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Chemosymbiotic bivalves from the mud volcanoes of the Gulf of Cadiz, NE Atlantic, with descriptions of new species of Solemyidae, Lucinidae and Vesicomyidae

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Abstract

The chemosymbiotic bivalves collected from the mud volcanoes of the Gulf of Cadiz are reviewed. Of the thirteen species closely associated with chemosynthetic settings two Solemyidae, *Solemya* (*Petrasma*) *elarraichensis* **sp. n.** and *Acharax gadirae* **sp. n.**, one Lucinidae, *Lucinoma asapheus* **sp. n.**, and one Vesicomyidae, *Isorropodon megadesmus* **sp. n.** are described and compared to close relatives of their respective families. The biodiversity and distribution of the chemosymbiotic bivalves in the Gulf of Cadiz are discussed and compared to the available information from other cold seeps in the Eastern Atlantic and Mediterranean. Although there is considerable similarity at the genus level between seep/mud volcano fields in the Eastern Atlantic and Mediterranean, there is little overlap at the species level. This indicates a high degree of endemism within chemosymbiotic bivalve assemblages.

Keywords

Bivalvia, chemosymbiotic, taxonomy, Gulf of Cadiz

Introduction

Chemosynthetic bivalves are prominent constituents of the fauna of cold seeps and are represented in that setting by five families: Solemyidae, Lucinidae, Vesicomyidae, Thyasiridae and Mytilidae (Sibuet and Olu 1998, Sibuet and Olu-Le Roy 2002, Sahling et al. 2003, Levin 2005). Recently the presence of bacteria in the gills in species of *Nucinella* and *Huxleyia* has been demonstrated (Oliver and Taylor in preparation), confirming the previous inclusion of the Manzanellidae in this group (Cosel and Bouchet 2008).

The occurrence of chemosymbiotic bivalves in the extensive mud volcano fields of the Gulf of Cadiz was first reported by Pinheiro et al. (2003). Then followed a series of more in-depth studies: on polychaete commensals of solemyid hosts (Ravara et al. 2007); on the distribution and taxonomy of Thyasiridae (Rodrigues et al. 2008); on the phylogenetic relationships of *Bathymodiolus mauritanicus* Cosel (Génio et al. 2008) and on the molecular characterization of chemosymbiotic endosymbionts of solemyids, lucinids, thyasirids and mytilids (Rodrigues et al. 2010, Rodrigues and Duperron 2011).

Chemosynthetic bivalve faunas have been discovered elsewhere in the Eastern Atlantic, notably off tropical West Africa (Cosel and Salas 2001, Olu-Le Roy et al. 2007, Cosel and Olu 2009) and in the eastern Mediterranean (Salas and Woodside 2002, Olu-Le Roy et al. 2004, Carlier et al. 2010). For the Vesicomyidae, at the species level, these faunas are considered disparate (Cosel and Salas 2001, Krylova and Sahling 2010). Conversely studies on *Bathymodiolus* suggest that the species occurring in the Gulf of Cadiz is not only found along the West African margin but is amphi-Atlantic (Génio et al. 2008). The biogeographic patterns within these faunas are currently unresolved and require further taxonomic characterization, which this paper begins to address.

This paper intends to provide the taxonomic basis for the chemosynthetic bivalves in the Gulf of Cadiz and includes the description of two new species of Solemyidae, one new species of Lucinidae and one new species of Vesicomyidae. Notes on the biogeography of these taxa in the Atlantic are given with special emphasis on the relationships between the Eastern Mediterranean, Gulf of Cadiz and West Africa.

Materials and methods

Study area. The Gulf of Cadiz is located in the NE Atlantic Ocean between 34°N and 37°15'N and 6°W to 9°45'W. It is enclosed by the southern Iberian and northern Moroccan margins, west of Gibraltar Strait. The geological history of the Gulf of Cadiz is intimately related to plate tectonic interaction between Southern Eurasia and North Africa and is driven by two major mechanisms: a) subduction associated with the westward emplacement of the Gibraltar Arc and formation of the Gulf of Cadiz accretionary wedge, probably not active at present and b) oblique lithosphere collision between Iberia and Nubia, active at present and causing active thrusting (Zitellini et al. 2009). It is now well established that the whole area is under compressive deformation and that mud volcanism and processes associated with the escape of hydrocarbon-rich fluids sustain a broad diversity of chemosynthetic assemblages. This extensive area encompasses over forty mud volcanoes (here after as MV), at depths ranging from 200 to 4000m (confirmed by coring) (Mazurenko et al. 2002, Pinheiro et al. 2003, Magalhães 2007), and active methane seepage has been documented on several locations (Kenyon et al. 2000, Gardner 2001, Niemann et al. 2006, Stadnitskaia et al. 2006, Hensen et al. 2007). Biological samples were available from 30 sites but for this study only thirteen MVs and one structure (PDE) yielded chemosymbiotic bivalves (Fig. 1).

In the shallow Moroccan margin the El Arraiche field encompasses Renard Ridge (including Pen Duick Escarpment), Vernadsky Ridge and several mud volcanoes (e.g.

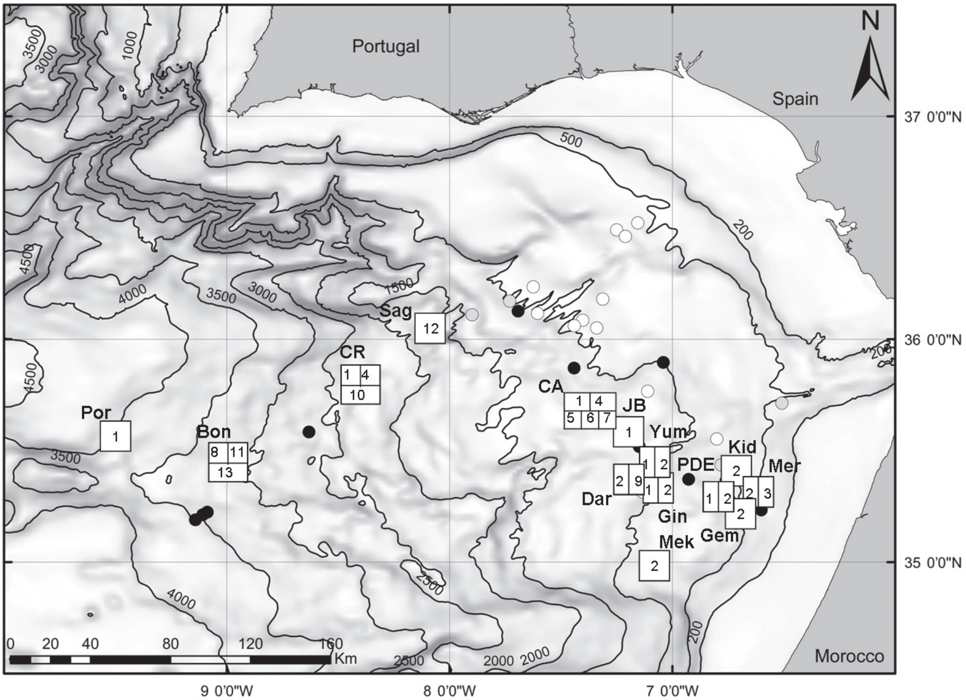

Figure 1. Map of the study area (Gulf of Cadiz) and location of sampling sites. **squares with numbers**, mud volcanoes with chemosymbiotic bivalves: **full black circles**, mud volcanoes visited during the study but bivalves not found: **grey circles**: mud volcanoes and other structures not visited during the study. **Bon**, Bonjardim MV; **CA**, Captain Arutyunov MV; **CR**, Carlos Ribeiro MV; **Dar**, Darwin MV; **Gem**, Gemini MV; **Gin**, Ginsburg; **JB**, Jesus Baraza MV; **Kid**, Kidd MV; **Mek**, Mèknes MV; **Mer**, Mercator MV; **PDE**, Pen Duick Escarpment; **Por**, Porto MV; **Sag**, Sagres MV; **Yum**, Yuma MV. The numbers inside the squares indicate the presence of the following species. **1** *Acharax gadirae* **2** *Petrasma elarraichensis* **3** *Lucinoma asapheus* **4** *Thyasira vulcolutre* **5** *Spinaxinus sentosus* **6** *Isorropodon megadesmus* **7** *Isorropodon* sp. indet. **8** *Christineconcha* cf. *regab* **9** *Bathymodiolus mauritanicus* **10** *Idas* sp. **11** *Laubiericoncha chuni* (empty shells only) **12** *Callogonia cyrili* (empty shells only) **13** *Pliocardia* sp. (empty shells only).

Al Idrisi, Mercator, Fíuza, Gemini, Kidd MVs) located at depths from 200 to approximately 600m depth: The proximity to the euphotic zone and to the African coast adds to the great productivity observed in the area. Dead cold-water scleractinean coral reefs, carbonate crusts and exposed carbonate chimneys characterize the Renard and Vernadsky Ridges. Carbonate crusts, rock blocks and clasts are often found in the craters of the shallow mud volcanoes where mild seepage activity has been recorded (Van Rensbergen et al. 2005). Mercator MV, one of the shallowest mud volcanoes differs significantly from the other mud volcanoes by the high chloride enrichment of its pore water (Van Rensbergen et al. 2005). The top of Mercator MV shows patches of disturbed sediments from which gas venting is occasionally observed. Solitary corals (*Caryophyllia* sp.), accompanied by Cidaridae echinoids and Onuphidae polychaetes (*Hyalinoecia*) are the most conspicuous organisms seen during video surveys of the Mercator MV crater.

The western Moroccan field comprises several mud volcanoes (e.g. Meknès, Student, Yuma, Ginsburg, Jesus Baraza, Darwin MVs) at intermediate depths (700–1200m) located along an extensive province of carbonate and mostly dead cold-water coral mounds. The widespread presence of authigenic carbonates and also extensive *Neptunea* and *Bathymodiolus* graveyards (usually within the crater of the mud volcanoes) suggest that this was a very active seepage area in the past. Darwin MV differs from the others in this area because its crater is completely covered by large carbonate slabs and crusts. The fissures among slabs and depressions with scattered crust are filled with abundant shell ash and occasionally small clumps of living *Bathymodiolus mauritanicus* Cosel, 2002. Meknès MV is the southernmost Moroccan mud volcano rising isolated among an extensive field of small coral mounds. The crater is formed by stiff, sometimes heavily disturbed, green mud breccia with scattered clasts and a striking large number of empty shells of the gastropod *Neptunea*. Except for a few *Paromola* individuals, living megafauna is rarely sighted in the crater

The deep-water field (1300–4000m), mostly within the Portuguese margin includes several mud volcanoes (e.g. Captain Arutyunov, Carlos Ribeiro, Bonjardim and Porto MVs) that are aligned along major crustal strike–slip faults associated with the African-Eurasian plate boundary (Duarte et al. 2005). Gas hydrates were recovered from these mud volcanoes and the methane concentrations yield the highest records from the Gulf of Cadiz (Kenyon et al. 2000, 2001, 2002, 2003, 2006; Akhmetzhanov et al. 2007, 2008). Video surveys of these deeper mud volcanoes often show conspicuous siboglinid fields (e.g. Porto MV) in the active craters and exuberant sponge and gorgonian patches at the crater rim and upper flank (e.g. Carlos Ribeiro MV).

Sampling. Samples were collected between 2002 and 2006 during TTR (Training Through Research) 12, TTR 14, TTR15 and TTR16 cruises onboard RV Prof. Logachev and MSM.01-03 cruise onboard RV Maria S. Merian (IFM–GEOMAR). The material was collected using TV-assisted grabs or USNEL box-corers. Occasionally faunal specimens were also recovered from Reineck box-corer, multiple corer or lander samples that were carried out for different purposes. Whenever possible the specimens were sorted onboard and preserved in 70 or 96% ethanol (the latter preserved for molecular analysis).

Deposition of samples. The majority of specimens are deposited in the Biological Research Collection of the Department of Biology, University of Aveiro but the holotype; some paratypes and selected specimens are deposited in the National Museum of Wales.

Institutional abbreviations. DBUA, Department of Biology, University of Aveiro (Biological Research Collection); IFM–GEOMAR, Institut für Meereskunde - Forschungszentrum für marine Geowissenschaften; IOC–UNESCO, Intergovernmental Oceanographic Commission – United Nations Educational, Scientific and Cultural Organization; NMW.Z, National Museum of Wales, Cardiff, Great Britain.

Measurements. All measurements were made using Sylvac™ vernier calipers accurate to 0.01mm but are given to the nearest tenth.

Systematics

Order Solemyoida Dall, 1889
Superfamily Solemyoidea Gray, 1840
Family Solemyidae Gray, 1840

Solemyids are among the most ancient bivalves dating from the Paleozoic (Métivier and Cosel 1993). They are protobranch in organization and characterized by an elongate shell (up to 220mm) with a posteriorly situated toothless hinge. The thick brown periostracum is much larger than the calcified part of the valve and is folded inwards by the muscular mantle edge upon closing of the valves (Métivier and Cosel 1993).

Solemyidae taxonomy is complex. Taylor et al. (2008) and Kamenev (2009) recognized two extant genera, *Solemya* and *Acharax*, with a further four subgenera within *Solemya*, namely *Petrasma, Austrosolemya Solemyarina* and *Zesolemya.* Solemyids, other than *Acharax* are generally found at continental shelf and upper-slope depths (0 to 600m), although Solemya (*Petrasma*) *pervernicosa* has been recorded at 1500m (Kamenev 2009). *Acharax* is generally restricted to deep-sea settings from ~400m on the continental slope to the deepest sites of the Japan Trench (Neulinger et al. 2006). Shallow dwelling solemyids live in sediments with high organic matter content, often at reduced oxygen concentrations. Hydrogen sulphide is frequently present due to sulphate reduction coupled with organic matter degradation (Conway et al. 1992). In contrast, the genus *Acharax* has been recovered from cold seep locations (see review by Sibuet and Olu 1998) and in sediments influenced by hydrothermal venting (Juniper et al. 1992, Métivier and Cosel 1993). *Acharax* species are morphologically similar but molecular data suggests a degree of cryptic speciation (Neulinger et al. 2006).

Superficially, all solemyids appear so similar that specimens discovered at various deep-sea sites might have been misclassified as *Solemya* (see review by Sibuet and Olu 1998).

Genus *Solemya* Lamarck, 1818

Subgenus *Petrasma* Dall, 1908

Type species. *Solemya borealis* Totten, 1834

Definition. As given by Taylor et al. 2008. Ligament wholly internal, supported by a buttress and lacking posterior or lateral extensions.

Solemya (*Petrasma*) *elarraichensis* sp. n.

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http://species-id.net/wiki/Solemya_(Petrasma)_elarraichensis
Figs 2, 3A–D, 4

Material examined. *Holotype*: one specimen, TTR14, stn AT528GR, El Arraiche field, Kidd MV, 35°25.304'N, 06°43.972'W, 489m, 03 August 2004, NMWZ.2010.4.1

Paratypes: ten specimens, same data as holotype, NMWZ.2010.4.2; seven specimens, TTR15, stn AT569GR, El Arraiche field, Mercator MV, 35°17.917'N, 06°38.717'W, 358m, 25 July 2007, DBUA.

Other material examined: eight juveniles specimens, same data as holotype; two specimens, TTR12, stn AT407GR, El Arraiche field, Pen Duick Escarpment, 35°17.695'N, 06°47.082'W, 560m, 15 July 2002; three specimens, TTR14, stn AT560B, El Arraiche field, Kidd MV, 35°25.306'N, 06°43.976'W, 498m, 8 August 2004; one specimen, TTR15, stn AT586GR, Western Moroccan field, Meknès MV, 34°59.146'N, 07°04.380'W, 701m, 28 July 2005; four specimens, TTR16, stn AT-604GR, Western Moroccan field, Yuma MV, 35°25.820'N, 07°06.330'W, 1030m, 29 May 2006; two specimens, TTR16, stn AT607GR, Western Moroccan field, Ginsburg MV, 35°22.677'N, 07°04.979'W, 983m, 29 May 2006.

Measurements (in mm)

	Station	Length	Height	Posterior Length
Holotype	AT528GR	33.8	14.1	9.8
Paratype	AT528GR	29.2	10.5	8.0
Paratype	AT528GR	25.6	10.0	6.2
Paratype	AT528GR	23.1	8.4	6.0
Paratype	AT528GR	14.7	5.3	3.6
Paratype	AT528GR	22.0	7.9	6.0
Paratype	AT528GR	11.6	4.7	2.5
Paratype	AT528GR	17.6	6.8	5.2

Description. *Shell* (Figs 2, 3): to 35mm in length. Fragile. Equivalve. Inequilateral, beaks situated at 1/4 length of shell from posterior margin. Outline subcylindrical, compressed, length about 2.6 times height, slightly deeper towards the anterior, dorsal and ventral margins subparallel, anterior margin more broadly rounded than ante-

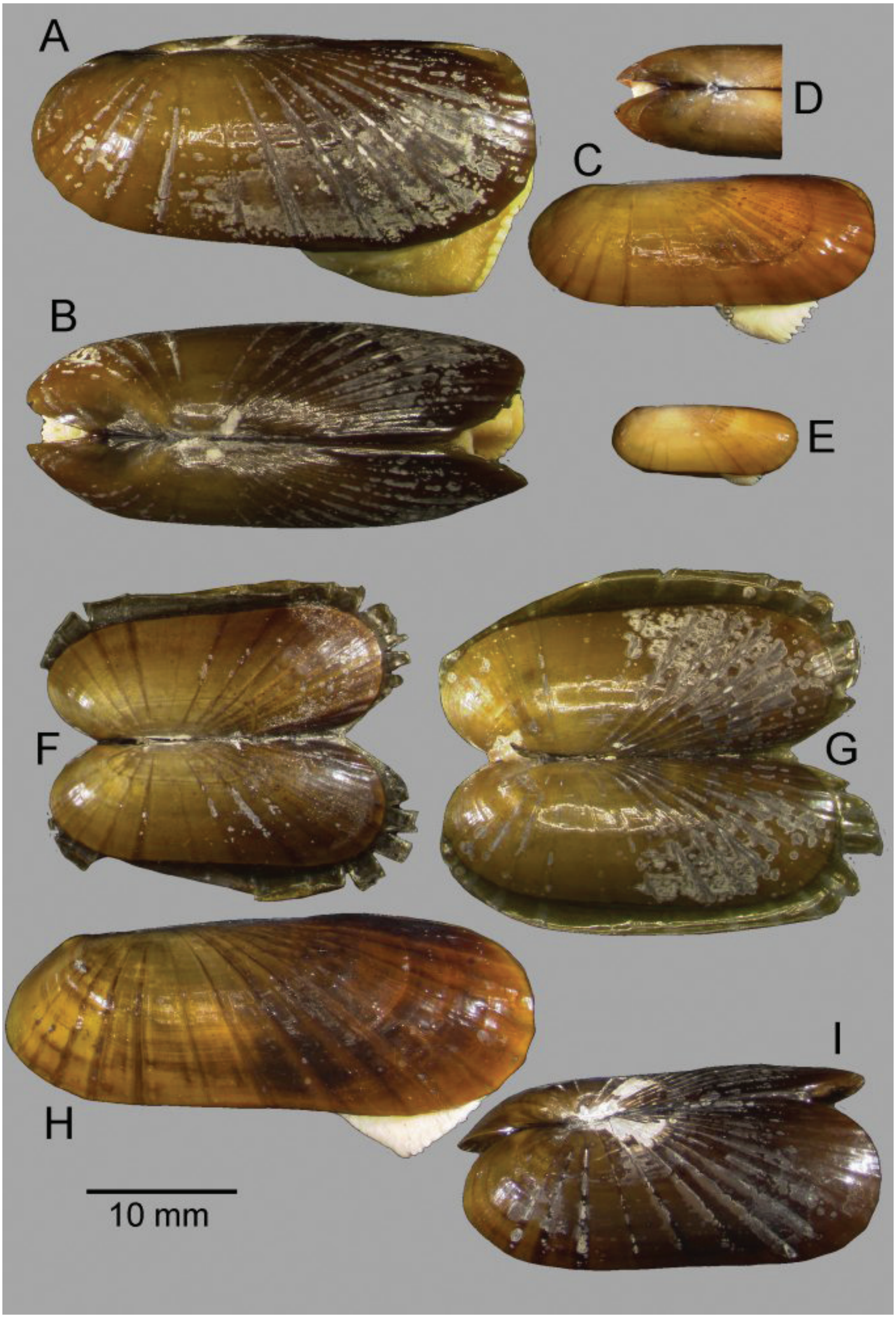

Figure 2. *Solemya* (*Petrasma*) *elarraichensis* sp. n. **A–E** from Kidd MV; **A–B** lateral and dorsal views of holotype **C–D** lateral and dorsal views of medium sized paratype **E** lateral view of small paratype. **F** paired valves from Pen Duick Escarpment **G** paired valves from Mercator MV **H** lateral view of specimen from Meknès MV **I** lateral view of shell from Yuma MV.

rior, posterior dorsal margin projecting a little. Beaks indistinct, umbos sunken. Hinge teeth absent. Ligament primarily internal, supported by a prominent chondrophore that extends only slightly as a chondrophore ridge around the posterior adductor, lacking posterior and anterior extensions but a small roughly heart shaped area is present in front of the chondrophore and this is also visible externally just behind the beaks. Periostracum persistent and extending well beyond the shell margin, initially yellowish brown in colour but darkening with growth to a dark chestnut brown. Sculpture of weak radial ridges, 5–6 over the posterior and 10–12 over median and anterior. Adductor scars impressed, dorsal part of posterior scar angulate where bounded by chondrophore ridge, anterior adductor scar larger, spatulate in outline.

Anatomy (Figs 3G, 4): The posterior siphonal opening is surrounded by a series of papillae: A single large dorsal papilla (dp) lies above two smaller but still large papillae (dmp) on the dorsal margin of the opening, below these is a short smooth section (sa) followed by a series of papillae increasing in size towards the ventral margin, there are 6 primary papillae (psp) on either side and a single ventral median papillae, between these on the inner side are smaller papillae (ssp); a pair of subsiphonal ridges (ssr) are present below the siphonal crown.

The mantle edge is fused from the posterior siphon for half the length of the ventral margin where there is a large anterior pedal gape. The mantle edge surrounding the rear of the foot bears a few tabulate papillae (pgp). The anterior dorsal mantle edge is prominently papillate (dap) and there is a single papilla on the junction of the mantle edge anterior of the anterior adductor muscle (admp). The foot is very large with a broad oval sole, this fringed by large papillae, all equal in size. The ctenidium is large with numerous laminar filaments attached to a prominent gill axis. The palps are short, twisted and flattened with cup shaped terminations. The gut is present but difficult to examine due to its small dimensions but the hind gut and rectum were easily visible.

Distribution. *Solemya* (*P.*) *elarraichensis* is presently only known from the mud volcano fields in the Gulf of Cadiz, Eastern Atlantic. The majority of specimens have been taken from the El Arraiche field off the coast of Morocco in Kidd, Fíuza and Mercator MVs and the Pen Duick Escarpment at depths between 358–560m. A few specimens have been taken from the Western Moroccan field at the Meknès, Yuma, Ginsburg and Darwin MVs at the slightly deeper range of 700–1115m.

Etymology. *elarraichensis*, denoting the geographic origin of the type locality; the El Arraiche field.

Remarks. The form of the ligament, which is primarily internal, supported by a chondrophore and lacks any lateral or anterior extensions, confirms the placement of *S.* (*P.*) *elarraichensis* in the subgenus *Petrasma* Dall, 1908 (Taylor et al. 2008). Some of the specimens carry an initial identification of "cf. *Solemya togata*" as might be expected from the proximity to the Mediterranean but the ligament of *Solemya s.s.* has prominent anterior extensions (Fig. 3E).

