenulate polychaetes (*Siboglinum* sp). The shell remains of *S. elarraichensis* were also collected in other samples taken with the box-corer at the summit of this MV, indicating a patchy distribution of living populations (Table 1). Two large living individuals (> 30 mm shell length) and shell remains were also collected at Almazán MV, using a benthic dredge that sampled the southwestern side and summit of this MV (Table 1). In this summit (829 m depth), the sediment ranged from hemipelagic muddy sand (0-0.05 mbsf) to mud breccia (0.05-0.15 mbsf) and the redox potential changes from positive (Eh 91 mV) to negative (Eh -124 mV), respectively. In these sediments, the frenulate polychaete *Siboglinum* sp. represents one of the top dominant invertebrates.

Three living individuals of the solemyid *Acharax gadirae* were collected in Aveiro MV, using a beam-trawl that sampled the summit as well as the northwestern and southeastern flanks of this MV (1115-1151 m depth) (Table 1). These individuals were small in size, with shell lengths between 6.8 and 10.5 mm. The sediment at the summit of Aveiro (1069 m depth) is composed of hemipelagic muddy sand down to 0.05 mbsf and mud breccia at deeper intervals (0.05-0.2 mbsf) with a redox potential already negative at the surface (Eh -46.9 mV) and rather lower at deeper sediment intervals (Eh -164 mV at 0.15-0.20 mbsf). The benthic community in the Aveiro MV is dominated by other chemosymbiotic organisms such as frenulate polychaetes (*Siboglinum* spp.) as well as non-chemosymbiotic ones that are common in the bathyal mud bottoms such as the bamboo coral *Isidella elongata* (Esper,

1788), and the sponges *Pheronema carpenteri* (Thomson, 1869) and *Thenea muricata* (Bowerbank, 1858). Shell remains of adult specimens (up to 54.7 mm shell length) were also collected in different areas (seafloor depression, flanks and summit) of the Almazán MV (Table 1).

The mytilid *Bathymodiolus* sp. was collected at the summit of the Almazán MV, but only represented by shell remains (Table 1). In this MV, the benthic community was composed of other chemosymbiotic organisms such as *Siboglinum* spp. and *S. elarraichensis* and non-chemosymbiotic ones that usually colonise soft (*Isidella elongata, Radicipes* sp.) and hard bottoms (*Leiopathes* sp., *Petrosia* sp.). The sediment is hemipelagic muddy sand to mud breccia with a sharp redox potential change from positive to negative, respectively, and described in more detail in the previous paragraphs.

Discussion

Six individuals of the lucinid *Lucinoma asapheus* were collected in the Mercator MV (358 m depth), located in El Arraiche field (Morocco), representing the first and only known population for this species until now (Oliver *et al.,* 2011). A low frequency of occurrence was also found, being present in just one of the twenty sampled MV (Oliver *et al.,* 2011). Our record extends the distribution range of the species to the Spanish margin of the Gulf of Cádiz, representing the first record for the European waters, and also extends the known bathymetric range of this species (down to 457 m depth in Anastasya MV). The presence of the remains of this lucinid in the other MV over a wide bathymetric range (334-940 m depth) may indicate that the species could be more frequent than previously believed or that earlier seepage conditions favoured that species in those MV. The latter may be the case of Gazul MV where no chemosymbiotic species have been found alive, so far, after intensive sampling and the benthic community is nowadays dominated by hard-bottom sessile filter feeders, including cold water corals, mainly *Madrepora oculata* Linnaeus, 1759, black corals (e.g. *Leiopathes, Anthipathella*), gorgonians (e.g. *Viminella, Acanthogorgia, Placogorgia*) and sponges (*Asconema setubalense* Kent, 1870 and *Petrosia* cf. *crassa*) (Fernández-Zambrano *et al.,* 2012). These organisms are favoured by the presence of hard bottoms with authigenic carbonates (e.g. chimneys, slabs) that are common on the seafloor of mud volcanoes with latent conditions (León *et al*., 2007).