The subgenus *Petrasma* is not known from the North-East Atlantic but is represented in the Western Atlantic by three species. Two species are known from near shore waters off the northeast coast of the USA: *S.* (*P.*) *velum* (Say, 1822) and *S.* (*P.*) *borealis*

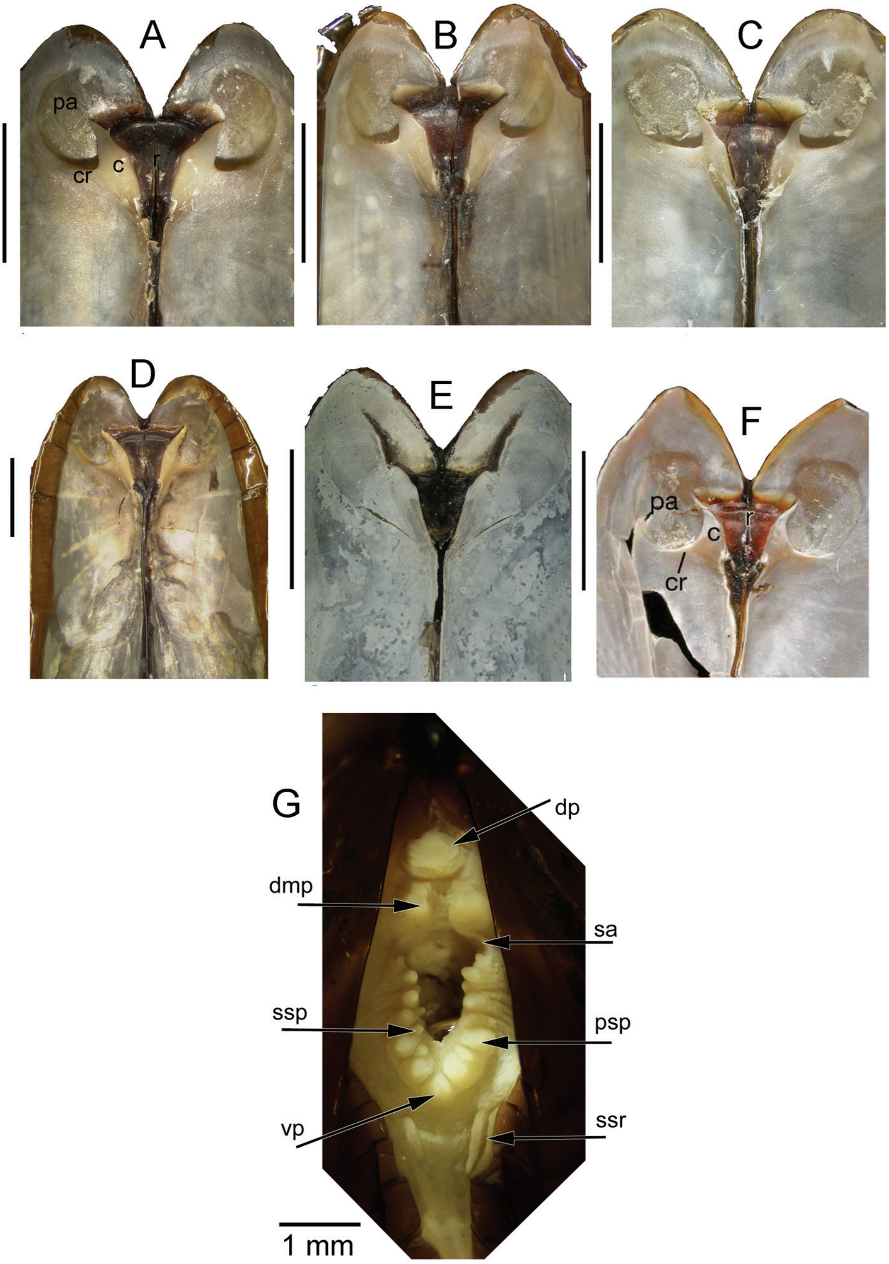

Figure 3. A–F Internal views of ligament, scale bars = 5mm. **A–D** *Solemya* (*Petrasma*) *elarraichensis* sp. n. from **A** Kidd MV **B** Pen Duick Escarpment **C** Mercator MV **D** Yuma MV. **E** *Solemya togata*, Mediterranean **F** *S.* (*P.*) *velum*, Rhode Island (from Taylor et al. 2009). c, chondrophore; cr chondrophore ridge; pa, posterior adductor scar; r, resilium. **G** posterior siphon of S. (*P.*) *elarraichensis*. dp, dorsal papilla; dmp, dorsal marginal papillae; psp, primary siphonal papillae; sa, smooth area; ssp, secondary siphonal papillae; ssr, subsiphonal ridge; vp, ventral papilla.

(Totten, 1834). The third, *S.* (*P.*) *occidentalis* (Deshayes 1857) is known from the warm waters of Florida, Caribbean and S. America (Mikkelsen and Bieler 2008).

We note that the curvature of the chondrophore and chondrophore ridge is circular in *S.* (*P.*) *velum* (Fig. 3F) but angular in *S.* (*P.*) *elarraichensis* (Figs 3A–D). Furthermore, the siphonal papillae of *S.* (*P.*) *velum* are by comparison less in number and reduced in development (Morse 1913, Taylor et al. 2008).

Abbott (1974) following Morse (1913) noted that the siphon of *S.* (*P.*). *borealis* differed markedly from that of *S.* (*P.*) *velum*, confirming that siphonal characters were important for distinguishing species. In *S.* (*P.*) *borealis,* the ventral-most dorsal marginal papillae are very large, and as big as the dorsal papilla, and much larger than any of the ventral papillae. This contrasts with the condition in *S.* (*P.*) *elarraichensis* where the ventral-most dorsal marginal papillae are smaller than the dorsal papilla and where the ventral papillae are fewer in number and distinctly increasing in size ventrally, with the ventral-most papillae equal in size to the dorsal marginal papillae. Conway et al. (1992), following Barnard (in Reid 1980) suggested that *S.* (*P.*) *borealis* lacked a gut and this would be in contrast with *S.* (*P.*) *velum* and *S.* (*P.*) *elarraichensis*.

The character of the ligament and chondrophore are rather similar in *S.* (*P.*) *elarraichensis* and *S.* (*P.*) *borealis.*

Abbott (1974) and Mikkelsen and Bieler (2008) note that *S.* (*P.*) *occidentalis* lacks any perceptible chondrophore ridge and give this as the main characteristic separating *S.* (*P.*) *occidentalis* from *S.* (*P.*) *velum* and, therefore, also from *S.* (*P.*) *elarraichensis*.

Ecologically *S.* (*P.*) *velum* and *S.* (*P.*) *borealis* are very different from *S.* (*P.*) *elarraichensis* in that they are not associated with deep-water methane seeps. In contrast they are found in sublittoral or shallow shelf settings with high organic enrichment (Morse 1913 in Conway et al. 1992). Mikkelsen and Bieler (2008) give a similar habitat for *S.* (*P.*) *occidentalis* (Deshayes, 1857) noting its occurrence in mangrove channels and around sewage outfalls.

Given the above differences in habitat and form we conclude that none of the Atlantic species is amphi-Atlantic, unlike *S.* (*P.*) *pervernicosa* Kuroda, 1948, which is considered to be amphi-Pacific by Kamenev (2009). If any of the Western Atlantic species were amphi-Atlantic it is unclear why, in the Eastern Atlantic, they should be absent from their typical settings (which are plentiful) and found only in deep water methane seeps.

Other North Atlantic species referred to as *Solemya*, *S. grandis* Verrill and Bush, 1898 and *S. caribbaea* Vokes, 1970 are excluded here because both belong to the genus *Acharax* (Abbott 1974).

A solemyid living at a pockmark, at a depth of 1607m, has been reported from the Eastern Mediterranean (Rodrigues et al. 2011). Unfortunately the small, single specimen was damaged and its taxonomic affinities remain unclear.

In conclusion, there are sufficient morphological and ecological grounds for considering the Gulf of Cadiz species of *Petrasma* to be new to science.

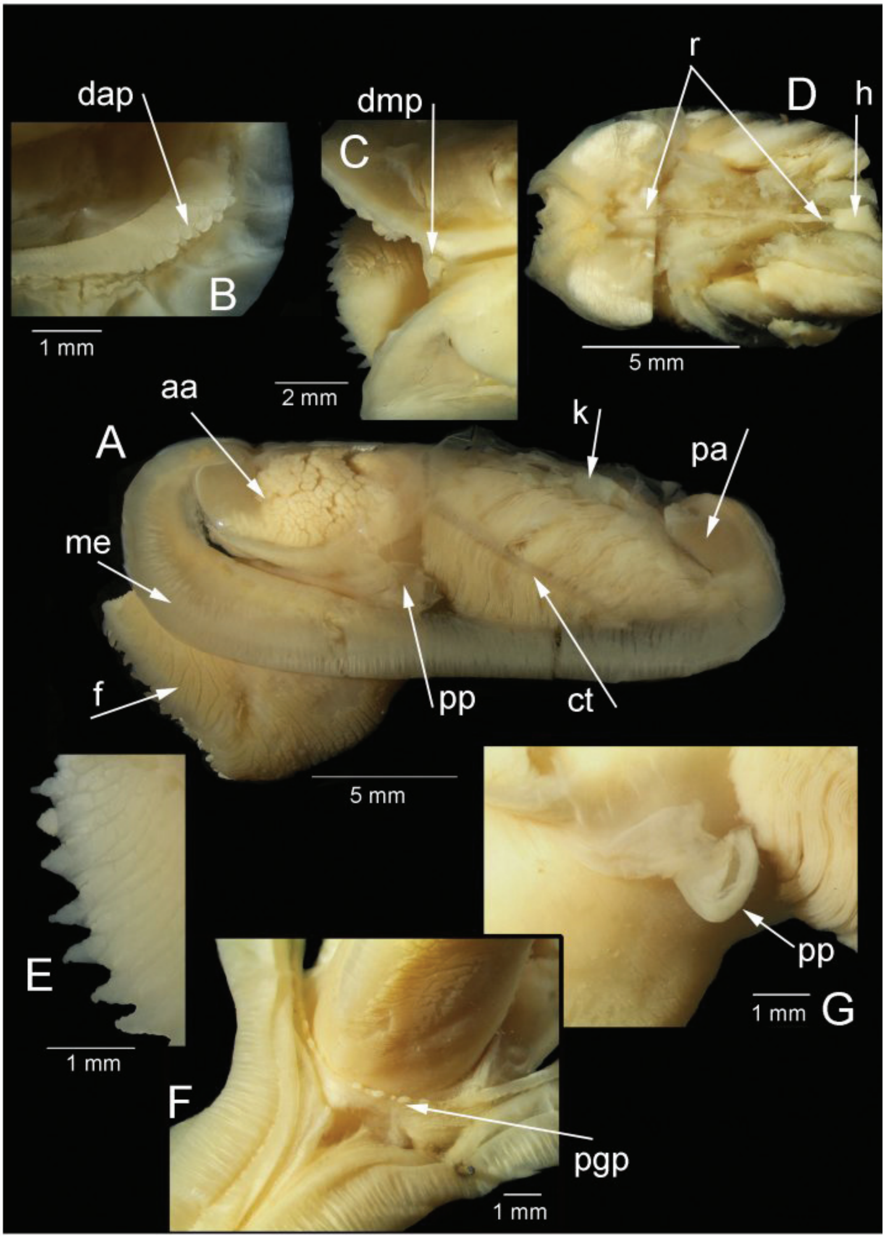

Figure 4. *Solemya* (*Petrasma*) *elarraichensis* sp. n., Pen Duick, stn. AT407GR, 560m. Anatomy. **A** whole animal viewed from left side **B** papillae on dorsal anterior mantle edge **C** single, large papilla in dorsal median position **D** posterior dorsal dissection showing rectum passing through heart **E** marginal papillae on foot **F** papillae on mantle edge surrounding pedal gape **G** palp. aa, anterior adductor muscle. ct, ctenidium. dap, dorsal anterior papillae. dmp, dorsal median papilla. f, foot. h, heart. k, kidney. me, mantle edge. pa, posterior adductor muscle. pgp, papillae surrounding pedal gape. pp, palp. r, rectum.

Genus *Acharax* Dall, 1908

Type species: *Solemya johnsoni* Dall, 1891

Definition. As given by Taylor et al. 2008. Ligament external, as a high arched band.

Acharax gadirae sp. n.

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http://species-id.net/wiki/Acharax_gadirae
Figs 5–6

Type material. *Holotype*: one specimen, TTR12, stn AT391GR, Western Moroccan field, Jesus Baraza MV, 35°35.439'N, 07°12.264'W, 1105m, 09 July 2002, NMWZ.2010.4.3.

Paratypes: one specimen, same data as holotype, DBUA; one shell, TTR 12, stn AT392G, deep-water field, Captain Arutyunov MV, 35°39.658'N, 07°20.018'W, 1320m, 9 July 2002, DBUA; one shell, TTR 16, stn AT607GR, Western Moroccan field, Ginsburg MV, 35°22.677'N, 07°04.979'W, 983m. 29 May 2006, NMWZ.2010.4.4.

Other material examined: one specimen, TTR16, stn AT602GR, El Arraiche field, Pen Duick Escarpment, 35°17.693'N, 06°47.089'W, 556m, 28 May 2006; one specimen, TTR16, stn AT604GR, Western Moroccan field, Yuma MV, 35°25.820'N, 07°06.330'W, 1030m, 29 May 2006; one specimen, TTR16, stn AT605GR, same locality, 35°25.046'N, 07°05.450'W, 975m, 29 May 2006; one specimen, TTR16, stn AT615GR, deep-water field, Carlos Ribeiro MV, 35°47.238'N, 08°25.272'W, 2200m, 31 May 2006; one specimen, TTR16, stn AT617K, same locality, 35°47.246'N, 08°25.303'W, 2230m, 31 May 2006; two specimens, MSM01.03, stn 145, deep-water field, Porto MV, 35°33.773'N, 09°30.416'W, 3902m, 3 June 2006.

Measurements (in mm)

	Station	Calcified Shell Length	Calcified Shell Height	Calcified Shell Posterior Length	Actual length	Anterior Ribs/ Posterior Ribs
Holotype	AT391GR	56.3	21.1	14.5	59.5	9/4
Paratype	AT391GR	60.0	22.0	14.2	65.4	8/4
Paratype	AT392GR	67.0	25.0	19.1	85.0	8/4
Paratype	AT607 GR	42.8	15.9	10.6	48.9	9//4

Description (Fig. 5) Calcified shell to 67mm in length, to 85mm including periostracal fringe. Robust. Equivalve. Inequilateral, beaks situated at 1/4 length of shell from posterior margin. Outline subcylindrical, compressed, calcified shell length about 3 times height, slightly deeper towards the anterior, dorsal and ventral margins subparallel, anterior margin more broadly rounded than anterior, posterior dorsal margin projecting a little. Including periostracal fringe, anterior appears greatly expanded compared with posterior. Beaks indistinct, umbos sunken. Hinge teeth absent. Ligament external, as a high arched band posterior of the beaks and supported by a thickened shell margin; an oval area of ligament is present immediately behind the beaks and visible internally, anterior of the beaks shell margins fused by periostracal material along entire dorsal margins. Periostracum persistent and extending well beyond the shell margin, initially yellowish brown in colour but darkening with growth to dark brown and black; periostracal frill thickened over ribs but entire. Sculpture of radial ridges, 4 closely spaced over the posterior; median area almost smooth with 2–3 low ribs; anterior with 8–9 deeply cut ribs. Adductor scars impressed, posterior scar subcircular, anterior adductor scar larger, spatulate in outline. Anterior inner shell margin scalloped corresponding to radial ribs.

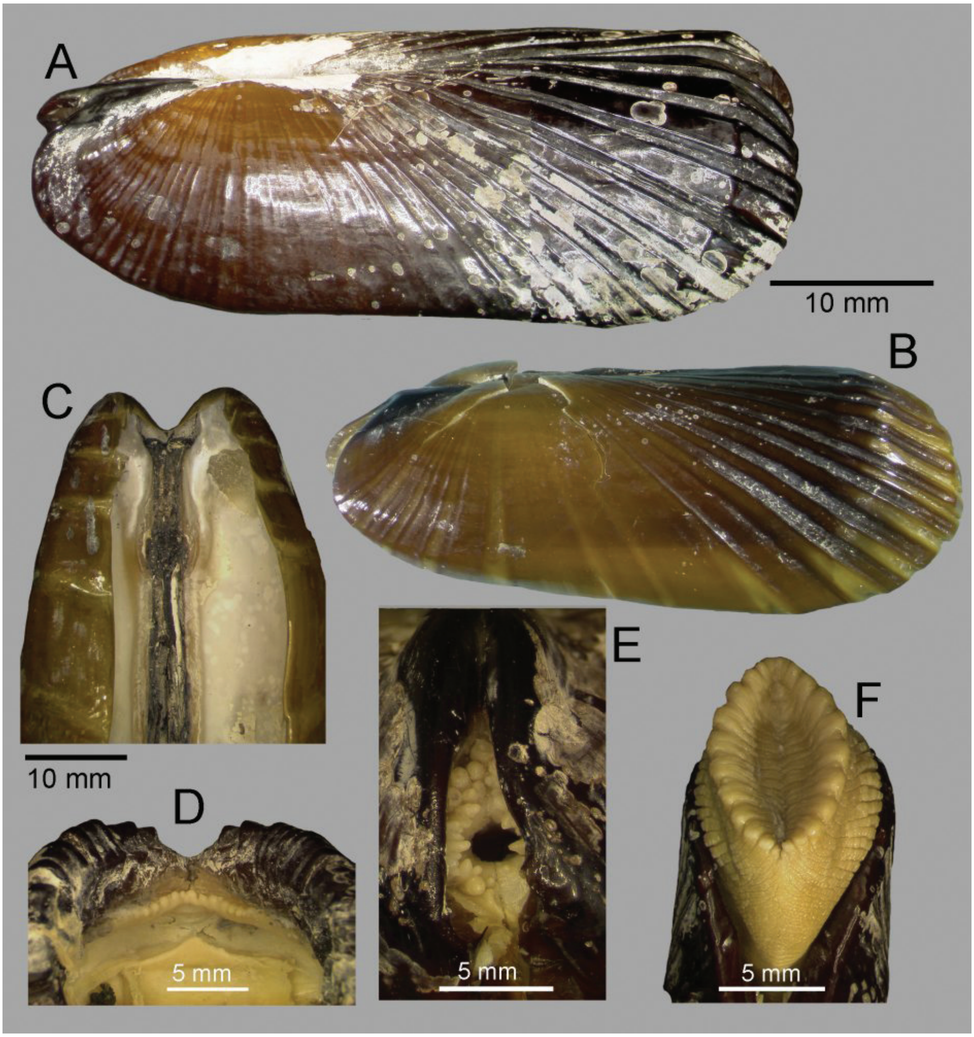

Figure 5. A–F *Acharax gadirae* sp. n. **A** Holotype, stn. AT391GR, Jesus Baraza MV **B** Paratype, stn. AT607GR, Ginsburg MV **C** interior view of posterior ligament, stn. AT392GR, Jesus Baraza MV **D** anterior dorsal mantle edge, st. AT391GR **E** posterior siphon, st AT391GR **F** foot, stn. AT391GR

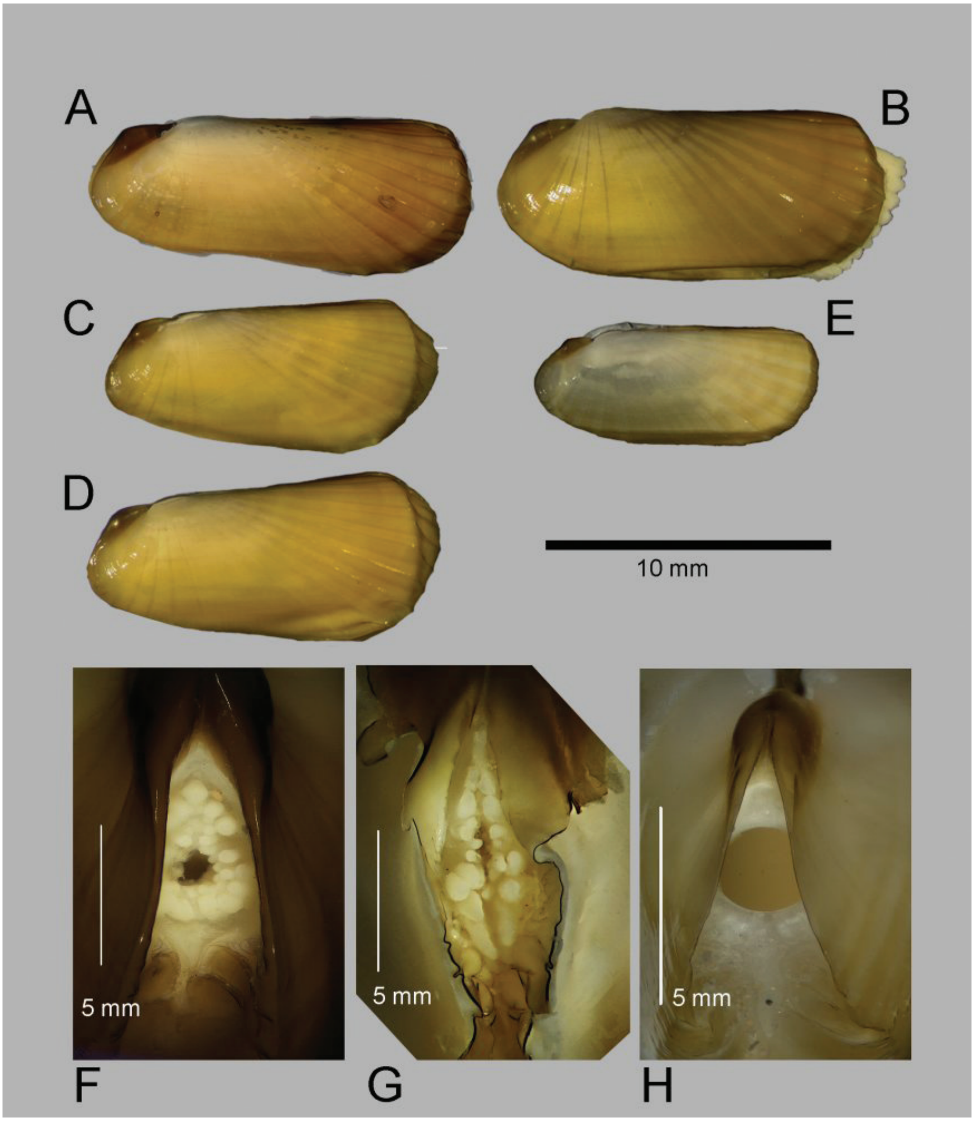

Figure 6. *Acharax gadirae* sp. n. **A** stn. AT602GR, Pen Duick Escarpment **B** stn. 145, Porto mud volcano **C–D** stns AT617GR & AT61GR, Carlos Ribeiro mud volcano **E** stn. 199, Capt Arutyunov mud volcano. **F–E** posterior siphon **F** specimen A **G** specimen D **H** specimen E.

Posterior siphonal opening surrounded by a series of papillae (Fig. 5E): A single large dorsal papilla (dp) lies above 2–3 pairs of slightly smaller papillae (dmp) on the dorsal margin of the opening, below these surrounding the opening is a series of approximately alternating large and small papillae with those most ventral the largest.

The mantle edge is fused from the posterior siphon for half the length of the ventral margin where there is a large anterior pedal gape. The mantle edge surrounding the rear of the foot is papillate. The anterior dorsal mantle edge is prominently papillate (Fig.

5D). The foot is very large with a broad oval sole, the margin interdigitates between large and small blunt papillae. The ctenidium is large with numerous laminar filaments attached to a prominent gill axis. The palps are short, twisted and flattened with cup shaped terminations. The presence or absence of a gut could not be confirmed.

Distribution. *Acharax gadirae* is presently only known from the mud volcano fields in the Gulf of Cadiz, Eastern Atlantic. The specimens have been taken from the Western Moroccan field at Yuma, Ginsburg and Jesus Baraza MVs, and from the deep-water field at Captain Arutyunov, Carlos Ribeiro and Porto MVs at depths between 975 to 3902m. A single specimen was recovered from the shallower El Arraiche field in Pen Duick Escarpment at 556m.

Etymology. *gadirae*, from the Phoenician "Gadir" the original name for Cadiz and meaning "walled fortification" and also the root of many Moroccan names such as Agadir. Named to indicate the widespread range across the Moroccan and Iberian margins.

Remarks. The genus *Acharax* is recognizable from the large external ligament and the generic placement of *A. gadirae* is confirmed.

The genus is rare in the Atlantic Ocean unlike the situation in the Pacific where species of *Acharax* are frequently recorded from chemosynthetic settings (Neulinger et al. 2006). Only two species are known from the Atlantic. *Acharax grandis* (Verrill and Bush 1898) is known only from the original material collected from depths between 548 and 2926m in the region of the New York Bight. *Acharax caribbaea* (Vokes 1970) again is only recorded from the original material collected from a depth of 350m off Colombia in the Caribbean Sea. However, the genus is recorded in recent studies from both the Gulf of Mexico (Carney 1994) and the Barbados prism (Olu et al. 1997) but the species are not identified.

Acharax grandis differs from both *A. gadirae* and *A. caribbaea* in being less inequilateral with the beaks distinctly more towards the mid-line. *Acharax caribbaea* differs from both *A. gadirae* and *A. grandis* in having very few (4) anterior ribs compared with the 6–8 on *A. grandis* and 8–9 on *A. gadirae* of similar size. Unfortunately, there are no anatomical data for either *A. grandis* or *A. caribbaea*, making a thorough comparison impractical.

There are no given ecological data for either *A. grandis* or *A. caribbaea*. The type locality for *A. grandis*, which is the region around the Hudson Shelf and Canyon, has no recorded seep or vent activity. In contrast the region around the type locality of *A. caribbaea* is known for a variety of chemosynthetic settings (Carney 1994).

The bathymetric range of *Acharax* in the Gulf of Cadiz is large, 556–3902m and specimens have been taken at many mud volcanoes raising the possibility that more than one species is involved. Unfortunately the specimens from the abyssal sites are all small about 10mm or less making comparison with the large specimens from the bathyal sites inconclusive. The specimens from Carlos Ribeiro MV (2200m) (Fig. 6C–D) are prominently wedge shaped in outline compared with the specimen from Porto MV (3902m) (Fig. 6B). The latter is not dissimilar to those from Capt. Arutyunov MV (1325m) (Fig. 6E) with the specimen from Pen Duick Escarpment (556m) (Fig. 6A) somewhat more elongate but not as wedge shaped as those from Carlos Ribeiro MV.

The specimens from the Capt Arutyunov MV are most problematic in that the siphonal opening appears to be devoid of any surrounding tentacles or papillae (Fig. 6H). This does not appear to be a function of size as similar specimens from other sites have siphonal papillae. Should this observation be confirmed in further material it would be appropriate to describe this as a separate species. Comparing the siphonal papillae of specimens from Pen Duick Escarpment (Fig. 6F) and Carlos Ribeiro MV (Fig. 6G) indicates a more complex arrangement in the latter but, with so few specimens, this is inconclusive.

Superfamily Lucinoidea Fleming, 1828

Family Lucinidae Fleming, 1828

The Lucinidae is, by far, the most disparate and species-rich family of chemosymbiotic bivalves and are thoroughly reviewed by Taylor and Glover (2006). Although they occupy a wide range of habitats, they are relatively infrequent in deep-sea settings (Taylor and Glover 2009). Some species are associated with cold seeps and mud volcanoes, oxygen minimum zones and a single species is known from a hydrothermal vent (Taylor and Glover 2006). Of most frequent occurrence in, but not exclusive to, deep-sea settings is the genus *Lucinoma* (Salas and Woodside 2002, Oliver and Holmes 2006a, Cosel 2006, Cosel and Bouchet 2008). Oliver and Holmes (2006a) and Cosel (2006) both comment on the considerable variation in shell form observed in some species, *L. gagei* Oliver and Holmes, 2006a and *L. myriamae* Cosel, 2006 respectively. This contrasts with the relatively small morphological differences cited for the discrimination of other species, e.g. *L. kazani* (Salas and Woodside 2002) and *L. vestita* (Dautzenberg and Fischer 1906 in Cosel 2006). In the latter instances geographic isolation and habitat preferences have played a major role in the in the interpretation of the significance of morphological variation.

Genus *Lucinoma* Dall, 1901

Type species. *Lucina filosa* Stimpson, 1851

Definition. As given by Oliver and Holmes (2006a)

Lucinoma asapheus sp. n.

urn:lsid:zoobank.org:act:E684B2EE-7C97-4FE9-9445-9B00CFE0259B
http://species-id.net/wiki/Lucinoma_asapheus
Fig. 7

Type material. *Holotype*; one complete specimen, live collected, TTR 15, stn AT-569GR, El Arraiche field, Mercator MV. 35°17.917'N, 06°38.717'W, 358m, 25 July 2005, NMWZ.2010.4.5.

Paratypes; five specimens, as holotype, NMWZ.2010.4.6.

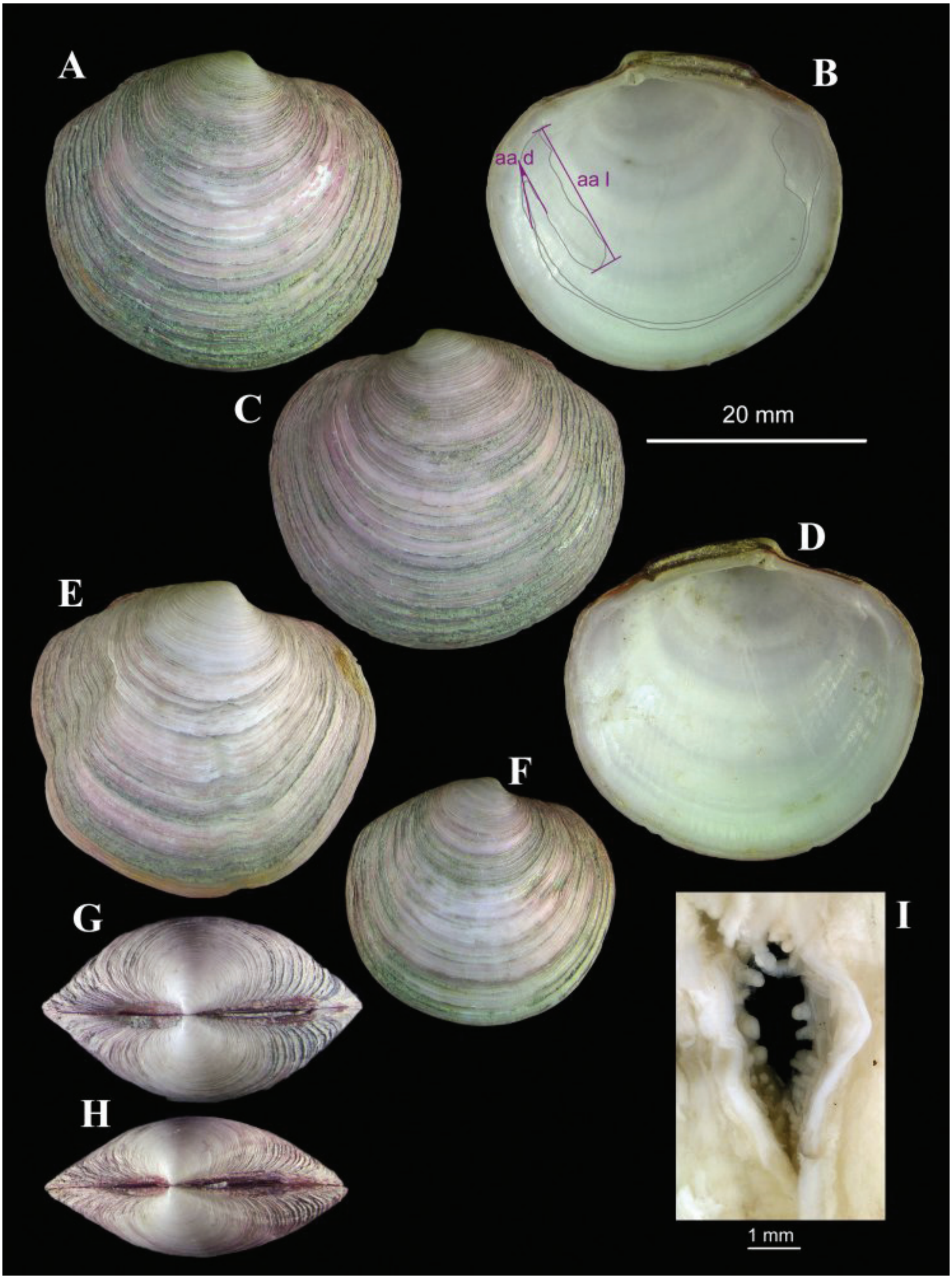

Figure 7. *Lucinoma asapheus* sp. n. stn. AT569GR, Mercator MV. **A–D** Holotype, aa d, angle of divergence of anterior adductor scar. aa l, length of anterior adductor scar **E** an aberrant specimen **F** a small specimen **G–H** two specimens showing variation in tumidity **I** the inhalant siphon.

Measurements (in mm)

	Length	Height	Width	Anterior scar length	Anterior scar angle	Lunule width
Holotype	33.3	30.1	15.9	15.0	15°	2.2
Paratype	25.0	23.0	10.8	11.3	17°	1.6
Paratype	30.7	27.6	18.0	14.6	16°	2.6
Paratype	28.8	27.1	13.5	13.0	15°	1.7
Paratype	32.1	29.2	16.4	16.4	18°	2.6
Paratype	31.7	29.4	15.4	16.4	15°	2.2

Description (Fig. 7). Shell to 34 mm in length. Solid. Equivalve. Equilateral. Tumidity variable (Fig. 7G, H) but mostly rather compressed. Umbos low, beaks pointing forward. Outline lenticular; posterior dorsal margin almost straight, sloping gently; posterior margin curved but less so than anterior; anterior dorsal margin short, a little concave. Escutcheon narrow, edges slightly raised, extending the length of the posterior dorsal margin; three-quarters filled by ligament, remainder smooth. Lunule distinct, width dependant on tumidity of shell; edges raised, sharp. Sculpture of numerous, low but erect, thin, concentric lamellae; between lamellae are weak concentric lines. Ligament external as a prominent, raised, arched band. Set on a narrow nymph. Hinge weak; two small cardinal teeth in each valve, RV anterior and LV posterior weakly bifid; anterior lateral protuberance distinct to obscure. Pallial line entire. Anterior adductor scar greatly elongate, approximately 3/4 free from pallial line. Shell white, periostracum thin but persistent, straw coloured (all material collected has been stained in Rose Bengal, thus the pink tinge).

The anatomy is essentially that described for *L. borealis* by Allen (1958) and for *L. kazani* by Salas and Woodside (2002). The inhalant siphon is surrounded by numerous short tentacles and papillae (Fig. 7I)

Variation. The shell can be rather compressed (Fig. 7H) or tumid (Fig. 7G) and this may be related to age rather than size as suggested by Oliver and Holmes (2006a) for *L. gagei* from the Arabian Sea. Some shells also show distortion with radial depressions developing abruptly (Fig. 7E).

Molecular data. Tissues were sent to Dr. John Taylor (NHM, London) for inclusion in his survey of Lucinidae and the 16S and CO1 genes were compared with those of *Lucinoma borealis*. The results although not entirely conclusive indicate that the two populations are not conspecific. More recently, John Taylor's group has demonstrated that *L. kazani* and *L. borealis* are distinct (J. Taylor pers. comm).

Distribution. Only found live at Mercator MV in the Gulf of Cadiz (358m).

Etymology. *asapheus* from *asaphes* Greek: meaning "indistinct" and "baffling", referring to the lack of distinctive morphological characters and the consequent unsettling taxonomic issues.

Remarks. A morphometric analysis was done comparing the Gulf of Cadiz shells with those of *L. borealis* from numerous localities from around the British Isles. This analysis could not demonstrate any statistically valid differences in the outline, the relative size of the anterior adductor scar (aa l on Fig. 7B) or the angle of divergence of this scar from the pallial line (aa d on Fig. 7B). It should be noted that the Cadiz sample size was small and that conclusive probability results were unlikely. However, the variation in tumidity and irregularity of some of the Cadiz shells is not found in samples of *L. borealis*. Anatomically *L. asapheus* and *L. borealis* are alike including the papillae that surround the inhalant aperture. Further evidence for the species level distinction between *L. asapheus* and *L. borealis* comes from the molecular data but here again the few specimens available curtails the analysis. Ecologically one might expect mud volcanoes and near shore sulphide enriched sediments to support different species. This argument was used by Salas and Woodside (2002) to support the distinction between *L. kazani* and *L. borealis*, but they also listed some morphological differences and this has been supported by molecular data (J. Taylor pers comm). Some of these, namely the tumidity of the valves, the width of the lunule and the expression of the lateral teeth are found here to be variable and therefore not conclusive. Similar variability was recorded for *L. gagei* (Oliver and Holmes 2006a) and *L. myriamae* (Cosel 2006) suggesting that small morphological differences in *Lucinoma* shells, especially if observed between small samples, may not be reliable taxonomic characters. The papillation of the inhalant siphon does appear to be much less developed in *L. kazani* compared with that in *L. borealis* and *L. asapheus*. The angle of divergence of the anterior adductor scar also shows a difference with that in *L. kazani* having a mean value of ca. 25° and both *L. borealis* and *L. asapheus* a mean value of ca. 15°. Given that *L. kazani* and *L. asapheus* both inhabit mud volcano settings one might expect them to be conspecific. However, accepting the morphological differences given by Salas & Woodside between *L. kazani* and *L. borealis* and that these also hold true for *L. asapheus* then the two must be considered distinct. This may be supported by the wide difference in depth range with *L. asapheus* coming from 358m in contrast to *L. kazani* from 1700 –2030m.

Other Eastern Atlantic species are *Lucinoma vestita* (Dautzenberg and Fischer 1906) from Cape Verde at 600m, *L. atalantae* Cosel, 2006 from Mauritania at c.2000m and *L. myriamae* Cosel, 2006 from the Angola margin at c.360m. Comparisons with *L. asapheus* are as follows. *Lucinoma vestita* is a smaller species not exceeding 16mm in length, more rounded in outline and with a weak sculpture of poorly developed (often absent) comarginal lamellae. *Lucinoma atalantae* has a distinctly longer and more steeply sloping anterior dorsal margin, a more angular posterior profile and irregular sculpture. *Lucinoma myriamae* is much larger reaching over 50mm in length and has a distinct angular anterior profile; in this respect, it resembles *L. saldanhae* Barnard, 1964 a species not considered by Cosel (2006).

Family Thyasiridae Dall, 1901

The Thyasiridae of the Gulf of Cadiz were reported on by Rodrigues et al. (2008) and of the seven species recognized only one was closely associated with active mud volcanoes, namely *T. vulcolutre* Rodrigues & Oliver, 2008). Here we report upon an additional species to the Gulf of Cadiz from the Captain Arutyunov MV. Since the publication of Rodrigues et al. (2008) thyasirids from the Eastern Mediterranean mud volcanoes and the REGAB site off West Africa have become available for study. These species will not be described here but are compared with *T. vulcolutre*.

Genus *Spinaxinus* Oliver & Holmes, 2006b

Type species. *Spinaxinus sentosus* Oliver & Holmes, 2006b

Definition. As given by Oliver and Holmes (2006b)

Spinaxinus cf. *sentosus* Oliver & Holmes, 2006b

http://species-id.net/wiki/Spinaxinus_sentosus
Fig. 8A–D

Material examined. One live collected specimen, MSM01.03, Stn 190, deep-water field, Captain Arutyunov MV, 35°39.665'N, 07°19.970'W, 1322m, 28 April 2006, NMWZ.2010.4.7

Description. (Fig. 8A–D). This specimen measures only 2.3 mm in length and is damaged. The outline agrees with that of *S. sentosus* in being extended anteriorly with a long lunule depression and in the presence of a long but shallow posterior sulcus. These features are in contrast to the juveniles of *Thyasira vulcolutre* (Rodrigues and Oliver 2008), which also occurs at similar depths and settings.

The periostracal spines are typical of *Spinaxinus* but are not seen in this specimen. The periostracum is coarse and the vestiges of lamellae and projections can be seen on the extreme edges of the shell especially on the ventral margin.

Remarks. Although the identification is not conclusive the likelihood that this shell is a juvenile *Spinaxinus* is high and as such represents the first finding of this species in a non-anthropogenic setting. The proximity of the type locality (off northern Portugal) to the Cadiz mud volcanoes makes this supposition more reasonable.

Thyasira vulcolutre Rodrigues & Oliver, 2008

http://species-id.net/wiki/Thyasira_vulcolutre

Comments. *Thyasira vulcolutre* belongs to a group of thyasirids with relatively large shells with weakly defined posterior sulci. It was concluded that it was most similar to *T. southwardae* (Oliver and Holmes 2006b) from the Anyas Garden site. At that time no thyasirid material had been found at cold seep sites along the West African margin. Consequently, unlike the situation for *Lucinoma* (Cosel 2006) and *Isorropodon* (Cosel and Salas 2001, Cosel and Olu 2008) no further biogeographic comparisons could be made. Recently we were able to examine a thyasirid (Fig. 8E–F) from the REGAB site (courtesy of Karine Olu) and although it superficially

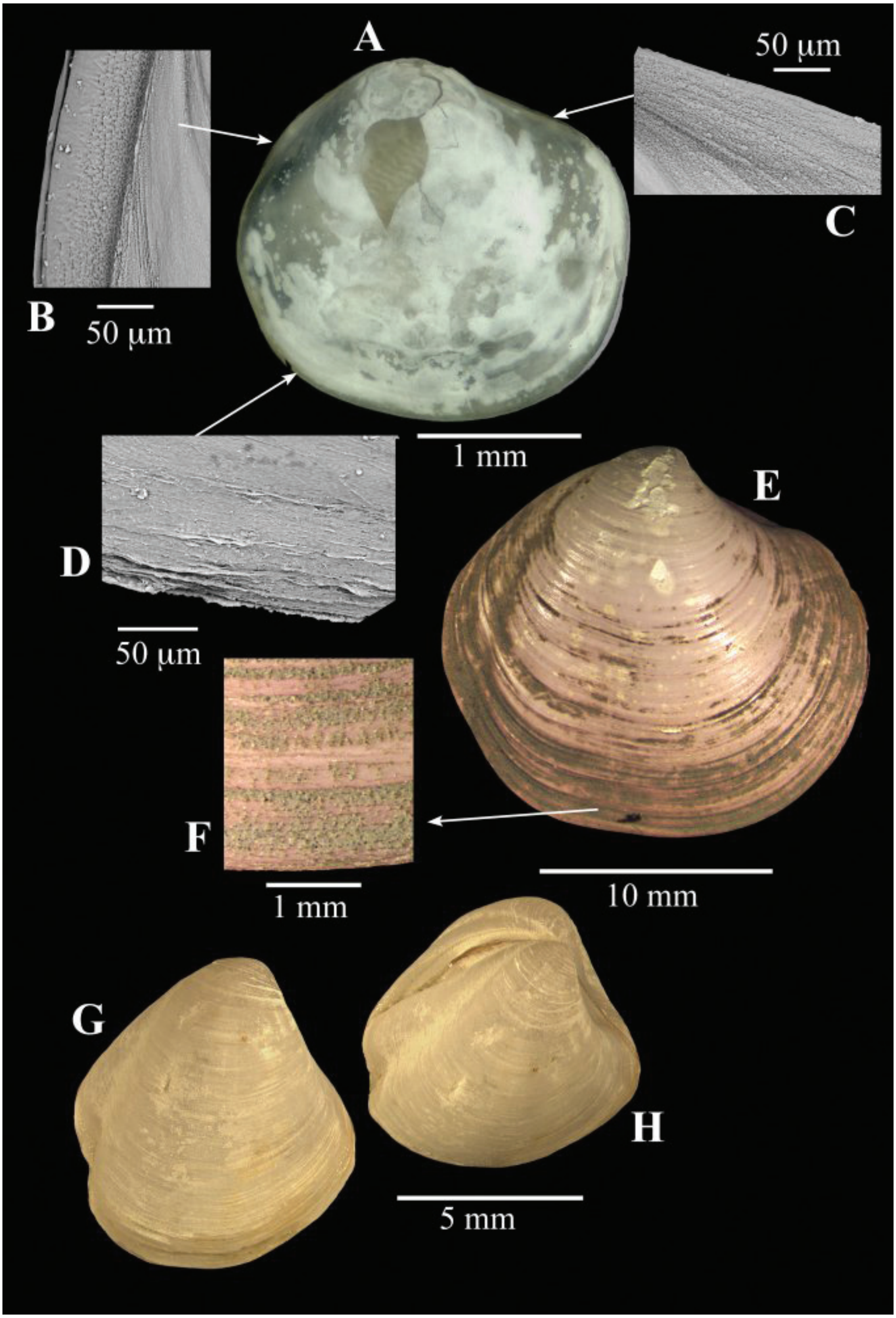

Figure 8. A–D *Spinaxinus* cf. *sentosus* Oliver & Holmes, 2006b. stn. 190, Captain Arutyunov MV. **A** digital image of right valve **B** SEM, periostracum on posterior margin **C** SEM, periostracum on anterior dorsal margin **D** SEM, periostracum on ventral margin. **E–F** *Thyasira* sp., Regab pock mark **E** external of right valve **F** periostracum. **G–H** *Thyasira striata*, Sturany, MEDINAUT, Eastern Mediterranean **G** external of right valve **H** oblique view showing posterior sulci.

resembles *T. vulcolutre* it significantly differs in having a minutely spicate periostracum (Oliver in prep).

Thyasira striata Sturany, 1896 has long been known from deep water in the eastern Mediterranean but was recently re-discovered at cold seep sites (Olu-Le Roy et al. 2004). Small specimens resemble *T. flexuosa* but larger examples (Fig. 8G–H) are very tumid with prominent lunule and deep posterior sulci quite unlike *T. vulcolutre*.

These new data suggest that those thyasirids closely associated with active cold seeps have restricted ranges within the eastern Atlantic/Mediterranean region.

Superfamily Glossoidea Gray, 1847
Family Vesicomyidae Dall & Simpson, 1901

The family Vesicomyidae has become familiar as a group of large chemosymbiotic clams associated with hot vents (Boss and Turner 1980, Tunnicliffe 1991) and cold seeps (Turner 1985, Okutani and Métivier 1986) and exemplified by *Calyptogena magnifica* Boss and Turner 1980. Not all taxa are large and the smallest, such as *Vesicomya atlantica*, are probably not chemosymbiotic (Allen 2001). Despite their conspicuous presence in many reducing environments, the taxonomy of vesicomyids is far from being settled, at both the species and supraspecific levels (Krylova and Sahling 2006). Different authors estimate that the family includes from 50 to more than 70 recent and fossil species and new species are constantly erected (Cosel and Salas 2001, Krylova and Sahling 2006, Krylova and Janssen 2006, Cosel and Olu 2009, Krylova et al. 2010). To date, fifteen chemosymbiotic species in the genera *Waisiuconcha, Isorropodon, Callogonia, Wareniconcha, Elenaconcha, Calyptogena, Christineconcha, Laubiericoncha and Abyssogena* have been reported from the eastern Atlantic (Krylova and Sahling 2010).

Genus *Isorropodon* Sturany, 1896

Type species. *Isorropodon perplexum* Sturany, 1896

Definition. As given by Cosel and Salas (2001)

Isorropodon megadesmus sp. n.

urn:lsid:zoobank.org:act:317BA11E-0B29-4396-8696-DEDCAF00B29F
http://species-id.net/wiki/Isorropodon_megadesmus
Figs 9, 10C–D

Material examined. *Holotype*: one complete specimen, live collected, MSM01.03, stn 218, deep-water field, Captain Arutyunov MV. 35°39.642'N, 07°20.049'W, 1321m, 30 April 2006, NMWZ.2010.4.8.

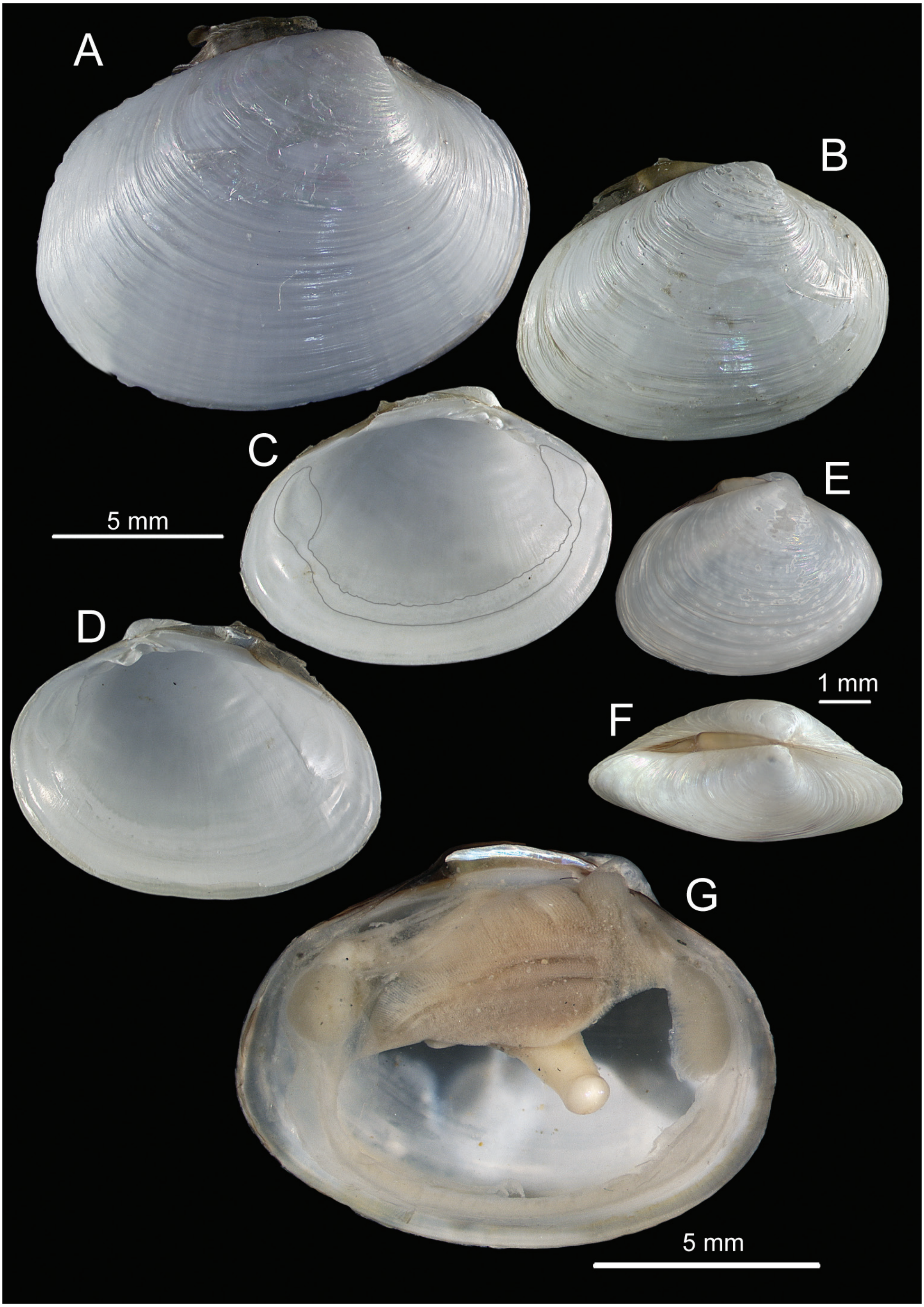

Figure 9. *Isorropodon megadesmus* sp. n. stn. 218, Captain Arutyunov MV. **A** large right valve, paratype **B–D** holotype, right valve external, left valve internal, right valve internal **E** small right valve, paratype **F** dorsal view, paratype **G** gross anatomy viewed after removal of right valve and mantle.