The solemyid *Solemya elarraichensis* appears to be the most widespread chemosymbiotic bivalve in the MVs of the Moroccan margin, as well as in those of the Spanish Margin. Oliver *et al*. (2011) found this species in 8 of 25 MVs studied in the Moroccan Margin, at depths between 358 and 1115 m, and our records represent the first ones for the European waters. Studies on other *Solemya* species have shown that they are dynamic in behaviour, moving up and down within the burrows between the oxidised and sulphide-rich anoxic sediment zones (Stanley, 1970; Reid, 1980). By contrast, the small size individuals appear to be less dynamic and do not burrow as deep in the sediment, probably because feeding is not restricted to the symbionts when the gut is still present in early stages (Cary, 1994). This may explain the vertical distribution pattern found for large and small size individuals of this species in the Anastasya MV.

According to Oliver *et al*. (2011), *A. gadirae* represents the second most widespread chemosymbiotic bivalve in the Gulf of Cádiz (7 mud volcanoes of 25 sampled) and has the widest depth range of occurrence (556-3902 m). Our record is the first one for the Spanish margin but not for the European waters, as the species was earlier reported for the MVs of the deep water field within the Portuguese margin (e.g. Porto, Carlos Ribero and Captain Arutyunov MVs). The presence of abundant shell remains in the Almazán MV may indicate the potential presence of this species in that MV, in which living individuals of *S. elarraichensis* were collected and anoxic bottoms also occur. Indeed, these two species have been found living together in 3 MVs of the Moroccan margin (Oliver *et al.,* 2011). Nevertheless, the calcified shell of *Acharax* may favour a higher presence in the bioclastic material when compared with the more fragile shells of *S. elarraichensis* with a low number of remains found in this study. The presence of deep-water cold seep solemyids in the European eastern Atlantic (Ivanov *et al.,* 2010; Oliver *et al.,* 2011) and the Mediterranean Sea (Rodrigues *et al.,* 2011, Taviani *et al.,* 2011) is very scarce and restricted to a few cold seeps, including the mud volcanoes and pockmarks (e.g. Southern Vøring Plateau, Gulf of Cádiz, Nile deep-sea fan). Unlike *Solemya*, living populations of *Acharax* are apparently absent from cold seeps and reducing habitats within the Mediterranean Sea, although they were widely distributed during the Neogene in this basin (Taviani *et al.,* 2011). Our finding and that of Oliver *et al.* (2011) represent the closest records to the Mediterranean Sea for living populations of *Acharax*.

In a previous study on the Gulf of Cádiz, living specimens of *Bathymodiolus* were only found in Darwin MV (1115 m depth) on the Moroccan margin, and attributed to the species *B. mauritanicus* after genetically determining the phylogenetic relationships (Génio *et al*., 2008). The type of mixed seafloor of Almazán MV with slabs and soft bottoms is similar to those of the Darwin MV, where this mytilid was found in the fissures and depressions with abundant shell remains (Génio *et al*., 2008). The absence of living individuals of this chemosymbiotic bivalve in our material complicates its identification to species level, especially when considering that several species occur in the cold seeps of the Atlantic Ocean, in-

cluding the Gulf of México and western Africa (Cosel, 2002; Olu-Le Roy *et al*., 2007). Nevertheless, as found in the MVs of the Moroccan and Portuguese waters by Oliver *et al*. (2011), the chemosymbiotic bivalves of the genus *Bathymodiolus* also appear to be one of the least frequently found in the MVs of the Spanish waters.

Acknowledgements

We would like to gratefully acknowledge the assistance of the Captains and all the crew members of the R/V Emma Bardán and R/V Cornide de Saavedra during the faunistic sampling cruises INDEMARES/CHICA 0610 and 0211, respectively. This study was supported by the INDEMARES/CHICA Project, EC contract INDEMARES-LIFE+ (07/NAT/E/000732) and FEDER funding assigned to the equipment of R/V Cornide de Saavedra (FICTS-2010-01). We deeply appreciate the comments made by two anonymous referees to the earlier drafts of this manuscript.

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