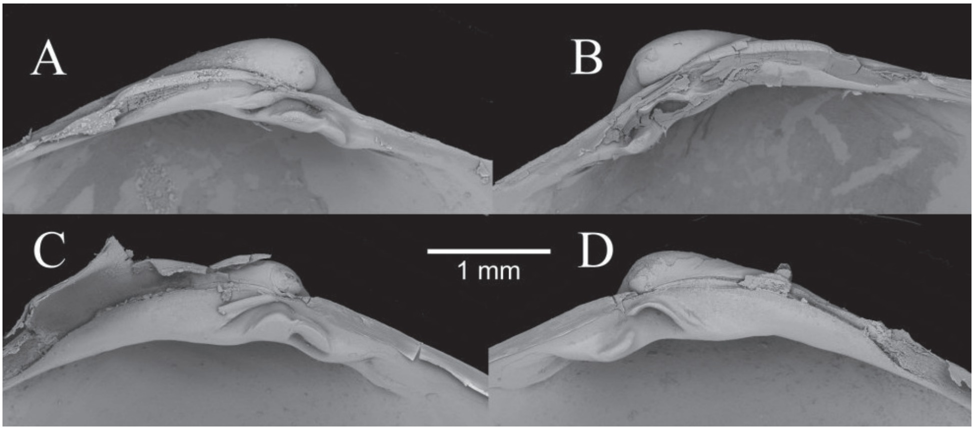

Figure 10. Comparison between the hinge teeth of *Isorropodon* sp. indet. (**A, B**) and *Isorropodon megadesmus* sp. n. (**C, D**).

Paratypes: ten specimens, four shells and one valve, same data as holotype, NMWZ.2010.4.9.

Other material examined: over thirty decalcified juvenile specimens, MSM01.03, stn 218, deep-water field, Captain Arutyunov MV. 35°39.642'N, 07°20.049'W, 1321m, 30 April 2006; one specimen, MSM01.03, stn 225, same locality, 35°39.707'N, 07°20.020'W, 1322m, 4 May 2006.

Measurements (in mm)

	Length	**Height**	**Tumidity** **One valve (paired)**	**Ratio (L/T)**
Holotype	11.2	8.2	2.3 (4.6)	2.4
Paratype	14.8	11.5	3.25 (6.5)	2.3
Paratype	10.6	7.7	2.3 (4.6)	2.3
Paratype	6.2	4.4	1.3 (2.6)	2.4
Paratype	6.6	4.5	1.3 (2.6)	2.5
Paratype	9.1	6.4	1.85 (3.7)	2.5
Paratype	9.6	7.2	incomplete	

Description. (Figs 9, 10C–D). To 15mm in length. Thin. Equivalve. Inequilateral, beaks in front of the midline. Compressed, length to tumidity ratio 2.3 to 2.5. Outline subovate, anterior rounded, posterior a little obliquely truncated; ventral curvature at its maximum well to the posterior of the mid line. Lunule indistinct, not depressed. Escutcheon narrow, deeply excavated but entirely occupied by ligament. Sculpture of dense concentric lines and irregular growth stops or wrinkles. Hinge plate prominent dominated by a long nymph supporting a very large external ligament; ligament rises well above the dorsal margin of the shell and extends posteriorly beyond the nymph to fill the escutcheon. Hinge teeth complex; RV with a single prominent anterior lateral tooth situated in front of the beak in the form of a narrow projecting peg with a flat or slightly excavated dorsal surface; below the beak is an arched laminar tooth its anterior end overlapping the lateral tooth, its posterior slopes steeply and ventrally and merges with a second ridge only noticeable by a weak notch mid way on this combined ridge. LV with a thin laminar posterior cardinal angled obliquely plus two combined cardinals in a horizontal orientation the posterior part larger than the anterior with a distinct notch between the two parts. Pallial line entire with a very small straightened section below the posterior adductor scar; adductor scars of about equal size; anterior pedal retractor scar deeply impressed, situated immediately in front of the hinge plate. Periostracum thin, persistent, glossy. Shell white.

Mantle thin, mantle edge unfused except for short inhalant and exhalant siphonal apertures; inhalant aperture with many papillae increasing in size dorsally, exhalant with papillae of equal size. Foot with a distinct finger-like toe and poorly developed heel, pedal retractors prominent, the anterior attached in a deep impression close to the hinge. Anterior adductor muscle oval in cross-section, posterior adductor muscle subcircular, smaller than the anterior one. Ctenidia of a large, single (inner) demibranch, ascending part approximately one half the height of the outer, filaments fine tightly connected.

Distribution. *Isorropodon megadesmus* is restricted to Captain Arutyunov MV (1321–1322m).

Etymology. *megadesmus* from the Greek *mega* meaning large and *desma* meaning bond; referring to the external ligament.

Remarks. The taxonomy of *Isorropodon* in the Atlantic and Mediterranean is complex and potentially confused (Cosel and Salas 2001, Cosel and Olu 2009). Cosel and Salas (2001) described two new species from the Eastern Atlantic, namely *I. bigoti* and *I. curtum*. They transferred a third from *Kelliella*, namely *I. elongatum* (Allen 2001). In discussing, the Mediterranean, *I. perplexum* Cosel and Salas (2001) stated that *Isorropodon* species are variable with regard to outline, tumidity and development of hinge teeth and this is illustrated in their figures 36–47 for *I. perplexum*. They noted similarities in shell morphology between the Eastern Mediterranean species *I. perplexum* and the West African *I. bigoti* but suggested that these taxa were isolated geographically and doubtfully could have gene flow between them. They further supported this argument by stating that *I. perplexum* had not been found in the Western Mediterranean or Ibero-Moroccan Gulf. In 2009, Cosel and Olu described another *Isorropodon* from West Africa (*I. atalantae*) and placed another vesicomyid in this genus (*I. striatum* Thiele and Jaeckel 1931). Therefore, before the discovery of *Isorropodon* in the Gulf of Cadiz there were already five west African species and one from the eastern Mediterranean. With the discovery of *Isorropodon* at the Capt. Arutyunov MV the assertion made by Cosel and Salas (2001) on genetic isolation can be questioned, as there is the possibility of gene flow between the seeps in the Mediterranean and around the east African coast.

In contrast to the variability given by Cosel and Salas (2001) for *I. perplexum,* all of the shells from station 218 examined here are constant with regard to features of outline, tumidity and hinge teeth. However, a single shell from station 180 is distinct, being inflated, having a distinct lunule, having a much smaller ligament and in the ventral margin being more convex. The shells from station 218 are distinct from all the shells of *I. perplexum* figured by Cosel and Salas (2001) in having a much longer nymph with the posterior teeth reaching only about one third of the ligament as opposed to the half distance given for *I. perplexum* by Cosel and Salas (2001). Furthermore, most of the shells illustrated by them have a more convex ventral margin than the shells from station 218. The single shell from station 180 shares more features with the Mediterranean shells in having the small ligament, convex ventral margin and being more inflated.

The outline of *I. bigoti* differs from all of the above in the narrower anterior and distinct angulation of the ventral curve, but is has a short nymph similar to *I. perplexum* and the shell from station 180.

Isorropodon atalantae has a more sunken lunule and more angular posterior profile than either of the Gulf of Cadiz taxa. *Isorropodon curtum* Cosel and Salas, 2001, from off Mauritania, is more circular in outline and *I striatum* Thiele and Jaeckel, 1931 from off Angola, is a much larger and more elongate form.

Cosel and Salas (2001) reassigned *Kelliella elongata* Allen, 2001 to the genus *Isorropodon*. Following examination of the type material in the Natural History Museum, London (BMNH 1998180) we conclude that it is not conspecific with any of the taxa discussed here. It is a small species not exceeding 2mm in any of the over 300 specimens listed by Allen (2001). It is inflated with a distinct lunule but the demarcating line illustrated by Allen (2001) is not so apparent. The hinge of the right valve has three distinct teeth including a small posterior tooth (4b in Allen 2001), which is not present in either of the species from the Cadiz mud volcanoes. Furthermore, the ligament is small and does not project as in *I. megadesmus*. From the ctenidial anatomy there is no indication that this species is chemosymbiotic. In addition to the morphological differences, *I. elongatum* has been collected from a wide geographical range, wide bathymetric range and associated with the typical oligotrophic deep-sea bivalve assemblage (derived from Allen 2008). It would appear that *I. elongatum*, if a chemosymbiotic species is not confined to seep/vent settings but as stated by Allen (2001) it is absent from the European basin and it was not present in the samples taken in the Gulf of Cadiz away from the vicinity of the mud volcanoes (Rodrigues 2009).

***Isorropodon* sp indet**

Figs 10A–B, 11

Material examined. One complete specimen, live collected, MSM01.03, stn 180, deep-water field, Captain Arutyunov MV. 35°39.740'N, 07°19.960'W, 1323m, 27 April 2006, NMWZ.2010.4.10.

Measurements. 6.4mm (L) × 5.3mm (H) × 3.6mm (T)

Description (Figs 10A–B, 11). 6.4 mm in length. Thin. Equivalve. Inequilateral, beaks in front of the midline. Inflated, length to tumidity ratio = 1.8. Outline subovate, anterior bluntly rounded, posterior a little obliquely truncated; ventral curvature at its maximum more or less at the midline. Lunule indistinct, slightly depressed. Es-

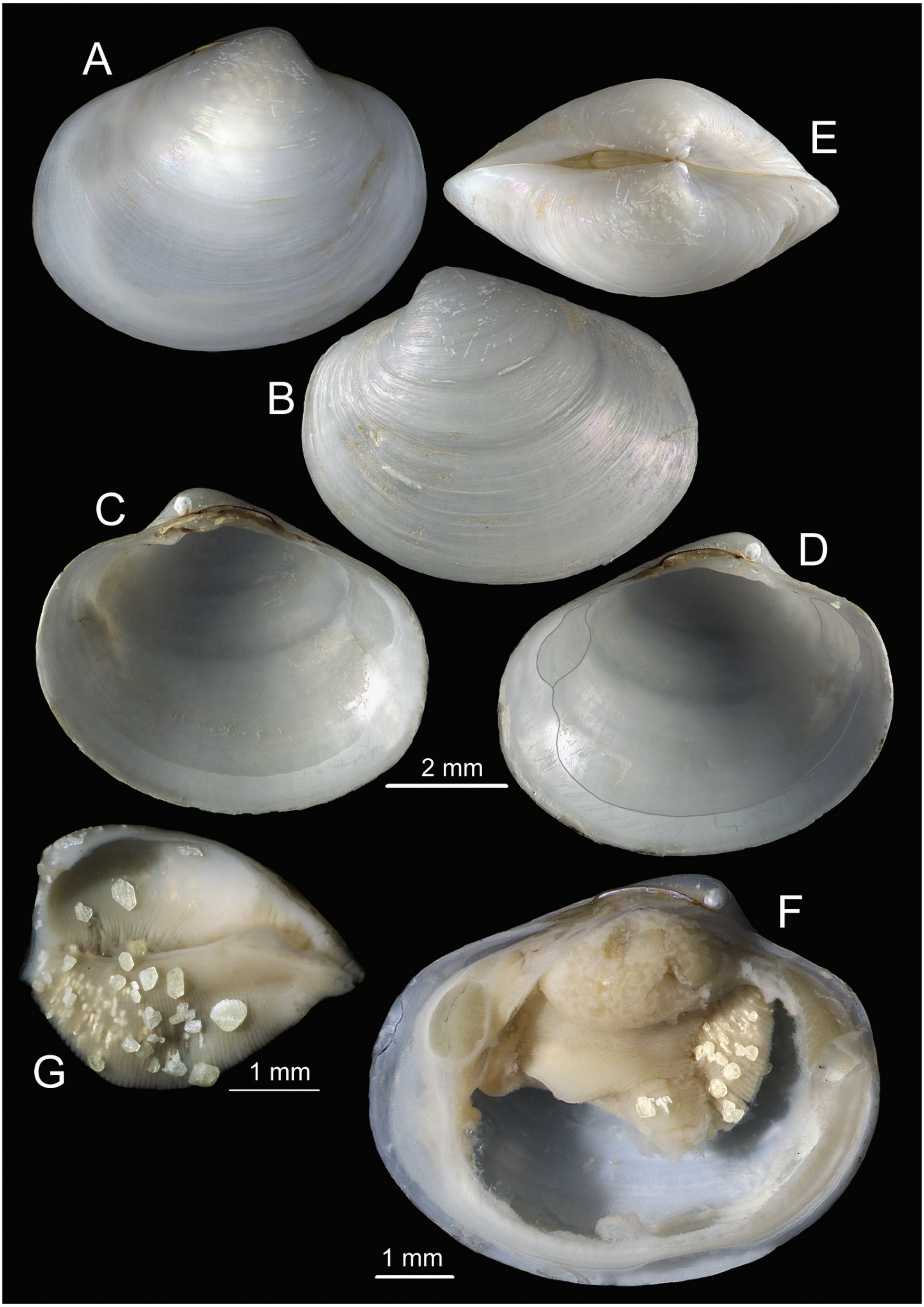

Figure 11. *Isorropodon* sp. indet., stn. 180 Captain Arutyunov MV. **A–D** External and internal views of right and left valves **E** dorsal view **F** gross anatomy viewed after removal of right valve and mantle **G** Excised ctenidium with crystalline artifacts.

cutcheon narrow, deeply excavated. Sculpture of dense, concentric, fine lines and few irregular growth stops. Hinge plate narrow, nymph supporting an external ligament; ligament scarcely rises above the dorsal margin of the shell and extends posteriorly to half the length of the escutcheon. Hinge teeth complex; RV with a single prominent anterior lateral tooth situated in front of the beak in the form of a narrow projecting peg with a flat or slightly excavated dorsal surface; below the beak is weakly arched laminar tooth its anterior end overlapping the lateral tooth, its posterior slopes steeply and ventrally and merges with a second ridge only noticeable by a weak notch mid way on this combined ridge. LV with a thin laminar posterior cardinal angled obliquely plus two combined cardinals in a horizontal orientation the posterior part only slightly larger than the anterior with a distinct notch between the two parts. Pallial line entire with a very small straightened section below the posterior adductor scar; adductor scars of about equal size; anterior pedal retractor scar deeply impressed, situated immediately in front of the hinge plate. Periostracum thin, persistent, glossy. Shell white

Mantle thin, mantle edge unfused except for short inhalant and exhalant siphonal apertures; inhalant aperture with few papillae increasing in size dorsally, the latter as short tentacles, exhalant with papillae of equal size. Foot with a blunt finger-like toe and poorly developed heel, pedal retractors prominent, the anterior attached in a deep impression close to the hinge. Anterior adductor muscle pyriform in cross-section, posterior adductor muscle subcircular, smaller than the anterior one. Ctenidia of a large, single (inner) demibranch, filling the majority of the mantle cavity, ascending part approximately one half the height of the outer, filaments fine tightly connected.

The numerous crystalline growths seen on and between the filaments are believed to be natural and not an artifact of preservation.

Distribution. *Isorropodon* sp indet is restricted to Captain Arutyunov MV (1323m).

Remarks. As discussed above for *I. megadesmus*.

Ecological Discussion

Symbiosis. Twelve bivalve species have been found in close association with chemosynthetic settings in the Gulf of Cadiz (this paper; Génio et al. 2008, Rodrigues et al. 2008, Rodrigues et al. 2010) with a thirteenth, *Callogonia cyrili* Cosel and Salas probable but not proven (Cosel and Salas 2001). The trophic status of these Solemyidae, Lucinidae, Thyasiridae and Vesicomyidae species has been confirmed by their gross anatomical features (e.g. gills for thyasirids, reduced gut for solemyids) and, in some cases, also by stable isotope analysis and/or molecular analysis (Rodrigues et al. 2010, Rodrigues and Duperron 2011). The $\delta^{13}C$ values for solemyids (*Acharax gadirae*, *Solemya* (*Petrasma*) *elarraichensis*), lucinid (*Lucinoma asapheus*) and thyasirid (*Thyasira vulcolutre*) bivalves were found to be in line with data for other bivalves known to host thiotrophic symbionts (Fisher 1990, Carlier et al. 2010). On the other hand, $\delta^{13}C$ and $\delta^{34}S$ values for the bathymodiolid species (*Bathymodiolus mauritanicus*) were compatible with the predominance of methanotrophy. Phylogenetic analysis of bacterial 16S rRNA gene sequences demonstrated that most bacteria were related to known sulfide-oxidizing endosymbionts found in other deep-sea chemosynthetic environments, with the co-occurrence of methane-oxidizing symbionts in *Bathymodiolus* specimens. The molecular results confirmed the thiotrophic nutrition for *S.* (*P.*) *elarraichensis*, *A. gadirae*, *L. asapheus* and *T. vulcolutre* and a dual symbiosis for *B. mauritanicus* (Rodrigues et al. 2010). Nutrition of *Isorropodon megadesmus* was not yet confirmed by either isotopic or molecular analyses but other studies suggest that *Isorropodon perplexum* contain sulphur-oxidizing bacteria (Olu-Le Roy et al. 2004), a phylotype also present in *Spinaxinus sentosus* (Oliver and Holmes 2006b).

Distribution patterns. The Gulf of Cadiz mud volcano field is comprised of over thirty seeps of various activity and spread over a bathymetric range of 200–4000m. Of the 25 mud volcanoes sampled, 13 have chemosymbiotic species, which indicates their importance in the structure of the seep assemblages (Fig. 1). Most of the thirteen, chemosymbiotic, species found in the Gulf of Cadiz are restricted to one or two mud volcanoes. This patchy distribution can result from physical or physiological constraints such as depth, distance and fluid flow rates. They were more frequent in the shallower mud volcanoes (200–1500m) but were especially diverse in Captain Arutyunov MV where five different species co-occur (Fig. 1). Some taxa are confined to single mud volcanoes whereas others are more widespread.

The family most frequently encountered in the chemosynthesis-based assemblages of the mud volcanoes from the Gulf of Cadiz, is the Solemyidae. The family is represented by two genera, *Solemya* (*Petrasma*) with a shallower distribution (358–1030m) and *Acharax* with a deeper distribution (556–3902m) but co-occurring at intermediate depths in the Western Moroccan field. Co-occurrence of these genera has not been reported elsewhere and may be explained by the apparent absence of the subgenus *Petrasma* from seep settings preferring reducing sediments and low oxygen conditions (Kamenev 2009).

This is the first record of this family in cold seeps from the North-east Atlantic, although *Solemya* (*Solemya*) *togata* is well known from shallow settings such as sea-grass beds in the Mediterranean. Why this species has not or been unable to colonize the shallow mud volcanoes is unknown. In contrast, the Pacific *Solemya* (*S.*) *tagiri* Okutani et al. (2003) is thought to be associated with seep settings (Kamenev 2009) suggesting that habitat is not linked to phylogeny.

Unlike *Solemya* (*Petrasma*), *Acharax* species are consistently associated with seep or vent settings and some species such as the Pacific *A. johnsoni* have extensive bathymetric ranges from 100 to over 5000m (Kamenev 2009). *Acharax* species are all rather morphologically similar and given the molecular data given by Neulinger et al. (2006) it may be found that there are more species than currently recognized. This is suggested here by the morphological differences in the posterior aperture between the shallow and deeper specimens. Some *Acharax* specimens (from Yuma, Ginsburg and Jesus Baraza MVs) host commensal polychaetes (Ravara et al. 2007).

In contrast to the solemyids, the lucinid *Lucinoma asapheus* has only been collected at Mercator MV, although video observations revealed presence of lucinids in other mud volcanoes from the Spanish field (MR Cunha, pers. comm.). *Lucinoma asapheus* is very similar morphologically to *Lucinoma kazani* from the Eastern Mediterranean (Salas and Woodside 2002) and both are almost identical with the widespread shelf species *Lucinoma borealis* that is abundant in the North-east Atlantic. Our preliminary molecular data separated *Lucinoma borealis* from *L. asapheus*, and further work on *L. kazani* confirms that it is also distinct from *L. borealis* (J Taylor, pers comm.). A more detailed study including a wider data set from *L. asapheus* should elucidate the relationship between it and *L. kazani*. The three species so far mentioned are similar but quite distinct from the much larger, deeper water SE Atlantic species such as *L. myriamae* and *L. saldanhae* and the Indo-Pacific species, *L. gagei* and *L. yoshidai* (Oliver and Holmes 2006a). It is therefore plausible that *L. asapheus, L. kazani* and *L. borealis* are a clade, but we cannot ascertain their sequence of appearance. Are the mud volcano species independently evolved from shallow water ancestors or are they descended from a common seep dwelling stock?

The thyasirid *Thyasira vulcolutre* was only found in the deep-water mud volcano field (Rodrigues et al. 2008). Despite the high number of thyasirid species present in the Gulf of Cadiz (eight) only one *Thyasira vulcolutre* is strictly associated with active mud volcanoes (Rodrigues et al. 2008). *Spinaxinus sentosus* has been discovered in the organic cargo of a sunken ship in the Atlantic Ocean (Oliver and Holmes 2006b) and is reported here for the first time associated to a cold seep site. Other chemosymbiotic thyasirids (Table 1) were found associated with Siboglinidae fields from the Hakon-Moseby MV (Gebruk et al. 2003) and from the eastern Mediterranean mud volcanoes (Olu-Le Roy et al. 2004).

Small vesicomyids including *Isorropodon megadesmus* and *Isorropodon sp.* were very abundant in Captain Arutyunov MV. The species *I. perplexum* is known only from the Eastern Mediterranean (Table 1), and was shown to harbour sulphur-oxidizing bacteria (Salas and Woodside 2002). During TTR17 (2008) a living specimen of *Calyptogena* was found for the first time in the Gulf of Cadiz. The specimen collected from Bonjardim has been identified as belonging to the species *Christineconcha regab* Cosel and Olu, 2009, recently found in the Regab region (E Krylova, pers. comm.). Other large vesicomyid shells but no living specimens of *Laubiericoncha chuni* (Thiele and Jaeckel 1931) and *Pliocardia* sp. were also collected at the same mud volcano. The vesicomyids *C. regab* and *L. chuni* dominate the faunal assemblages of Congo and Angola Basin cold seeps (Olu-Le Roy et al. 2007, Cosel and Olu 2008).

Bathymodioline mussels of the amphi-Atlantic species *Bathymodiolus mauritanicus* were only found living in Darwin MV although extensive graveyards of mussel shell ash are also found in other mud volcanoes of the western Moroccan field (Génio et al. 2008). Although confirmed as chemosymbiotic, *Bathymodiolus mauritanicus* was first collected during a commercial trawl in the Mauritanian margin where no hydrocarbon seeps have yet been discovered. Nevertheless owing to the repeated appearance of other typical seep molluscs it is likely the existence of seepage in this region might be confirmed in the future (Cosel 2002). Another small mytilid *Idas* sp. was collected living in a small wood fall retrieved by the ROV Isis at Carlos Ribeiro MV during JC10. *Idas*

Table 1. Distribution of chemosymbiotic taxa known from seep/mud volcano fields in the Eastern Atlantic and Mediterranean.

Taxon	**East. Mediterranean**	**Gulf of Cadiz**	**Mauritania Basin**	**Gulf of Guinea**
Solemyidae				
Acharax indet.				**X**
Acharax gadirae		**X**		
Solemya (*Petrasma*) *elarraichensis*		**X**		
Solemyidae Eastern Med	**X**			
Mytilidae				
Bathymodiolus mauritanicus		**X**	**X**	
B. aff. *boomerang*				**X**
Idas modiolaeformis	**X**			
Idas sp.	**X**	**X**		
Lucinidae				
Lucinoma asapheus		**X**		
Lucinoma kazani	**X**			
Lucinoma atalantae			**X**	
Lucinoma myriamae				**X**
Myrtea amorpha	**X**			
Graecina karinae				**X**
Joellina dosiniformis				**X**
Thyasiridae				
Thyasira vulcolutre		**X**		
T. striata	**X**			
T. sp. n.				**X**
Spinaxinus sentosus		**X**		
Vesicomyidae				
Isorropodon perplexum	**X**	**X**?		
I. megadesmus		**X**		
I. bigoti			**X**	**X**
I. curtum			**X**	
I. striatum				**X**
I. atalantae				**X**
Callogonia cyrili		**X**		
C. mauritanica			**X**	
Calyptogena valdiviae			**X**	**X**
Christineconcha regab		**X**?		**X**
Wareniconcha guineensis				**X**
Elenaconcha guiness				**X**
Laubiericoncha chuni		**X**?		**X**
Pliocardia sp.		**X**		
Abyssogena southwardae			**X**	
Totals ( ) shared species	**6** (1?) (0) (0)	(1?) **13** (1) (2)	(0) (1) **7** (2)	(0) (1) (2) **14**

specimens are present in the mud volcanoes from the Eastern Mediterranean (Table 1) and their symbioses have been studied in detail by Duperron et al. (2008).

When discussing *Isorropodon*, Cosel and Salas (2001) suggested that Mediterranean and SE Atlantic forms were unlikely to be the same species due to their geographic isolation. With the discovery of *Isorropodon* in the Gulf of Cadiz, this argument can be challenged. Here we review the distributions of all chemosymbiotic taxa found at seeps in the Eastern Atlantic/Eastern Mediterranean and consider the apparent levels of endemism. Table 1 presents the known taxa and their occurrence in the four major seep fields, namely Eastern Mediterranean, Gulf of Cadiz, off Mauritania and the Gulf of Guinea.

The Gulfs of Cadiz and Guinea are most diverse and almost equally so with 13 and 14 species respectively. The less sampled Mauritania basin has only 7 recorded species while the well studied Eastern Mediterranean has only 6 species. The number of species common to more than two fields is zero and the maximum number of shared species is two, that for the Gulfs of Cadiz and Guinea. These data suggest high levels of endemism within fields but where there is overlap, especially with the larger vesicomyids, that this occurs at deeper sites. The vesicomyids are the most diverse family but only one species has colonized the Eastern Mediterranean and they appear to be rare in the Gulf of Cadiz compared with the Gulf of Guinea. From a geological history perspective one can explain the poor diversity in the Eastern Mediterranean from the shorter period of time for colonization since the re-invasion of Atlantic waters post the hypersaline event. At this time there is no evidence to indicate the origins of the Eastern Atlantic faunas, either by dispersal or local speciation. Molecular data from the species rich genus *Isorropodon* may illuminate the relationships and sequence of speciation and we await the study in progress on the Vesicomyidae mentioned by Cosel and Olu (2008). Warén and Bouchet (2009) in discussing seep gastropods stated that the West African fauna was quite distinct from that of the Gulf of Cadiz at both species and generic levels and suggested that the latter was of more recent origin via local radiation. At the generic level, these findings appear at odds with the bivalve data where there are strong similarities between the Gulfs of Guinea and Cadiz. The exceptions are *Solemya* (*Petrasma*), which appears to have a NW Atlantic origin and *Lucinoma* species, where their origin may be local.

Warén and Bouchet (2009) further indicated strong affinities of the West African fauna with the Western Atlantic and Gulf of Mexico faunas. Relationships among the bivalves with the western Atlantic at the species level are few, if any; only with *Bathymodiolus mauritanicus* is there a suggestion of an amphi-Atlantic distribution (Génio et al. 2008). At the generic level there is considerable over-lap within the solemyids, lucinids and thyasirids but less so with the vesicomyids. This may be in part to the considerable number of new genera erected for eastern Atlantic taxa by Cosel and Olu (2008, 2009).

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References

Abbott RT (1974) American Seashells; the marine Mollusca of the Atlantic and Pacific Coasts of North. America. Van Nostrand Reinhold, New York, 663 pp.

Akhmetzhanov AM, Ivanov MK, Kenyon NH, Mazzini A (Eds) (2007) Deep water cold seeps, sedimentary environments and ecosystems of the Black and Tyrrhenian Seas and the Gulf of Cadiz. Preliminary results of investigations during the TTR-15 cruise of RV Professor Logachev, June-August, 2005. IOC Technical Series, 72.

Akhmetzhanov AM, Ivanov MK, Kenyon NH, Mazzini A (Eds) (2008) Deep water depositional systems and cold seeps of the Western Mediterranean, Gulf of Cadiz and Norwegian continental margins. Preliminary results of investigations during the TTR-16 cruise of RV Professor Logachev, May-July, 2006. IOC Technical Series, 76: 58 pp + Annexes.

Allen JA (1958) On the basic form and adaptations to habitat in the Lucinacea (Eulamellibranchia). Philosophical Transactions of the Royal Society B, 241: 421–484. doi:10.1098/rstb.1958.0010

Allen JA (2001) The family Kelliellidae (Bivalvia: Heterodonta) from the deep Atlantic and its relationship with the family Vesicomyidae. Zoological Journal of the Linnean Society 131: 199–226.

Allen JA (2008) Bivalvia of the Deep Atlantic. Malacologia 50(1–2): 57–173. doi:10.1111/j.1096-3642.2001.tb01315.x

Barnard KH (1964) Contributions to the knowledge of South African marine Mollusca. Part V. Lamellibranchiata. Annals of the South African Museum 47: 361–593. doi:10.4002/0076-2997-50.1.57

Boss KJ, Turner RD (1980) The giant white clam from the Galapagos Rift, *Calyptogena magnifica* species novum. Malacologia 20: 161–194.

Carlier A, Ritt B, Rodrigues CF, Olu K, Sarrazin J, Grall J, Clavier J (2010) Heterogeneous energetic pathway and carbon sources for deep eastern Mediterranean cold-seep communities. Marine Biology 157(11): 2545–2565. doi:10.1007/s00227-010-1518-1

Carney RS (1994) Consideration of the oasis analogy for chemosynthetic communities at Gulf of Mexico hydrocarbon vents. Geo-Marine Letters 14: 149–159. doi:10.1007/BF01203726

Conway NM, Howes BL, McDowell Capuzzo JE, Turner RD, Cavanaugh CM (1992) Characterization and site description of *Solemya borealis* (Bivalvia: Solemyidae), another bivalve-bacteria symbiosis. Marine Biology 112: 601–613. doi:10.1007/BF00346178

Cosel R von (2002) A new species of bathymodioline mussel (Mollusca, Bivalvia, Mytilidae) from Mauritania (West Africa) with comments on the genus Bathymodiolus Kenk and Wilson, 1985. Zoosystema 24(2): 259–271.

Cosel R von (2006) Taxonomy of tropical West African bivalves. VI. Remarks on Lucinidae (Mollusca: Bivalvia), with descriptions of six new genera and eight new species. Zoosystema 28(4): 805–851.

Cosel R. von, Bouchet P (2008) Tropical deep-water lucinids (Mollusca: Bivalvia) from the Indo-Pacific: essentially unknown, but diverse and occasionally gigantic. In: Héros V, Cowie R, Bouchet P (Eds) Tropical Deep Sea Benthos, volume 25. Mémoires du Muséum National d'Histoire Naturelle 196: 115–21.

Cosel R von, Salas C (2001) Vesicomyidae (Mollusca: Bivalvia) of the genera *Vesicomya*, *Waisiuconcha*, *Isorropodon* and *Callogonia* in the eastern Atlantic and Mediterranean. Sarsia 86: 333–366.

Cosel R von, Olu K (2008) A new genus and new species of Vesicomyidae (Mollusca, Bivalvia) from cold seeps on the Barbados accretionary prism, with comments on other species. Zoosystema 30(4): 924–944.

Cosel R von, Olu K (2009) Large Vesicomyidae (Mollusca: Bivalvia) from cold seeps in the Gulf of Guinea off the coasts of Gabon, Congo and northern Angola. Deep Sea Research Part II: Topical Studies in Oceanography 56 (23): 2350–2379. doi:10.1016/j.dsr2.2009.04.016

Dall WH (1891) On some new or interesting West American shells obtained from the dredgings of the U.S. Fish Commission steamer 'Albatross' in 1888, and from other sources. Proceedings of the United States National Museum 14: 173–191.

Dautzenberg P, Fischer H (1906) Mollusques provenant des dragages effectués à l'ouest de l'Afrique pendant les campagnes scientifiques de S.A.S. le Prince de Monaco. Resultats des Campagnes scientifiques du Prince Albert I 32: 1–125, pls 1–5.

Deshayes GP (1857) Note sur différents Mollusques de la Guadeloupe, Envoyés par M. Schramm. Journal de Conchyliologie 6: 137–143.

Duarte JC, Rosas F, Pinheiro LM, Matias LM, Carvalho AM, Terrinha P, Ivanov M (2005) Interpretation of recent sedimentary and tectonic structures off SW Iberia from multibeam bathymetry, seismic reflection and experimental modelling. Geophysical Research Abstracts 7, 07867.

Duperron S, Halary S, Lorion J, Sibuet M, Gaill F (2008) Unexpected co-occurrence of six bacterial symbionts in the gills of the cold seep mussel *Idas* sp. (Bivalvia: Mytilidae). Environmental Microbiology 10(2): 433–445. doi:10.1111/j.1462-2920.2007.01465.x

Fisher CR (1990) Chemoautotrophic and methanotrophic symbioses in marine invertebrates. Review Aquatic Science 2: 399–436.

Gardner JM (2001) Mud volcanoes revealed and sampled on the Western Moroccan continental margin. Geophysical Research Letters 28: 339–342.

Gebruk AV, Krylova EM, Lein AY, Vinogradov GM, Anderson E, Pimenov NV, Cherkashev GA, Crane K (2003) Methane seep community of the Haakon Mosby mud volcano (the Norwegian Sea): composition and trophic aspects. Sarsia 88: 394–403.

Génio L, Johnson SB, Vrijenhoek RC, Cunha MR, Tyler PA, Kiel SK, Little CTS (2008) New record of *"Bathymodiolus" mauritanicus* Cosel 2002 from the Gulf of Cadiz (NE Atlantic) mud volcanoes. Journal of Shellfish Research 27(1): 1–9.

Hensen C, Nuzzo M, Hornibrook E, Pinheiro LM, Bock B, Magalhães VH, Bruckmann W (2007) Sources of mud volcanoes fluids in the Gulf of Cadiz – indications for hydrothermal imprint. Geochimica et Cosmochimica Acta 71: 1232–1248.

Juniper SK, Tunnicliffe V, Southward EC (1992) Hydrothermal vents in turbidite sediments on a Northeast Pacific spreading centre: organisms and substratum at an ocean drilling site. Canadian Journal of Zoology 70: 1792–1809.

Kamenev GM (2009) North Pacific species of the genus *Solemya* Lamarck, 1818 (Bivalvia: Solemyidae), with notes on *Acharax johnsoni* (Dall, 1891). Malacologia 51(2): 233–261.

Kenyon NH, Ivanov MK, Akhmetzhanov AM, Akhmanov GG (Eds) (2000) Multidisciplinary study of geological processes on the North East Atlantic and Western Mediterranean Sea Margins. Preliminary results of geological and geophysical investigations during the TTR-9 cruise of R/V Professor Logachev June-July, 1999. Intergovernmental Oceanographic Commission (IOC) Technical Series 56, UNESCO, Paris, 102 pp.

Kenyon NH, Ivanov MK, Akhmetzhanov AM, Akhmanov GG (Eds) (2001) Interdisciplinary approaches to geoscience on the North East Atlantic Margin and Mid-Atlantic Ridge. Preliminary results of investigations during the TTR-10 cruise of RV Professor Logachev. July-August, 2000. IOC Technical Series 60. UNESCO, Paris, 104 pp.

Kenyon NH, Ivanov MK, Akhmetzhanov AM, Akhmanov GG (Eds) (2002) Geologica processes in the Mediterranean and Black Seas and North East Atlantic. Preliminary results of investigations during the TTR-11 cruise of RV Professor Logachev July-September, 2001. IOC Technical Series, 62, UNESCO, Paris, 89 pp.

Kenyon NH, Ivanov MK, Akhmetzhanov AM, Akhmanov GG (Eds) (2003) Interdisciplinary geoscience research on the Northeast Atlantic Margin, Mediterranean Sea and Mid-Atlantic Ridge. Preliminary results of investigations during the TTR-12 cruise of RV Professor Logachev June-August, 2002. IOC Technical Series 67, UNESCO, Paris, 112 pp.

Kenyon NH, Ivanov MK, Akhmetzhanov AM, Akhmanov GG (Eds) (2006) Interdisciplinary geoscience studies of the Gulf of Cadiz and Western Mediterranean basins. Preliminary results o investigations during the TTR-14 cruise of RV Professor Logachev July-September, 2004. IOC Technical Series 70, UNESCO, Paris, 115 pp.

Krylova EM, Janssen R (2006) Vesicomyidae from Edison Seamount (South Western Pacific: Papua New Guinea: New Ireland fore-arc basin) (Bivalvia: Glossoidea). Archiv für Molluskenkunde 135: 233–263.

Krylova EM, Sahling H (2006) Recent bivalve molluscs of the genus *Calyptogena* (Vesicomyidae). Journal of Molluscan Studies 72: 359–395. doi:10.1093/mollus/eyl022

Krylova EM, Sahling H (2010) Vesicomyidae (Bivalvia): Current taxonomy and distribution. Plos One 5(4): e9957. doi:10.1371/journal.pone.0009957

Krylova EM, Sahling H, Janssen R (2010) *Abyssogena*: a new genus of the family Vesicomyidae (Bivalvia) from deep-water vents and seeps. Journal of Molluscan Studies 76: 107–132.

Kuroda T (1948) Two new gigantic species of *Solemya*. Venus 15(1–4): 29–32. doi:10.1093/mollus/eyp052

Levin L (2005) Ecology of cold seep sediments interactions of fauna with flow chemistry and microbes. Oceanography and Marine Biology: An Annual Review 43: 1–46. doi:10.1201/9781420037449.ch1

Magalhães VH (2007) Authigenic carbonates and fluid escape structures in the Gulf of Cadiz. PhD thesis, Universidade de Aveiro, 422 pp.

Mazurenko LL, Soloviev VA, Belenkaya I, Ivanov MK, Pinheiro LM (2002) Mud volcano gas hydrates in the Gulf of Cadiz. Terra Nova 14: 321–329. doi:10.1046/j.1365-3121.2002.00428.x

Métivier B, Cosel R von (1993) *Acharax alinae* n. sp. Solemyidae (Mollusca: Bivalvia) géante du bassin de Lau. Académie des Sciences (Paris), Comptes Rendu de Seances (3- Sciences de la Vie) 316: 229–237.

Mikkelsen PM, Bieler R (2008) Seashells of Southern Florida - Living Marine Mollusks of the Florida Keys and Adjacent Regions: Bivalves. Princeton University Press, Princeton, New Jersey, 503 pp.

Neulinger SC, Sahling H, Süling J, Imhoff JF (2006) Presence of two phylogenetically distinct groups in the deep-sea mussel *Acharax* (Mollusca: Bivalvia: Solemyidae). Marine Ecology Progress Series 312: 161–168. doi:10.3354/meps312161

Niemann H, Duarte J, Hensen C, Omoregie E, Magalhães VH, Elvert M, Pinheiro LM, Kopf A, Boetius A (2006) Microbial methane turnover at mud volcanoes of the Gulf of Cadiz. Geochimica et Cosmochimica Acta 70: 5336–5355. doi:10.1016/j.gca.2006.08.010

Okutani T, Métivier B (1986) Descriptions of three new species of vesicomyid Bivalves collected by the Submersible Nautile from the Abyssal depths off Honshu, Japan. Venus 45: 147–160.

Okutani T, Hashimoto J, Miura T (2003) A new species of solemyid bivalve from near submarine fumaroles in Kagoshima Bay, Japan. Venus 62(3–4): 91–96.

Oliver PG, Holmes AM (2006a) A new species of *Lucinoma* (Bivalvia: Lucinoidea) from the oxygen minimum zone of the Oman Margin, Arabian Sea. Journal of Conchology 39 (1): 63–77.

Oliver PG, Holmes AM (2006b) New species of Thyasiridae (Bivalvia) from chemosynthetic communities in the Atlantic Ocean. Journal of Conchology 39 (2): 175–183.

Oliver PG, Taylor JD (submitted) Bacterial chemosymbiosis in the Nucinellidae (Mollusca: Bivalvia) with descriptions of two new species.

Olu K, Lance S, Sibuet M, Henry P, Fiala-Médioni A, Dinet A (1997) Cold seeps communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados accretionary prism. Deep-Sea Research I 44(5): 811–841. doi:10.1016/S0967-0637(96)00123-9

Olu-Le Roy K, Sibuet M, Fiala-Médioni A, Gofas S, Salas C, Mariotti A, Foucher JP, Woodside J (2004) Cold seep communities in the deep eastern Mediterranean Sea: composition, symbiosis and spatial distribution on mud volcanoes. Deep-Sea Research I 51: 1915–1936. doi:10.1016/j.dsr.2004.07.004

Olu-Le Roy K, Caprais JC, Fifis A, Fabri MC, Galeron J, Budzinsky H, Le Menach K, Khripounoff A, Ondreas H, Sibuet M (2007) Cold-seep assemblages on a giant pockmark off West Africa: spatial patterns and environmental control. Marine Ecology 28(1): 115–130. doi:10.1111/j.1439-0485.2006.00145.x

Pinheiro LM, Ivanov MK, Sautkin A, Akhamanov G, Magalhães VH, Volkonskaya A, Monteiro JH, Somoza L, Gardner J, Hamouni N, Cunha MR (2003) Mud volcanoes in the Gulf of Cadiz: results from the TTR-10 cruise. Marine Geology 195: 131–151. doi:10.1016/S0025-3227(02)00685-0

Ravara A, Cunha MR, Rodrigues CF (2007) The occurrence of *Natsushima bifurcata* (Polychaeta: Nautiliniellidae) in *Acharax* hosts from mud volcanoes in the Gulf of Cadiz (south Iberian and north Moroccan Margins). Scientia Marina 71(1).

Rodrigues CF (2009) Comunidades macrobentónicas dos vulcões de lama do Golfo de Cádis// Macrofaunal assemblages from mud volcanoes of the Gulf of Cadiz. Departamento de Biologia, Universidade de Aveiro, Aveiro, 348 pp.

Rodrigues CF, Duperron S (2011) Distinct symbiont lineages in three thyasirid species (Bivalvia: Thyasiridae) from the eastern Atlantic and Mediterranean sea. Naturwissenchaften, 98(4): 281–287. doi:10.1007/s00114-011-0766-3

Rodrigues CF, Duperron S, Gaudron SM (2011) First documented record of a living solemyid bivalve in a pockmark of the Nile Deep-sea Fan (eastern Mediterranean Sea). Marine Biodiversity Records 4: e10. doi:10.1017/S175526721100008X

Rodrigues CF, Oliver PG, Cunha MR (2008) Thyasiroidea (Mollusca: Bivalvia) from the mud volcanoes of the Gulf of Cadiz (NE Atlantic). Zootaxa 1752: 41–56.

Rodrigues CF, Webster G, Cunha MR, Duperron S, Weightman AJ (2010) Chemosynthetic bacteria found in Bivalvia species from mud volcanoes of the Gulf of Cadiz. FEMS Microbial Ecology 73(3): 486–499.

Sahling H, Galkin SV, Salyuk A, Greinert J, Foerstel H, Piepenburg D, Suess E (2003) Depth-related structure and ecological significance of cold-seep communities - a case study from the Sea of Okhotsk. Deep Sea Research I 50: 1391–1409. doi:10.1016/j.dsr.2003.08.004

Salas C, Woodside J (2002) *Lucinoma kazani* n. sp. (Mollusca: Bivalvia): evidence of a living benthic community associated with a cold seep in the eastern Mediterranean Sea. Deep-Sea Research Part 1 49: 991–1005. doi:10.1016/S0967-0637(02)00010-9

Say T (1822) An account of some of the marine shells of the United States. Journal of the Academy of Natural Sciences of Philadelphia 2: 221–248, 257–276, 302–325.

Sibuet M, Olu K (1998) Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep-Sea Research II 45: 517–567. doi:10.1016/S0967-0645(97)00074-X

Sibuet M, Olu-Le Roy K (2002) Cold Seep Communities on Continental Margins: Structure and Quantitative Distribution Relative to Geological and Fluid Venting Patterns. In: Weffer G, Billett D, Hebbeln D, Jorgersen BB, Van Weering TJ (Eds) Ocean Margin System, Springer Verlag, 235–251.

Stadnitskaia A, Ivanov MK, Blinova V, Kreulen R, van Weering TCE (2006) Molecular and carbon isotopic variability of hydrocarbon gases from mud volcanoes in the Gulf of Cadiz, NE Atlantic. Marine and Petroleum Geology 23: 281–296.

Stimpson, W (1851) A revision of the synonymy of the testaceous mollusks of New England. Phillips, Sampson & Company, Boston, 56 pp, 2 pls.

Sturany R (1896) Mollusken I (Prosobranchier und Opisthobranchier, Scaphopoden; Lamellibranchier) gesammelt von S. M. Schiff "Pola" 1890–94. Denkschriften der mathematisch-naturwissenschaftlichen Klasse der Kaiserlichen Akademie der Wissenschaften, Wien 63: 1–36, pls 1–2.

Taylor JD, Glover EA (2006) Lucinidae (Bivalvia) - the most diverse group of chemosymbiotic molluscs. Zoological Journal of the Linnean Society 148(3): 421–438. doi:10.1111/j.1096-3642.2006.00261.x

Taylor JD, Glover EA (2009) New lucinid bivalves from hydrocarbon seeps of the Western Atlantic (Mollusca: Bivalvia: Lucinidae). Steenstrupia 30(2): 127–140.

Taylor JD, Glover EA, Williams ST (2008) Ancient chemosynthetic bivalves: systematics of Solemyidae from eastern and southern Australia (Mollusca: Bivalvia). In: Davie PJF, Philips JA (Eds) Proceedings of the Thirteenth International Marine Biological Workshop, The Marine Fauna and Flora of Moreton Bay, Queensland. Memoirs of the Queensland Museum - Nature 54(1): 75–104.

Thiele J, Jaeckel S (1931) Muscheln der deutschen Tiefsee-Expedition. Wissenschaften Ergbnisse der Deutschen Tiefsee-Expedition auf der Dampfer "Valdivia" 1898–1899, 21: 161–268.

Totten JG (1834) Description of some new shells, belonging to the coast of New England American. Journal of Science and Arts 26: 366–369.

Tunnicliffe V (1991) The biology of hydrothermal vents: ecology and evolution. Oceanography Marine Biology 29: 219–407.

Turner RD (1985) Notes on mollusks of deep-sea vents and reducing sediments. American Malacological Bulletin (Special Edition) 1: 23–34.

Van Rensbergen P, Depreiter D, Pannemans B, Moerkerke G, Van Rooij D, Marsset B, Akhmanov G, Blinova V, Ivanov M, Rachidi M, Magalhães V, Pinheiro L, Cunha M, Henriet JP (2005) The El Arraiche mud volcano field at the Moroccan Atlantic slope, Gulf of Cadiz. Marine Geology 219 (1): 1–17. doi:10.1016/j.margeo.2005.04.007

Verrill AE, Bush KJ (1898) Revision of the deep-water Mollusca of the Atlantic coast of North America, with descriptions of new genera and species. Proceedings of the United States National Museum 20: 775–901.

Vokes HE (1970) Two new species of deep water bivalves from the Caribbean Sea. Veliger 12(3): 357–361.

Warén A, Bouchet P (2009) New gastropods from deep-sea hydrocarbon seeps off West Africa. Deep-Sea Research II 56: 2326–2349. doi:10.1016/j.dsr2.2009.04.013

Zitellini N, Gràcia E, Matias L, Terrinha P, Abreu MA, DeAlteriis G, Henriet JP, Dañobeitia JJ, Masson DG, Mulder T, Ramella R, Somoza L, Diez S (2009) The quest for the Africa-Eurasia plate boundary west of the Strait of Gibraltar. Earth and Planetary Science Letters 208(1–4): 13–50. doi:10.1016/j.dsr2.2009.04.013

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The dazed and confused identity of Agassiz's land tortoise, *Gopherus agassizii* (Testudines, Testudinidae) with the description of a new species, and its consequences for conservation

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Abstract

We investigate a cornucopia of problems associated with the identity of the desert tortoise, *Gopherus agassizii* (Cooper). The date of publication is found to be 1861, rather than 1863. Only one of the three original cotypes exists, and it is designated as the lectotype of the species. Another cotype is found to have been destroyed in the 1906 San Francisco earthquake and subsequent fire. The third is lost. The lectotype is genetically confirmed to be from California, and not Arizona, USA as sometimes reported. Maternally, the holotype of *G. lepidocephalus* (Ottley & Velázques Solis. 1989) from the Cape Region of Baja California Sur, Mexico is also from the Mojavian population of the desert tortoise, and not from Tiburon Island, Sonora, Mexico as previously proposed. A suite of characters serve to diagnose tortoises west and north of the Colorado River, the Mojavian population, from those east and south of the river in Arizona, USA, and Sonora and Sinaloa, Mexico, the Sonoran population. Species recognition is warranted and because *G. lepidocephalus* is from the Mojavian population, no names are available for the Sonoran species. Thus, a new species, *Gopherus morafkai* **sp. n.**, is named and this action reduces the distribution of *G. agassizii* to only 30% of its former range. This reduction has important implications for the conservation and protection of *G. agassizii*, which may deserve a higher level of protection.

Keywords
Lectotype, *Xerobates*, *Gopherus lepidocephalus*, desert tortoise, recovery units, California, Arizona, Mexico

> *Been dazed and confused for so long, it's not true*
> Jake Grier Holmes, Jr. 1967 (not Jimmy Page 1968)

Introduction

Often, systematics and taxonomy are clear cut. Species are described and they persist in recognition, either as being valid taxa or buried in a synonymy. That said, taxonomic chaos also occurs, often with respect to generic allocation, the validity of subspecies (Frost and Hillis 1990), and the recognition of species themselves. The taxonomy of the desert tortoise, or Agassiz land-tortoise, is engulfed in errors. Some errors have now persisted for almost 150 years, and others are more recent in origin.

Berry et al. (2002) summarize data suggesting that the desert tortoise, *Gopherus agassizii* (Cooper), of the southern United States and northwestern mainland Mexico is a composite of at least two and possibly four species. They note that much work remains to be accomplished before formally recognizing any new species. This task is more complex than originally imagined, in part because of a convoluted taxonomy plagued with uncertainties and problems. Our reviews of several conundrums obtain the background data required to untangle a knot of confusion and make some decisions and recommendations. The greatest problem concerns the identities of true *G. agassizii* and the enigmatic *G. lepidocephalus* (Ottley et Velázques Solis).

Date of publication of Cooper's name *Xerobates agassizii*

The discovery of *Gopherus agassizii* was first presented by James G. Cooper, MD (Fig. 1) as a new genus and species, *Xerobates agassizii*, the "Agassiz Land-Tortoise," at the California Academy of Natural Sciences meeting of 7 July 1861 (Leviton and Aldrich 1997:

Figure 1. Portrait of Dr. James Graham Cooper, M.D. who discovered and described *Xerobates agassizii* (courtesy of the Archives of the California Academy of Sciences).

53). Shortly thereafter, in 1861, the description was published as a separate issue (termed a signature) of the Proceedings of the Academy. However, there is confusion about the date of publication. The collected Proceedings, series 1, volume 2 spanned the years 1858–1862 but was closed in 1863 when a dated title page, table of contents, and index were issued. The closing date has long been used in error for the description of *Xerobates agassizii* (e.g. Van Denburgh 1897; Cochran 1961; Auffenberg and Franz 1978; Crumly 1994; Reynolds et al. 2007), and this error may have been started by True (1881), who states the description was "…issued in 1863." Curiously, in the same issue of the Proceedings, Cooper (1861) described *Athene whitneyi* (=*Micrathene whitneyi*, the elf owl) and *Helminthophaga luciae* (=*Oreothlypis luciae*, Lucy's warbler), and historically these descriptions have been correctly credited to 1861, as have a series of botanical papers credited to Albert Kellogg (Leviton et al. 2010: 235–236). Although originally named as the Agassiz land-tortoise, and not Agassiz's land tortoise as given by Cooper (1870: 67) and quoted in error by True (1881), it was also once also called Agassiz's Gopher (Yarrow 1883). Today the species is commonly referred to as the desert tortoise, a transliteration of *Xerobates* (xeros, Gr. dry; bates, Gr., one that walks, treads, haunts) that dates back to Van Denburgh (1897). This common name is also applied to other tortoises in the genus *Testudo*. Unlike Latinized names, common names do not enjoy precedence.

Documentation of the publication date has required a venture into the history of the Proceedings of the California Academy of Natural Sciences (see Leviton and Aldrich 1997, 2010). The Proceedings, started in September 1854, were initially issued in four or eight-page signatures, later expanding to 16-page signatures. Printing required about one to four months and the signatures were distributed by the Academy usually within four days of receipt from the printer. Volume 1 included signatures published between 4 September 1854 and 31 January 1858. Volume 2 contains contributions from 22 February 1858 to 15 December 1862, and it was closed in 1863. This volume is comprised of 15 numbered, 12- or 16-page signatures, the first eight of which are not dated but they were printed shortly after the last included dated meetings. For example, signature number 1 (pp. 1–16), which included all materials presented at meetings held between 22 February 1858 and 26 September 1859, but was otherwise undated, was printed between 26 September 1859 and, at the latest, 26 January 1860, but in all likelihood within days of 26 September 1859. This was followed by signature 2 (pp. 17–32) which reported on activities, including the text of papers presented, for the period 26 September to 24 October 1859, and so forth to signature 8 (pp. 110–124), which covered the meetings held between 15 April to 21 July 1861, the last, undated signature to be included in volume 2. After that, signatures were print-dated. Signature 9 (pp. 125–140), for the period 21 July to 19 August 1861, was dated December 1861; signature 10 (pp. 141–156), for the period 19 August through 1 December 1861, was dated April 1862, and so forth. Thus, signature 8, which contained the pages bearing Cooper's original description of *Xerobates agassizii*, was printed and available for distribution no earlier than four days following the last meeting reported on in the signature, i.e., 21 July 1861, but no later and most likely weeks earlier than the print date of signature 9, which is given as December 1861. We propose that the official date of publication should be 25 July 1861.

Type locality of *Xerobates agassizii*

Credited to multiple places, the type locality of *G. agassizii* has been thoroughly confused. *Xerobates agassizii* was described on the basis of three "young" cotypes collected from the "mountains of California, near Fort Mojave" (Cooper 1861). In late 1860, Cooper, a medical officer in the Army, was assigned to report for duty at Fort Mojave, an Army fort located on the east bank of the Colorado River in northern Arizona (see Coan 1981: 100–106 for more details). In early December 1860, Cooper travelled to the Fort via a Quartermaster's wagon train departing from Los Angeles. The group traveled via Cajon Pass and then across the Mojave Desert, reaching the Fort on 20 December. His time at the Fort was to be truncated by the onset of military action in the East—the Civil War—which led to the abandonment of the Fort on 28 May 1861. However, before leaving California for Fort Mojave, Cooper had already contacted Josiah Dwight Whitney, director of the California State Geological Survey. During his return trip from Fort Mojave to the Pacific Coast, beginning on 29 May 1861, Cooper prepared a report for Whitney describing the conditions of some of the areas through which he passed, including Pah-Ute Spring, Rock Spring, and Soda Lake (Soda Playa), which during his trip from Los Angeles to Fort Mojave he described as "in December the only warm part of the route east of Cajon Pass..." (Coan 1981: 104).

When Cooper finally reached Los Angeles, sometime during the second half of June 1861, he found a letter waiting for him from Spencer Fullerton Baird, then Assistant Secretary of the Smithsonian Institution, informing him that Whitney had expressed an interest in hiring him, Cooper, as the State Survey's zoologist. Cooper arrived in San Francisco on 4 July 1861, met with Whitney, and then sent a letter to Baird stating that he had encountered two new birds at Fort Mojave as well as a new species of tortoise (Coan 1981: 105). In the letter, Cooper also informed Baird that he planned to describe the new tortoise in the Academy's Proceedings, and perhaps jokingly asked, "… who shall I name it for, Agassiz?" (Cooper to Baird, 14 July 1861) (Coan 1981: 105).

By the time Cooper described the new tortoise, he had already been hired by Whitney as the California State Geological Survey's zoologist. Cooper was also a member of the California Academy of Sciences, where he held the title Curator of Zoology in 1862. Curiously, the date of Cooper's Academy membership has been as enigmatic as the information associated with some of the specimens he collected. According to the Academy's membership list, Cooper became a member on 18 February 1867, at least six years after the dates with which we are concerned. But not only did Cooper attend the Academy's meeting in the latter half of 1861, at one of which (7 July 1861) he presented his paper describing new species of Californian animals, in early 1862 he was elected Curator of Zoology, which could only have happened if he were already a member (Leviton and Aldrich 1997: 54).

Irrespective of Academy membership, Cooper's collection near Fort Mojave was made before he was employed by the Survey. Once employed by the Survey, all specimens collected thereafter were treated as Survey property. Regardless, sometime after 1861, Cooper sent one of the cotypes, a juvenile, to Baird at the United States National Museum, Smithsonian Institution (USNM 7888). Although the specimen supposedly was collected by Cooper in March 1861, the collecting locality has been credited to multiple places. Cochran (1961), and Auffenberg and Franz (1978) gave the locality of this cotype as "Utah Basin, Mojave River." However, according to

Cochran (1961) the catalog gives the locality as “Solado Valley, California.” Reynolds et al. (2007: 32) provide some clarification. The USNM catalogue states: “The original parchment label attached to USNM 7888 lists the locality as Soda Valley, but the original catalog record has Solado Valley.” Further, an old label in the jar with the specimen and Yarrow (1883) give the locality as Solado Valley. Regarding Utah Basin, Reynolds et al. (2007) further state “This information is not in the catalog record, and we have been unable to determine why she (Cochran) included it in the locality for this specimen.” Certainly, the type locality occurs within the “Mountains of California, near Fort Mojave” (Cooper 1861), and most likely in Soda Valley (today also known as Soda Playa). The USNM catalogue states that the specimen was collected in March 1861 (Reynolds et al. 2007). Cooper passed through the area on at least two occasions, first in early December 1860 and again in early June 1861 enroute to and on his return from Fort Mojave, Arizona, about 83 km. Unfortunately, none of Cooper’s writings for that period have survived.

Fate of the two other cotypes

The fate of the remaining two specimens Cooper had collected is also confused. Reynolds et al. (2007: 32) state “Two other syntypes were originally in the collection of the California State Geological Survey and later deposited in the California Academy of Sciences”, and that “CAS 7141 and CAS 7142 … were the likely syntypes …” However, these two tortoises were collected on 11 March 1905 by John Carlson, and thus could not have been the two missing cotypes of *Xerobates agassizii* Cooper, 1861. Given that most of the records of the California Academy of Sciences were destroyed in the San Francisco earthquake and subsequent fire of 1906, no written record of a transfer of the cotypes to the Academy exists, although the catalogue of the herpetological specimens, started by Van Denburgh in 1894, was saved and exists today (see below).

One of the three possible cotypes was likely deposited in the herpetological collections of the Academy. The herpetological collections that accumulated between 1853 and 1894 were not cataloged until 1894, when John Van Denburgh came to the Academy and initiated the formal catalog of amphibians and reptiles. Possible cotype CAS 3567, catalogued as “*Gopherus agassizii*,” was collected by Cooper. The specimen was likely catalogued in 1896, well after Stejneger (1893) made the generic change from *Xerobates* to *Gopherus*. The specimen was undated and the locality was originally recorded as being “Arizona.” However, a note by Van Denburgh in the Department’s catalog states that Cooper said the tortoise came from the Mojave Desert, California. This specimen may or may not have been one of the three cotypes. Regardless, tortoise CAS 3567 was destroyed in the earthquake and subsequent fire in 1906 as were three other specimens, CAS 3568, 3269, and 3570, all shown as *Gopherus agassizii*. Of the latter three, CAS 3568, listed as a shell, was collected at “Crater Summit, Mojave Desert,” by Oscar Brown, but without a date. Numbers CAS 3569, a shell, and CAS 3570, a skeleton, have “original numbers”, but it is not known whether these numbers were either field numbers or numbers from an earlier cataloging effort, the records of which no longer exist; no other data exist. If CAS 3567 was one of the cotypes, then the whereabouts of the third juvenile specimen remains a mystery. In this scenario, it is possible that the third cotype was retained by the California State Geological Survey and was subsequently lost or destroyed. There is a remote possibility that a syntype was deposited elsewhere. For instance, some of the Survey's paleontological collections formed the nucleus of the Museum of Paleontology's collections at the University of California, Berkeley (UCMP) (Lipps 2004: 220), but part of the collection went to Harvard (MCZ) when Survey Director Whitney returned to the university, and a portion went to the Academy of Natural Sciences in Philadelphia (ANSP) with William Gabb. However, none of these collections have specimens of *Gopherus agassizii* collected in the 1860s or otherwise transferred by Cooper. Of course, it is also possible that the specimen was shipped elsewhere, but that seems unlikely.

Description of *Xerobates lepidocephalus* and taxonomic views on its validity.

Ottley and Velázques Solis (1989) described a new species, *X. lepidocephalus*, from the Cape Region of Baja California Sur, Mexico. Ecologically, the species occurs on sloped or hillside areas and it is not reported to live in burrows. This habitat choice closely resembles that of tortoises living in the Sonoran Desert, specifically those tortoises occurring east and south of the Colorado River. These tortoises, called Sonoran desert tortoises (Van Devender 2002), differ substantially from tortoises in the Mojave Desert. In general, Sonoran tortoises live in rock crevices on steep slopes and hill tops (Riedle et al. 2008) and Mojave desert tortoises live in burrows in valleys and on alluvial fans (Berry et al. 2002). Morphologically, *G. lepidocephalus* is most similar to tortoises on Tiburon Island off the coast of Sonora, Mexico and the species was considered to be a junior synonym of *G. agassizii* by Crumly and Grismer (1994).

Generic instability

The generic allocation of the Agassiz land-tortoise, the desert tortoise, has occasionally changed. Cope (1875) transferred *X. agassizii* to the genus *Testudo*, as *T. agassizii,* in his checklist of North American amphibians and reptiles but without comment or justification. Presumably, this determination followed the generic allocation of Gray (1870) and certainly this was not an oversight as Cope (1880) repeated the generic allocation for *G. berlandieri*. The next taxonomic change was made by Stejneger (1893) in his discussion of the fauna of Death Valley. He considered the Californian tortoise to be distinct from *G. berlandieri* and to belong to the North American genus *Gopherus* Rafinesque 1832, as "*G. agassizii* (Cooper)" (Stejneger 1893: 160). This generic allocation was stable for almost 100 years.

Nomenclatural stability for *G. agassizii* was maintained until Bramble (1982) revised the genus using both extant and extinct species. He discovered two groups and erected the genus *Scaptochelys* for *G. agassizii* and *G. berlandieri*. The type species was designated as *Xerobates agassizii* Cooper, 1863 [sic]. Thus, *G. agassizii* was referred to as *S. agassizii*. Shortly thereafter, Bour and Dubois (1984) reported that *Scaptochelys* was a junior synonym of genus *Xerobates* Agassiz, 1857, whose type species was *X. berlandieri* Agassiz, 1857, by subsequent designation (Brown 1908). Because Bramble (1982) resolved *S. agassizii* as the sister group of *X. berlandieri*, Bour and Dubois (1984) referred *S. agassizii* back to *Xerobates agassizii* Cooper, 1863 [sic].

In terms of generic allocation, Crumly (1994) aptly notes that the genus *Xerobates* cannot be diagnosed morphologically owing to intraspecific variation. Thus, he refers *Xerobates agassizii* back to *Gopherus agassizii* (Cooper, 1861). Symplesiomorphies are used by Bramble (1982) to define *Scaptochelys*, a practice that contravenes the principles of phylogenetic systematics. Although morphological evidence does not unite *G. agassizii* and *G. berlandieri*, molecular evidence does (Lamb and Lydeard 1994). And although it is possible to recognize *Xerobates* for the extant species *G. agassizii* and *G. berlandieri*, the phylogenetic relationships among extinct species (Reynoso and Montellano-Ballesteros 2004) preclude monophyly of the two genera. Thus, *Xerobates* should not be recognized.

Species instability

Mertens and Wermuth (1955) and Wermuth and Mertens (1961) were unimpressed by the extent of morphological differentiation among North American *Gopherus* and impressed by the reports of hybrids. While recognizing long-term isolation, they recognized only one species, *G. polyphemus*, stating “Da sich die einzelnen Formen der Gopherschildkröten äußberlich nur wenig unterscheiden, deutlich geographisch vikariieren und mehreren Veröffentlichungen zufolge auch zu verbastardieren scheinen, sind sie hier als Unterarten aufgeführt” (Wermuth and Mertens 1961: 172). In doing so, they considered *G. agassizii* to be a subspecies of *G. polyphemus*, *G. p. agassizii* (Cooper). Their taxonomic arrangement was rarely, if ever, followed.

More than one species

Berry et al. (2002) summarize evidence for the existence of at least two species of desert tortoises, and support is still mounting (Table 1). Evidence includes either fixed or statistically significantly differences in microsatellite DNA alleles (Murphy et al. 2007; Engstrom et al. 2007; Edwards et al. 2011), differences in maternal lineages as evidenced by mitochondrial DNA (Lamb et al. 1989; Lamb and Lydeard 1994; Edwards 2007), significant behavioral and ecological differences (Berry et al. 2002), and perhaps significant differences in longevity and growth strategies (Curtin et al. 2009). The exception is

Table 1. Summary of morphological, physiological, and ecological characteristics that differ between populations of desert tortoises from the Mojave and Sonoran deserts.

Character	Mojave Desert	Sonoran Desert	Reference
Morphology-shell shape			
Width of shell at mid-bridge	significantly wider shell		Germano 1993
Length of gular scutes	Significantly longer gular scutes		Germano1993
		Significantly shorter length of projection of anal scutes	Germano 1993
General shape of shell	California: box-like, high-domed; Utah: box-like, low-domed, shorter plastron	Flatter, pear-shaped	Weinstein and Berry 1989
Geographical distribution	North and west of the Colorado River	South and east of the Colorado River	
Habitats occupied Topography	Predominantly valleys and alluvial fans	Predominantly slopes and rocky hillsides	U.S. Dept. of the Interior, Fish and Wildlife Service 1994; Van Devender 2002
Vegetation types	Mojave Desert: Saltbush scrub, creosote bush scrub, desert scrub, tree yucca woodland	Sonoran Desert: Arizona upland, thornscrub, desert grassland	U.S. Dept. of the Interior, Fish and Wildlife Service 1994, 2010b; Van Devender 2002
Egg production			
Mid-line carapace length (mm MCL) at first reproduction	176 (Germano), 178 (Turner et al. 1987)	220	Turner et al. 1984, 1986, 1987; Germano 1994b; Henen 1994; Averill-Murray 2002, Averill-Murray et al. 2002, Curtin et al. 2009
Oviposition time (range)	April to mid-July	Early June to early August	Turner et al. 1986; Averill-Murray et al. 2002
Number of clutches/yr	0–3	0–1	Turner et al. 1986; Averill-Murray et al. 2002
Number of eggs per year	5–16	1–12, avg. ~5	Turner et al. 1986, 1987; Henen 1994; Karl 1998; Mueller et al. 1998; Wallis et al. 1999
Proportion of females ovipositing/yr	0.67–1.0; typically 1.0	0.36–0.80; typically < 1, based on one study (Averill-Murray)	Turner et al. 1986; Henen 1997; Mueller et al. 1998; Wallis et al. 1999; Averill-Murray 2002

one small, geographically restricted zone where the two forms of tortoises hybridize (Fig. 2; McLuckie et al. 1999; Edwards et al. unpublished data). The two forms are thought to have been isolated from 5 to 6 Ma (Lamb and Lydeard 1994; Lamb and McLuckie 2002) As currently conceived (Fritz and Havaš 2007), *G. agassizii* is best viewed as a composite of at least two and possibly as many as four species (Berry et al. 2002).

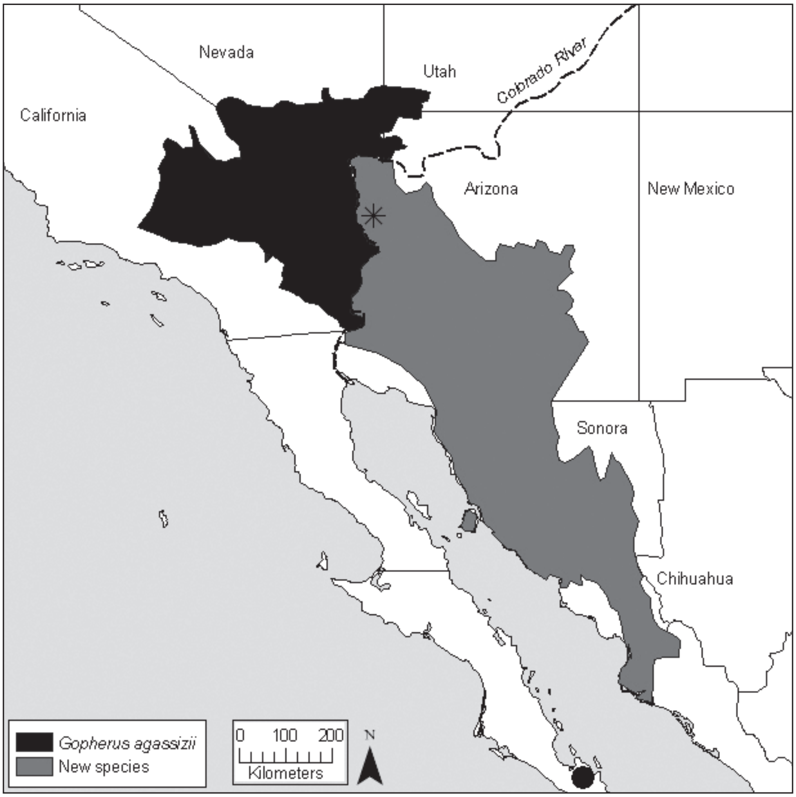

Figure 2. Distribution of the desert tortoises aligned with *Gopherus agassizii*. The locality of BYU 39706 from Baja California Sur is shown as a black dot. The location of the hybrid population described in McLuckie et al. (1999) is shown as a star.

Two species of desert tortoise can be recognized after a nomenclatural conundrum is solved. The population in the Mojave Desert that occurs north and west of the Colorado River, and the population in the Colorado Desert of California (Fig. 2; Berry et al. 2002), will bear the name *Gopherus agassizii* (Cooper, 1861) unless unequivocal data proves otherwise. Tortoises that occur east and south of the Colorado River will require at least one name. The true identity of *G. lepidocephalus* remains a problem. It is possible, albeit seemingly unlikely, that this species is native and endemic to the Cape Region of Baja California, yet it is also possible that the tortoise represents a translocation from mainland Mexico, and perhaps from northern Sonora, Mexico (Crumly and Grismer 1994), or elsewhere. If the population in Baja California Sur is native to northern Sonora, Mexico, then the name *G. lepidocephalus* (Ottley & Velázques Solis, 1989) will apply to tortoises south and east of the Colorado River currently known as *G. agassizii* and irrespective of the type locality being non-native. Further, it is possible that another species is associated with tropical deciduous forests in southern Sonora and northern Sinaloa, Mexico (Lamb et al. 1989), and, if true, then it is also possible that the name *G. lepidocephalus* applies to this potential species. Finally, and of some concern, it is possible that the species is a translocated hybrid population because hybrid individuals are exceptionally common in the ex situ, captive population (Edwards et al. 2010).

To evaluate the validity of *G. lepidocephalus* and to confirm the geographic origin of *G. agassizii*, we obtained mitochondrial DNA sequences from both type specimens. This kind of analysis could not detect hybrids because the mitochondrial genome is inherited only maternally. However, if *G. lepidocephalus* has its origin in the Mojave Desert, then the name will persist as a junior synonym of *G. agassizii* regardless of whether it is a hybrid or not. Alternatively, if the maternal lineage is from a Sonoran desert tortoise, then the possible hybrid state would create another problem to be solved. Finally, if the lineage was new and divergent, then perhaps *G. lepidocephalus* was native to the peninsula.

Materials and methods

Tissue samples (leg muscle) were dissected from the lectotype of *Gopherus agassizii* (Cooper, 1861) (USNM 7888) and the holotype of *G. lepidocephalus* (Ottley & Velázques Solis, 1989) (Brigham Young University [BYU] 39706). Genomic DNA was extracted from approximately 10 mg of tissue. The lectotype of *G. agassizii* was likely preserved in ethanol yet the holotype of *G. lepidocephalus* was initially well-preserved in formalin. Subsequently, both specimens were stored in 70% ethanol. To remove fixatives, tissues were washed twice in PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl) as recommended in the DNA Easy Extraction Kit (Qaigen) for tissue exposed to formalin. Subsequently, higher yields of DNA were achieved using our standard extraction method, rather than the DNA Easy Extraction Kit, as follows: digestion of the tissue was carried out at 52 °C in a lysis buffer (Tris 6.06g, Na_2EDTA 0.93g, NaCl 5.85g and SDS 1.0g, 500ml ddH2O, pH 8.5) and spiked daily with 12.5 µl of proteinase K (Roche) until the tissue sample was completely digested (5–7 days). Purification used two washes with phenol:chloroform:isoamyl alcohol followed by a final wash of chloroform:isoamyl alcohol.

Primer design

Using the alignment of Murphy et al. (2007), primers were designed for a 423 bp fragment that was diagnostic for haplotypes of *G. agassizii*. The forward primer GoCytL (5'-CAATTCGATTCTTCCTAGTAGC-3') was located in the NADH3 gene and reverse primer GoCytH (5'- GGCTGAGAAGGATAGTATTAGTATTGG-3') located on ND4. Attempts to amplify the holotype sample of *G. lepidocephalus* (BYU 39706) failed after numerous attempts using these two primers. Because DNA exposed to formalin is prone to degradation and fragmentation (Bucklin and Allen 2004), several internal primers were designed and used in various combinations until amplification was successful. Eventually we amplified a 225 bp fragment using the original GoCytL forward primer and a new internal reverse primer (LepidoNd3h3: 5'-TTGGTGTCATTTTGATAGCCGTGAAG-3') that straddles the tRNA^{Arg} and ND4L genes; one bp was not confidently resolved.

PCR amplification

Each PCR was carried out in 25ul volume on a DNA Engine PTC-200 (MJ Research). Due to a low concentration of the template (2–5ng/µL), 30µl of the DNA extraction was concentrated via standard ethanol precipitation. Subsequently, the reagents for the PCR were used to resuspend the pellet. The reagents included 0.8µl of 10mM dNTP, 1µL of each 10µM primer, 2.5 µl 1x PCR buffer (1.5 mM $MgCl_2$; Fisherbrand), and 0.75 U Taq DNA polymerase (Fisherbrand). Cycling parameters were 94 °C for 2 min, 39 cycles of 94 °C for 30s, 55 °C for 45s, 72 °C for 45s and a final extension at 72 °C for 7 min.

To verify amplicons, a 25 µl of the PCR product was run out in a 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light. Bands were excised and purified by spinning in a filter tip (Sorenson; 75–30550T) that was set in a 1.7µl Eppendorf tube. Samples were centrifuged at 16.1G for 10 min. We used 4µl of the cleaned PCR product for sequencing on an ABI3100 (Applied Biosystems) using a ¼ reaction of Big Dye 3.1 recommended by ABI (Applied Biosystems).

Negative controls

DNA was extracted a minimum of three times for the lectotype of *G. agassizii* and once for the holotype of *G. lepidocephalus*. To avoid any possibility of cross contamination, final extractions were done in isolation of one another. Amplification and sequencing were also done independently for both strands. Desert tortoise sequences were confirmed using a BLAST search of the NCBI database.

Sequence analysis

The sequence data were aligned by eye using CLUSTALW (Thompson et al. 1994) against fragments used by Murphy et al. (2007), which were downloaded from GenBank (accession No. DQ649394–DQ649409), because (1) the target region mostly contained encoding sequences, and (2) the length of the fragment precluded the necessity of computer-assisted alignments. Nucleotide divergences against the most similar sequence were merely counted. The fragment was too short and the levels of divergence too small to be used for meaningful tree constructions.

Results

We resolved a 224 bp fragment for *G. lepidocephalus* and 401 aligned nucleotides from the lectotype of *G. agassizii* including 4bp that were not confidently resolved. Attempts to sequence a larger fragment from *G. lepidocephalus* failed. The shorter sequence was located completely within the larger. The aligned sequences were identical (Fig. 3). A BLAST search in GenBank revealed that the sequences were identical to the most common haplogroup in tortoises from the Mojave Desert—group A in Murphy et al. 2007 (e.g. GenBank Acc. No. DQ649394; Fig. 3). Group A was detected throughout the Mojave Desert except in the Northeastern Mojave Recovery Unit. The sequences of the lectotype of *G. agassizii* (USNM 7888) and holotype of *G. lepidocephalus* differed from group B (e.g. GenBank Acc. No. DQ649398; Fig. 3) by only 2 of 224 bp (0.9%); the longer sequence of the lectotype differed at the same 2 nucleotide positions only (0.5%) from group B (Fig. 3). In contrast, the sequence of the lectotype of *G. agassizii* (USNM 7888) differed from Sonoran desert tortoises (e.g. GenBank Acc. No. DQ649406; Fig. 3) by 16 of 397 bp (4.0%). The shorter fragment from both the lectotype of *G. agassizii* and the holotype of *G. lepidocephalus* (BYU 39706) differed from Sonoran desert tortoises by 11 of 224 bp (4.9%). Thus, at least the maternal lineages of both type specimens were from the Mojave Desert, and not the Sonoran Desert. This discovery did not exclude the possibility of *G. lepidocephalus* being a hybrid individual.

Several observations suggested the absence of DNA contamination. First, amplification of DNA from the two type specimens resulted in differing fragment lengths. Primers used for the lectotype of *G. agassizii* did not amplify DNA from the holotype of *G. lepidocephalus*. Thus, it is exceptionally unlikely that contamination occurred between these two species. Neither type specimen had DNA extracted along with other samples of *Gopherus*; all comparative samples were downloaded from GenBank. Thus, cross-contamination outside of this project was not possible. Finally, DNA extracted in isolation of the other type precluded the possibility of contamination. Consequently, all evidence suggested that the sequence data were obtained from the respective specimens.

Given that only one of the cotypes is known, we propose the following designation for *G. agassizii*:

***Gopherus agassizii* (Cooper, 1861)**
Agassiz's Desert Tortoise
http://species-id.net/wiki/Gopherus_agassizii
Figs 4–8

Lectotype. USNM (National Museum of Natural History, Smithsonian Institution) 7888; terra typica restricta: California, San Bernardino County; Mountains of California, near Fort Mojave; Soda Valley (very approximately 35° 6' N, 116° 6' W). We restrict the type locality to that published by Cooper (1861) and the parchment tag

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            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                5          15         25         35         45         55         65         75
DQ649394    AATCTTGTTC CTCCTATTTG ATTTAGAAAT CGCACTATTA CTTCCATTAC CATGAGCAAT TCAGCTCCCA CACCCAACCA
DQ649398    ....C..... .......... .......... .......... .......... .......... .......... ..........
BYU 39706   .......... .......... .......n.. .......... .......... .......... .......... ..........
USNM 7888   .......... .......... .......... .......... .......... .......... .......... ..........
DQ649406    .......... .......... .......... .......... .....G.... .......... ...A...... ..T.......

            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                85         95         105        115        125        135        145        155
DQ649394    AATCTTTCAC CTGGGCTTTT ATCATTTTGC TACTACTAAC GTTGGGCCTT ATATACGAAT GAATTCAAGG AGGCCTCGAG
DQ649398    .......... .......... .......... .......... .......... .......... .......... ..........
BYU 39706   .......... .......... .......... .......... .......... .......... .......... ..........
USNM 7888   .......... .......... .......... .......... .......... .......... .......... ..........
DQ649406    .......... .......... ......C... .......G.. A..A..T... .......... .......... .......A..

            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                165        175        185        195        205        215        225        235
DQ649394    TGGGCAGAGT AGATAACTAG TCTAACACAA GACAACTAAT TTCGACTTAG TCAATCGTGA CTAAACTTCA CGGCTATCAA
DQ649398    .......... .......... .......... .......... .......... .......... T......... ..........
BYU 39706   .......... .......... .......... .......... .......... .......... .....nnnnn nnnnnnnnnn
USNM 7888   .......... .......... .......... .......... .......... .......... .......... ..........
DQ649406    ........A. .......... .......... .......... .......... .......... T......... ..........

            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                245        255        265        275        285        295        305        315
DQ649394    AATGACACCA ACACATTTCA GCTACTACTC TGCCTTTATC ATCAGCATTA CAGGCCTCTC ACTACATCGA ACCCACCTAA
DQ649398    .......... .......... .......... .......... .......... .......... .......... ..........
BYU 39706   nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
USNM 7888   .......... .......... .......... .......... .......... .......... .......... ..........
DQ649406    .......... .......... ....T..... .........T .......... .......... .T.G...... ..........

            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| .
                325        335        345        355        365        375        385        395
DQ649394    TCTCAACTTT ATTGTGTCTT GAGGGGATAA TATTATCCCT ATTTGTTGCC CTATCCATAT GACCAATTCA ACTACAAACC T
DQ649398    .......... .......... .......... .......... .......... .......... .......... .......... .
BYU 39706   nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn n
USNM 7888   .......... .......... ..nnnn.... .......... .......... .......... .......... .......... .
DQ649406    .......... .......... .......... .......... ....A..... .......... .......... .......... .
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Figure 3. Implied alignment of the mitochondrial DNA sequence data spanning the partial genes NADH3, tRNA^{Arg} and ND4L from tortoises of the *Gopherus agassizii* complex. BYU 39706 is the holotype of *G. lepidocephalus*. USNM 7888 is the lectotype of *G. agassizii*. GenBank sequence DQ649394 is the sequence of *G. agassizii* in widespread group A of Murphy et al. (2007), DQ649398 is from narrowly distributed group B, and DQ649406 is a specimen of *G. morafkai* from Tucson, Arizona. “n” indicates unresolved or ambiguous base pairs.

Figure 4. Dorsal view of the lectotype of *Gopherus agassizii*, USNM 7888. Black bar is 3 cm.

associated with the specimen. References to other localities, including Solado Valley, an apparent synonym of Soda Valley, should be considered to be in error.

The evolutionary species concept (Simpson 1961; Wiley 1978) suggests that the Sonoran population of the desert tortoise should be recognized as a new taxon. Frost and Hillis (1990) effectively argue that subspecies should not be recognized for continuously distributed species; we agree. Given these two observations, at least two species of desert tortoise should be recognized. The DNA sequence data exclude application of the available name *G. lepidocephalus* for the Sonoran Desert population of *Gopherus* that occurs west and south of the Colorado River and they confirm that the lectotype of *G. agassizii* is from the Mojave Desert, and not Arizona. Because no names are available for the tortoise population occurring in the Sonoran Desert south and east of the Colorado River, we describe it as a new species.

Gopherus morafkai, sp. n.

Morafka's Desert Tortoise
urn:lsid:zoobank.org:act:B4A14033-BD75-4D90-BFD0-CA3C76FB17EF
http://species-id.net/wiki/Gopherus_morafkai
Figs 9–15

Xerobates agassizii Cooper 1861 (partim)
Testudo agassizii (Cooper 1861) (partim). Generic reassignment by Cope (1875)

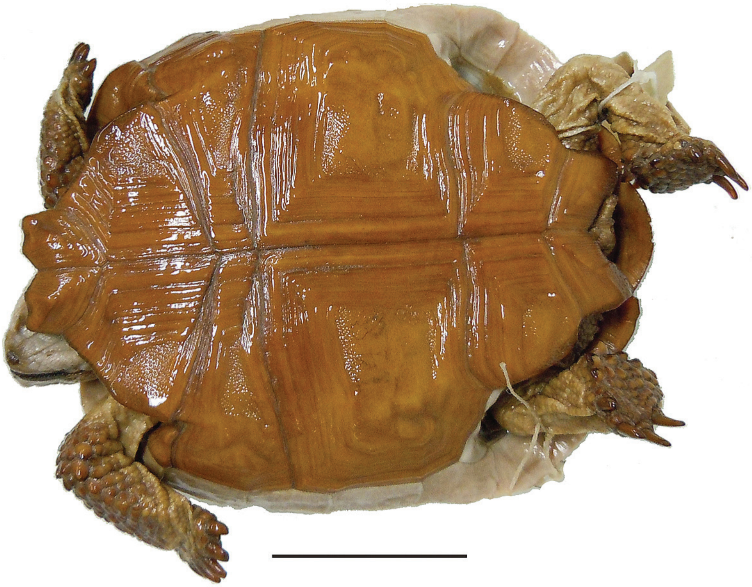

Figure 5. Ventral view of the holotype of *Gopherus agassizii*, USNM 7888. Black bar is 3 cm.

Gopherus agassizii (Cooper 1861) (partim). Generic reassignment by Stejneger (1893)
Scaptochelys agassizii (Cooper 1861) (partim). Generic reassignment by Bramble (1982)
Xerobates lepidocephalus Ottley et Velázques Solis 1989. In error by Crumly and Grismer (1994)

Holotype. CAS (California Academy of Sciences) 33867; juvenile from Tucson (approximate location 32° 7' N, 110° 56' W, elevation 948 m), Pima County, Arizona, U.S.A, collected on 9 July 1912 by H. Brown and preserved in ethanol.

Paratypes. ROM (Royal Ontario Museum) 47501, formerly CAS 13165, an immature tortoise collected by H. Brown from 20 miles (32 km) west of Tucson, (presumably the Roskruge Mountains, Pima County) Arizona, USA (approximate location 32° 7' N, 111° 18' W, where tortoises occur today), on 9 March 1908, received at CAS alive on 23 March 1908, and died 8 July 1908; CAS 34263, a juvenile collected by J.R. Slevin in the Catalina Mountains (Santa Catalina Mountains), foothills at west end of mountains, Pima County, Arizona, USA on 15 May 1912 (approximate location 32° 21' N, 110° 57' W). Specimens are preserved in ethanol.

Diagnosis. All of the species of *Gopherus* and their hybrids can be easily diagnosed using molecular data. Morphologically, *G. morafkai* can be separated from both *G. flavomarginatus* and *G. polyphemus* in having relatively smaller front feet. Whereas the distance from the bases of the first to fourth claws is the same on all feet in *G. morafkai*, in the latter two species the distance from the bases of the first and third claws on the forelimb is about the same as the distance between the bases of the first and fourth claws on the hindlimb (Auffenberg and Franz 1978). The diagnosis of living specimens

Figure 6. Anterior view of the holotype of *Gopherus agassizii*, USNM 7888. Black bar is 3 cm.

of *G. morafkai, G. berlandieri* and *G. agassizii* can be impossible in captive tortoises because of extensive hybridization (Edwards et al. 2010) and because of abnormalities in shell, head and limb integument from poor nutrition (Donoghue 2006). However, in non-hybrid individuals, *G. morafkai* can be separated from *G. berlandieri* in having a rounded snout when viewed from above as opposed to a wedge-shaped snout in *G. berlandieri* (Auffenberg and Franz 1978). Further, in *G. morafkai* the gular projections do not normally diverge, and it has a single axillary scale preceding each bridge, yet in *G. berlandieri* the gular projections often diverge and the axillary scales are often paired. Morphologically, *G. morafkai* can be separated from *G. agassizii* in having a relatively narrower shell, shorter gular scutes, shorter projections of the anal scutes and in having a flatter, pear-shaped carapace (Table 1). Ecologically, whereas *G. agassizii* predominantly occurs in valleys and alluvial fan topography, *G. morafkai* prefers slopes and rocky hillsides (Riedle et al. 2008), including animals of the isolated population in northwestern Arizona (McLuckie et al. 1999).

Description of holotype. A juvenile, with straight-line carapace length at midline (MCL) = 86.5 mm, maximum carapace length is 88.5, curved carapace length from free edge of nuchal scute to that of supracaudal scute = 118 mm, maximum plastron plastron length from tip of gular horn to tip of anal scutes = 86 mm, midline plastron length from gular notch to anal notch = 78 mm, maximum height of shell at 3rd vertebral scute = 40 mm, width at 3rd marginal scute = 64 mm, maximum midbody width = 69, maximum width at 7th marginal scute = 73 mm, and head length from tip of snout to posterior edge of supraoccipital condyle = 25 mm (Figs 8, 12). Eleven marginal scutes present on both right and left edges of carapace. Supracaudal scute single, undivided. Five toenails present on each forelimb, four toenails on each hind limb (Fig. 9). Third nail of each hind limb longer than others. Two enlarged, raised scales present on anterior ventral surface of foreleg of which the ventral-most scale is larger,

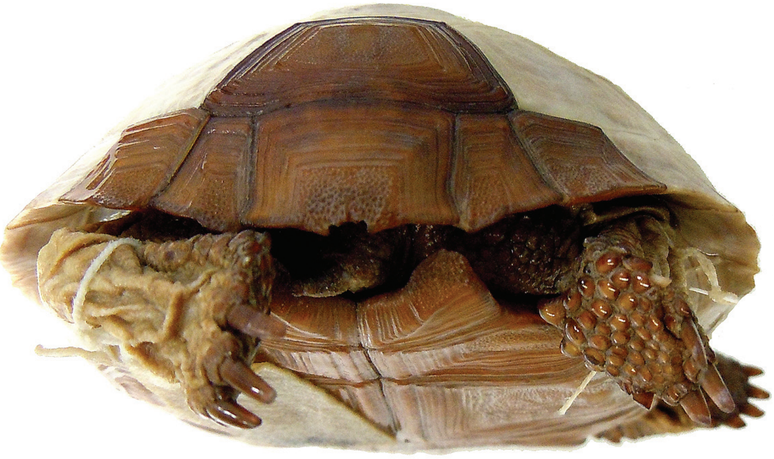

Figure 7. Posterior view of the holotype of *Gopherus agassizii*, USNM 7888.

more protruding than others. Scales on head smooth, asymmetrical, larger anteriorly at snout, becoming much smaller in temporal area (Fig. 12). Areolae and 7 to 8 growth laminae present on all scutes. In alcohol, the color of areolae and adjacent two growth laminae on carapacial scutes (Figs 8, 11) predominantly dark reddish brown grading to reddish black on laminae at or near seams between scutes. Small areas of areolae on 2nd and 3rd vertebral scutes and left 1st costal scute yellowish brown or copper. Color of areolae on plastron light olive brown grading to dark yellowish brown on 2nd through 4th laminae. Laminae at and adjacent to the seams dark reddish brown with a few areas of dark red. Head and neck multi-colored (Figs 8, 9, 13): neck and throat very pale yellowish brown and very pale brown. Dorsal and lateral surfaces of head darken from parietal to frontal scales (Fig. 12). Skin in the axillary and inguinal areas also lighter in coloration, becoming reddish brown to dark reddish brown on lower limbs and pads of feet (Fig. 10). Nails golden brown at tips.

Coloration of the species in life. Coloration of *G. morafkai* varies considerably by size and age as well as by location. Adult tortoises generally have hues and chromas of the integument in dark colors, e.g., very dark greyish brown, dark brown, very dark brown, olive brown, dark olive brown, reddish brown, dark reddish brown, dark grey, black, and occasionally to rarely xanthic tones (GretagMacbeth 2000). Neonates and young juveniles tend to be bi-colored, with orange to reddish areolae and reddish brown to dark brown laminae. As the juveniles age, they become darker. Coloration of limb scales tends to mirror that of the shell. Based on observations of the authors, the protected skin in axillary and inguinal areas is generally in lighter colors for all sizes and ages of tortoises.

Variation. Variation in coloration and morphology deserve further research with respect to location, vegetation and soil types, as well as by size, sex, and age of the tortoise. All future studies should include genetic documentation of non-hybrid specimens.

Figure 8. Left, lateral view of the holotype of *Gopherus agassizii*, USNM 7888.

Distribution. *Gopherus morafkai* occurs naturally east and south of the Colorado River in Arizona, as well as in Sonora, including Tiburon Island, and Sinaloa on the west side of the Sierra Madre Occidental, Mexico (Berry et al. 2002). The species appears to have been recently introduced from Sonora into at least one home in La Paz, Baja California Sur, Mexico as pets, where it successfully reproduced (Patricia Galina, personal communication to RWM). It likely occurs as introduced individuals or populations in North America and possibly elsewhere, although in this case many individuals are likely hybrids of *G. morafkai* x *agassizii*.

Natural history. *Gopherus morafkai* occurs in upland habitats in the Sonoran Desert scrub (Brown et al. 1979) with rocky outcrops and palo verde-saguaro cactus communities and ecotonal desert grasslands (Van Devender 2002). Within these habitats, *G. morafkai* is generally found along rocky slopes, or bajadas, of desert mountain ranges, with breeding populations occurring as high as 1,420 m elevation and individual observation records occurring to 2,380 m (Flesch et al. 2010). The species typically occupies excavated or eroded burrows underneath rocks or boulders. Consequently, geology and resultant burrow availability among mountain ranges is an important determinant in regulating population density (Averill-Murray et al. 2002a, b). Low density populations of *G. morafkai* also occur along alluvial fans and in intermountain valleys, where individuals utilize desert washes and associated caliche caves as shelter sites (Riedle et al. 2008; Grandmaison et al. 2010). These peripheral populations provide important genetic linkages between disjunct mountain ranges (Edwards 2003; Edwards et al. 2004; Averill-Murray and Averill-Murray 2005).

Gopherus morafkai exhibits both a spring (mid-March to May) and a late summer activity period (late July to late September). Activity patterns are rainfall-dependent, with increased activity related to increased precipitation during the late summer monsoons (Averill-Murray et al. 2002b). Monsoonal storms within the range of G.

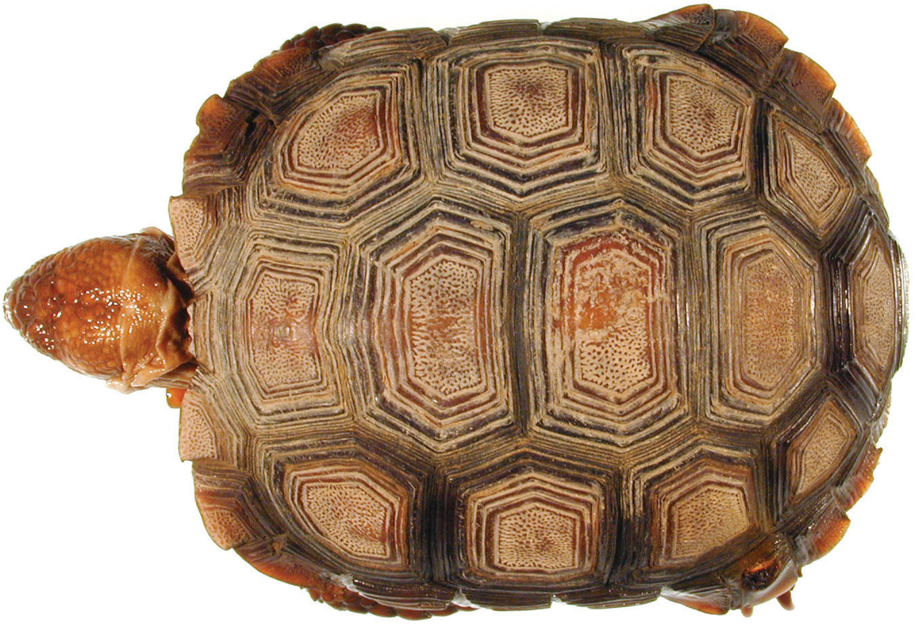

Figure 9. Dorsal view of the holotype of *Gopherus morafkai*, CAS 33867.

morafkai result from warm season winds pushing tropical moisture northwards from the Pacific Ocean and northern Mexico (Turner and Brown 1994). Female activity begins earlier than male activity in the spring, possibly because females might need to forage to develop shelled eggs before oviposition in June and July (Averill-Murray et al. 2002a). Activity is higher for both sexes during late summer monsoons, with courtship and breeding occurring in July–September (Averill-Murray et al. 2002a). Females develop ovarian follicles before entering brumation in the fall (Henen et al. 2000). The follicles probably mature in the spring with oviposition shortly afterwards (Henen et al. 2000; Averill-Murray 2002). Clutch size ranges from 1–12 eggs with a mean of 5.7 eggs (Averill-Murray 2002).

Female *G. morafkai* mature at larger sizes (220 mm carapace length) (Averill-Murray 2002) than does *G. agassizii* (176–190 mm carapace length) (Turner et al. 1986; Germano 1994a; Karl 1998). Clutch sizes between the two species are similar (Averill-Murray 2002), but *G. morafkai* only produces 1 clutch every 1–2 yr (Averill-Murray 2002) while *G. agassizii* may produce 1–3 clutches every year (Turner et al. 1986; Wallis et al. 1999). Harsher, more arid climates in the Mojave Desert may have led to increased female reproductive investment to offset hatchling and juvenile mortality (Heppell 1998; Hellgren et al. 2000), but information is limited for juvenile tortoises of both species.

Annual survivorship for juvenile *G. morafkai* at three sites in Arizona ranged from 0.84 to 0.93 (Averill-Murray et al. 2002a). Adult survivorship was high (0.89–0.97).

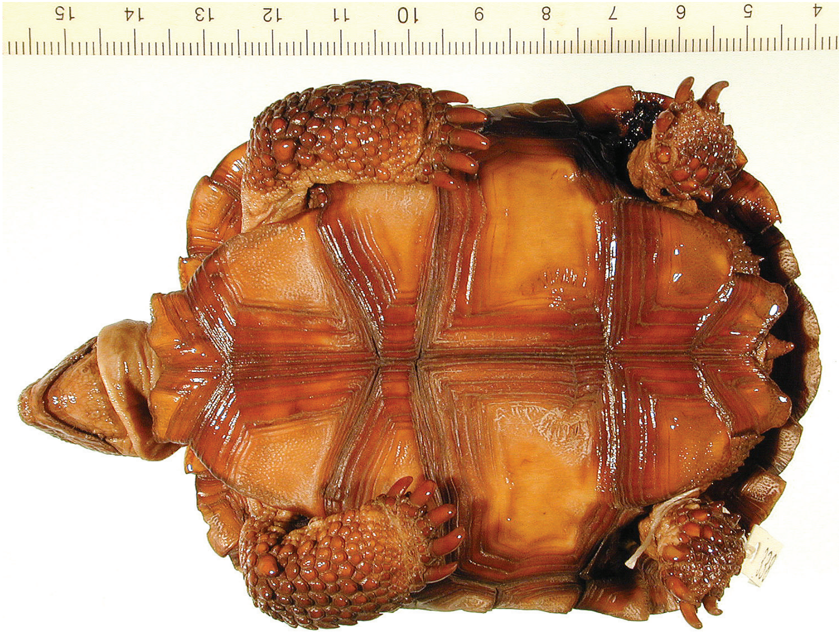

Figure 10. Ventral view of the holotype of *Gopherus morafkai*, CAS 33867.

Seasonal differences in mortality reflected seasonal differences in activity patterns (Riedle et al. 2010). Adult survivorship was similar between both species (Table 1), although little was determined about hatchling or juvenile survivorship. Primary sources of mortality for *G. morafkai* in Arizona included the following: 1) falls related to steep rocky habitat; 2) being overturned during combat and mating rituals; and 3) predation by mountain lions, *Puma concolor* (Riedle et al. 2010). Prehistorically, Native Americans ate Mojave and Sonoran tortoises (Schneider and Everson 1989) and historically, Native Americans and Mexicans hunted the tortoise for food (Cooper 1861; Cox 1881), although Cooper (in Cronise 1868: 480; see True 1881) reported that they were "not very well flavored."

Etymology. The new species is a patronym for the late Professor David Joseph Morafka in recognition of his many contributions to the biology and conservation of the species of *Gopherus* and his unsurpassed way of facilitating research, even among researchers with very different perspectives.

Discussion

Few paratypes

We designated only two of many possible paratypes to exclude the possibility of hybrid individuals in the type series. Hybrid animals would confound the identity of *G.*

Figure 11. Anterior view of the holotype of *Gopherus morafkai*, CAS 33867.

morafkai (Edwards et al. 2010). To this end, we specifically selected individuals collected from near the turn of the 19th Century from one of the oldest western North American herpetological collections, the California Academy of Sciences. The intent was to select paratypes collected before the development of major trans-desert highways that followed mass-produced automobiles, which in turn facilitated interspecific translocations. The future documentation of variation in the species, which should be accomplished within the context of geographic and habitat variation, must be restricted to wild-caught individuals genetically confirmed to be non-hybrids. Unfortunately, this may exclude the use of many formalin-fixed animals in museum collections. Such investigations could delineate morphological characters useful in identifying F_1 and other hybrid individuals.

Common names

Common names do not enjoy precedence and they can create much unnecessary confusion. Historically, the species of *Gopherus* were commonly referred to simply as gophers, a word that normally refers to mammals. Now that *G. morafkai* is recognized, the desert tortoise requires two common names. *Gopherus agassizii* could be referred to as the Mojavian desert tortoise, yet this is inaccurate because the species also occurs within the Sonoran Desert of California. Therefore, we prefer to call it Agassiz´s desert tortoise. This name also serves to retain the original designation of Cooper (1861). Similarly, *G. morafkai* occurs in the Mojave Desert of Arizona, the Sonoran Desert of

Figure 12. Posterior view of the holotype of *Gopherus morafkai*, CAS 33867.

Arizona, USA and Sonora, Mexico and in Sinaloan thornscrub, but not in the Mojave and Sonoran deserts of California. Therefore, the term Sonoran desert tortoise is inaccurate. Consequently, we prefer to call this species Morafka´s desert tortoise. These common names will serve to exclude the species from other desert tortoises in the genus *Testudo*.

Implications for conservation of western Gopherus

The most important implication of describing *G. morafkai* is that Arizona and Mexico can no longer be considered to harbor a genetic reservoir for the Mojavian population of the desert tortoise, now exclusively defined as *G. agassizii*. The recognition of *G. morafkai* reduces the geographic range of *G. agassizii* to about 30% of its former range (Van Devender 2002, Fig. 1.2); *G. agassizii* now occupies an estimated 83,124 km^2 of habitat (Fig. 2, also see model in U.S. Dept. of the Interior, Fish and Wildlife Service 2010a). *Gopherus agassizii*, which can now be referred to as Agassiz's desert tortoise, has suffered tremendous population declines in the past 30 years (U.S. Dept. of the Interior, Fish and Wildlife Service 1994, 2010a). And much of the Mojave Desert

Figure 13. Detail of head scales of the holotype of *Gopherus morafkai*, CAS 33867.

does not offer habitat suitable for *G. agassizii* (Hagerty et al. 2011). The taxonomic reduction of the species' distribution can have dire consequences. Whereas species with broad distributions may survive population declines, those that have small distributions are far more likely to become extinct (MacArthur and Wilson 1963, 1967; Gilpin and Soulé 1986; Saccheri et al. 1998; O'Grady et al. 2006). Agassiz's desert tortoise, currently listed as threatened under the Endangered Species Act of 1973 (as amended) (U.S. Dept. of the Interior, Fish and Wildlife Service 1990), may require a higher level of protection to ensure the level of management that would maximize its chances of survival.

A Recovery Plan was prepared for the Mojavian population (Agassiz's desert tortoise) in 1994 (U.S. Dept. of the Interior, Fish and Wildlife Service 1994). Six recovery units were described in this Recovery Plan in an effort to capture ecological and genetic variation. The writers of the Recovery Plan also noted evidence of important ecological substructuring within the Western Mojave Recovery Unit, the largest and most heterogenous of the recovery units in terms of climate, vegetation and topography (U.S. Dept. of the Interior, Fish and Wildlife Service 1994). In an analysis of

Figure 14. Right, lateral view of the head of the holotype of *Gopherus morafkai*, CAS 33867.

genetic differences within the Mojavian population, Murphy et al. (2007) confirmed that genetic substructuring existed within the Western Mojave Recovery Unit, with boundaries similar to those described in the 1994 Recovery Plan for western, central, and southern regions. The boundaries followed a major river, the Mojave River, as well as other climatic and ecological differences. Hagerty et al. (2011) confirmed the pattern reported by Murphy et al. (2007), although Hagerty and Tracy (2010) speculated that patterns reported by Murphy et al. (2007) were due to sampling bias. We think that the new genetic information from Murphy et al. (2007) and Hagerty et al. (2011) provide important support for updating recovery planning in the future.

Conservation status of G. morafkai

Population declines for *G. morafkai* within the USA appear to mirror those of *G. agassizii* (Arizona Interagency Desert Tortoise Team 1996). In 2010, the U.S. Fish and Wildlife Service issued a determination that federal listing of the Sonoran population

Figure 15. Detailed view of the anal scutes of the holotype of *Gopherus morafkai*, CAS 33867.

as threatened in the USA is warranted but precluded by other, higher priority species (U.S. Dept. of the Interior 2010b). The recognition of *G. morafkai* is likely to hasten federal listing of the new species, *G. morafkai*, in the USA. The Mojave population can no longer be considered to be a genetic reservoir for *G. morafkai* or vice versa, and, unfortunately, the hybrid ex situ population involves a significant portion of tortoises presumed to be *G. morafkai* (Edwards et al. 2010). These hybrids involve not only *G. agassizii* but also *G. berlandieri*. The genetic integrity of *G. morafkai* may now be threatened by intentional release and escape of captive hybrids. As noted previously, natural hybrids occur in a limited portion of northwestern Arizona where Mojave and Sonoran ecosystems interdigitate (McLuckie et al. 1999). The hybrid zone appears to occur only in this area (Fig. 2). Little is known about the effects of hybridization on the native population, a topic that deserves attention. Adding to the concerns, *G. morafkai* may contain two cryptic taxa in Mexico (Lamb et al. 1989). All species and populations in both Mexico and the USA would benefit from aggressive conservation action because of the potential for additional cryptic species in Mexico.

Remaining problems

The questioned identity of *G. lepidocephalus* has now been sufficiently answered to address its taxonomic status. The name is a junior synonym of *G. agassizii*. Whether the holotype is a hybrid or not is taxonomically irrelevant because the maternal lineage had an origin in the Mojave Desert population. Nevertheless, three questions remain. First, is the holotype of *G. lepidocephalus* a hybrid individual? This could explain its uniqueness (Ottley and Velázques Solis 1989) as well as it association with Sonora, Mexico (Crumly and Grismer 1994). Second, the question remains as to whether *G. morafkai* consists of two forms that warrant recognition at the species level: Morafka's desert tortoise and a potentially new Sinaloan thornscrub tortoise (Lamb et al. 1989). Currently, we are examining the spatial overlap of several genotypes at the eastern and southern boundaries of Sonoran desert scrub in Sonora, Mexico to better understand the evolutionary drivers responsible for shaping the genetic diversity of *G. morafkai*, and to evaluate the possibility that the species is a composite of two cryptic species. Finally, it is critical to evaluate ontogenetic development in both species. This may vary geographically within species as well as with nutrition and other environmental parameters.

Conclusion

Our investigation of the taxonomy of Agassiz's land tortoise resolved many issues. The publication date has been given in error as 1863 since its first citation. The type series was likely collected by Cooper from near Soda Lake, California, and not elsewhere. Only one of the three original cotypes exists, USNM 7888, and it was designated as the lectotype. Our mtDNA sequence data from the lectotype confirmed that it was from California, not Arizona. Further, mtDNA sequence data from the holotype of *G. lepidocephalus* placed its origin to the Mojavian population, rather than the Sonoran Desert of either Arizona or Mexico. Genetic, morphological and ecological data confirmed the existence of at least two species contained within *G. agassizii*. The Sonoran population is named as a new species, *G. morafkai*, Morafka`s desert tortoise. The recognition of *G. morafkai* reduces the range of *G. agassizii* to occupying about 30% of its former range. Given drastic population declines in *G. agassizii* during the past few decades, it might be endangered.

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References

Arizona Interagency Desert Tortoise Team (1996) Management plan for the Sonoran Desert population of the desert tortoise in Arizona. Arizona Interagency Desert Tortoise Team and Arizona Game and Fish Department, Phoenix, 55 pp.

Auffenberg W, Franz R (1978) *Gopherus agassizii*. Catalogue of American Amphibians and Reptiles 212: 1–2.

Averill-Murray RC (2002) Reproduction of *Gopherus agassizii* in the Sonoran Desert, Arizona. Chelonian Conservation and Biology 4: 295–301.

Averill-Murray RC, Averill-Murray A (2005) Regional-scale estimation of density and habitat use of the desert tortoise (*Gopherus agassizii*) in Arizona. Journal of Herpetology 39: 65–72.

Averill-Murray RC, Martin BE, Bailey SJ, Wirt EB (2002a) Activity and behavior of the Sonoran desert tortoise in Arizona. In: Van Devender TR (Ed) The Sonoran Desert Tortoise: Natural History, Biology, and Conservation. University of Arizona Press and Arizona-Sonora Desert Museum, Tucson, 135–158.

Averill-Murray RC, Woodman AP, Howland JM (2002b) Population ecology of the Sonoran desert tortoise in Arizona. In: Van Devender TR (Ed) The Sonoran Desert Tortoise: Natural History, Biology, and Conservation. University of Arizona Press and Arizona-Sonora Desert Museum, Tucson, 109–134.

Berry KH, Morafka DJ, Murphy RW (2002) Defining the desert tortoise(s): our first priority for a coherent conservation strategy. Chelonian Conservation and Biology 4: 249–262.

Bour R, Dubois A (1984) *Xerobates agassizii*, 1857, synonyme ancient de *Scaptochelys* Bramble, 1982 (Reptilia, Chelonii, Testudinidae). Bulletin Mensuel de la Société Linnéenne de Lyon 53: 30–32.

Bramble DM (1982) *Scaptochelys*: generic revision and evolution of gopher tortoises. Copeia 1982: 852–867.

Brown AE (1908) Generic types of Nearctic Reptilia and Amphibia. Proceedings of the Academy of Natural Sciences of Philadelphia 60: 112–127.

Brown DE, Lowe CH, Pase CP (1979) A digitized classification system for the biotic communities of North America, with community (series) and association examples for the Southwest. Journal of the Arizona–Nevada Academy of Science 14, Supplement: 11–16.

Bucklin A, Allen LD (2004) MtDNA sequencing from zooplankton after long-term preservation in buffered formalin. Molecular Phylogenetics and Evolution 30: 879–882.

Coan E (1981) James Graham Cooper, Pioneer Western Naturalist. University Press of Idaho, Idaho Research Foundation, Moscow, Idaho. 255 pp.

Cochran DM (1961) Type specimens of reptiles and amphibians in the U.S. National Museum. Bulletin of the U.S. National Museum 220: 1–291.

Cooper JG (1861) New Californian animals. Proceedings of the California Academy of Sciences (ser. 1) 2: 118–123.

Cooper JG (1870) The fauna of California and its geographical distribution. Proceedings of the California Academy of Sciences (ser. 1) 4: 61–81.

Cope ED (1875) Check-list of North American Batrachia and Reptilia. Bulletin of the U.S. National Museum 1: 1–104.

Cope ED (1880) On the zoölogical position of Texas. Bulletin of the U.S. National Museum 17: 1–51.

Cox ET (1881) The tortoises of Tucson. American Naturalist 15: 1003–1004.

Cronise TF (1868) The Natural Wealth of California: Comprising Early History; Geography, Topography, and Scenery; Climate; Agriculture and Commercial Products; Geology, Zoology, and Botany; Mineralogy, Mines, and Mining Processes; Manufactures; Steamship Lines, Railroads, and Commerce; Immigration, Population and Society; Educational Institutions and Literature; Together with a Detailed Description of Each County; its Topography, Scenery, Cities and Towns, Agricultural Advantages, Mineral Resources, and Varied Productions. H.H. Bancroft & Company, San Francisco.

Crumly CR (1994) Phylogenetic systematics of North American tortoises (Genus *Gopherus*): Evidence for their classification. In: Bury RB, Germano DJ (Eds), Biology of North American Tortoises. Fish and Wildlife Research 13: 7–31.

Crumly CR, Grismer LL (1994) Validity of the tortoise *Xerobates lepidocephalus* Ottley and Velazquez [sic] in Baja California. In: Bury RB, Germano DJ (Eds), Biology of North American Tortoises. Fish and Wildlife Research 13: 32–36.

Curtin AJ, Zug GR, Spotila JR (2009) Longevity and growth strategies of the desert tortoise (*Gopherus agassizii*) in two American deserts. Journal of Arid Environments 73: 463–471.

Donoghue S (2006) Nutrition. In: Mader R (Ed) Reptile Medicine and Surgery. 2nd ed. Saunders Elsevier, St. Louis, Missouri, 251–298.

Edwards T (2003) Desert tortoise conservation genetics. MSc thesis, Tucson, Arizona, University of Arizona.

Edwards T, Schwalbe CR, Swann DE, Goldberg CS (2004) Implications of anthropogenic landscape change on inter-population movements of the desert tortoise (*Gopherus agassizii*). Conservation Genetics 5: 485–397.

Edwards T, Jarchow CJ, Jones CA, Bonine KE (2010) Tracing genetic lineages of captive desert tortoises in Arizona. Journal of Wildlife Management 7: 801–807.

Edwards T, Lathrop A, Ngo A, Choffe K, Murphy RW (2011) STR/microsatellite primers for the desert tortoise, *Gopherus agassizii*, and its congeners. Conservation Genetics Resources 3: 365–368. DOI: 10.1007/s12686-010-9363-6.

Engstrom TN, Edwards T, Osentoski MF, Myers EM (2007) A compendium of PCR primers for mtDNA, microsatellite, and other nuclear loci for freshwater turtles and tortoises. In: Shafer HB, FitzSimmons NN, Georges A, Rhodin AGH (Eds) Defining Turtle Diversity: Proceedings of a Workshop on Genetics, Ethics, and Taxonomy of Freshwater Turtles and Tortoises. Chelonian Research Monographs 4: 124–141.

Flesch AD, Swann DE, Turner DS, Powell BF (2010) Herpetofauna of the Rincon Mountains, Arizona. The Southwestern Naturalist 55: 240–253.

Frost DR, Hillis DM (1990) Species in concept and practice: herpetological applications. Herpetologica 46: 87–104.

Fritz U., Havaš P (2007) Checklist of chelonians of the world. Vertebrate Zoology 57: 149–368.

Germano DJ (1993) Shell morphology of North American tortoises. American Midland Naturalist 129: 319–335.

Germano DJ (1994a) Growth and age at maturity of North American tortoises in relation to regional climates. Canadian Journal of Zoology 72: 918–931.

Germano DJ (1994b) Comparative life histories of North American tortoises. In: Bury RB, Germano DJ (Eds), Biology of North American Tortoises. Fish and Wildlife Research 13: 175–185

Gilpin ME, Soulé ME (1986). Minimum viable populations: the processes of species extinctions. In: Soulé M (Ed) Conservation Biology: The Science of Scarcity and Diversity. Sinauer Associates, Sunderland Mass., 13–34.

Grandmaison DD, Ingaldi MF, Peck C R (2010) Desert tortoise microhabitat selection on the Florence Military Reservation, south-central Arizona. Journal of Herpetology 44: 581–590.

Gray, JE (1870) Supplement to the catalogue of shield reptiles in the collection of the British Museum. Part I. Testudinata (tortoises). Taylor and Francis, London.

GretagMacbeth (2000) Munsell® Soil Color Charts, rev. ed. GretagMacbeth, New Windsor, New York.

Hagerty BE, Tracy CR (2010) Defining population structure for the Mojave desert tortoise. Conservation Genetics 11: 1795–1807.

Hagerty BE, Nussear KE, Esque TC, Tracy CR (In press) Making molehills out of mountain landscape genetics of the Mojave desert tortoise. Landscape Ecology. DOI: 10.1007/s10980-010-9550-6.

Hellgren EC, Kazmaier RT, Ruthven DC, III Synatzske DR (2000) Variation in tortoise life history: demography of *Gopherus berlandieri*. Ecology 81: 1297–1310.

Heppell SS (1998) Application of life history theory and population model analysis to turtle conservation. Copeia 1998: 367–375.

Henen BT (1994) Seasonal and Annual Energy and Water Budgets of Female Desert Tortoises (*Gopherus agassizii*) at Goffs, California. PhD thesis. Los Angeles, California, University of California.

Henen, BT (1997) Seasonal and annual energy budgets of female desert tortoises. Ecology 78: 283–296.

Henen BT, Averill-Murray RC, Kluge CM (2000) Egg follicles and yolks of Sonoran desert tortoises (*Gopherus agassizii*). Abstracts: 25th Annual Meeting of the Desert Tortoise Council, April 212-–4, 2000, 1 pp. Available at http:// deserttortoise.org/.

Karl AE (1998) Reproductive Strategies, Growth Patterns, and Survivorship of a Long-Lived Herbivore Inhabiting a Temporally Variable Environment. PhD thesis, Davis, California, University of California.

Lamb T, Avise JC, Gibbons JW (1989) Phylogeographic patterns in mitochondrial DNA of the desert tortoise (*Xerobates agassizii*), and evolutionary relationships among the North American gopher tortoises. Evolution 43: 76–87. doi:10.2307/2409165

Lamb T, Lydeard C (1994) A molecular phylogeny of the gopher tortoises, with comments on familial relationships within the Testudinoidea. Molecular Phylogenetics and Evolution 3: 283–291. doi:10.1006/mpev.1994.1036

Lamb T, McLuckie AM (2002) Genetic differences among geographic races of the desert tortoise. In: Van Devender TR (Ed) The Sonoran Desert Tortoise: Natural History, Biology, and Conservation. University of Arizona Press and Arizona-Sonora Desert Museum, Tucson, 67–85.

Leviton AE, Aldrich ML (1997) Theodore Henry Hittell's The California Academy of Sciences 1853–1906. Memoirs of the California Academy of Sciences 22: i–xv, 1–623.

Leviton AE, Aldrich ML (2010) Introduction and historical overview. In: Leviton AE, Wogan GOU, Aldrich ML (Eds) Scientific Publications of the California Academy of Sciences, 1854–2010. Occasional Papers of the California Academy of Sciences 158: vii–xiii.

Leviton AE, Wogan GOU, Aldrich ML (2010) Scientific publications of the California Academy of Sciences, 1854–2010. Occasional Papers of the California Academy of Sciences 158: i–xiii, 1–568 pp.

Lipps JH (2004) Success story: the history and development of the Museum of Paleontology at the University of California. In: Leviton AE, Aldrich ML (Eds) Museums and Other Institutions of Natural History: Past, Present, and Future. Proceedings of the California Academy of Sciences, ser. 4, 55 (Suppl. I, 9): 209–243.

MacArthur RH, Wilson EO (1963) An equilibrium theory of insular zoogeography. Evolution 17: 373–387. doi:10.2307/2407089

MacArthur RH, Wilson EO (1967) The Theory of Island Biogeography. Princeton University Press, Princeton, New Jersey.

McLuckie AM, Lamb T, Schwable CR, McCord RD (1999) Genetic and morphological assessment of an unusual tortoise (*Gopherus agassizii*) population in the Black Mountains of Arizona. Journal of Herpetology 33: 36–44. doi:10.2307/1565541

Mertens R, Wermuth H (1955) Die rezenten Schildkröten, Krokodile, und Brückenechsen. Eine kritische Liste der heute lebenden Arten und Rassen. Zoologische Jahrbuecher 83: 323–440.

Mueller JM, Sharp KR, Zander KK, Rakestraw DL, Rautenstauch KR, Lederle PE (1998) Size-specific fecundity of the desert tortoise (*Gopherus agassizii*). Journal of Herpetology 32: 313–319. doi:10.2307/1565443

Murphy RW, Berry KH, Edwards T, McLuckie AM (2007) A genetic assessment of the recovery units for the Mojave population of the desert tortoise, *Gopherus agassizii.* Chelonian Conservation and Biology 6: 229–251. doi:10.2744/1071-8443(2007)6[229:AGAOTR]2.0.CO;2

O'Grady JJ, Brook BW, Reed DH, Ballou JD (2006) Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation 133: 42–51. doi:10.1016/j.biocon.2006.05.016

Ottley JR, Velázques Solis VM (1989) An extant, indigenous tortoise population in Baja California Sur, Mexico, with the description of a new species of *Xerobates* (Testudines: Testudinidae). Great Basin Naturalist 49: 496–502.

Reynolds RP, Gotte SW, Ernst CH (2007) Catalog of type specimens of Recent Crocodilia and Testudines in the National Museum of Natural History, Smithsonian Institution. Smithsonian Contributions to Zoology 626: (v), 1–49, 1 table.

Reynoso V-H, Montellano-Ballesteros M (2004) A new giant turtle of the genus *Gopherus* (Chelonia: Testudinidae) from the Pleistocene of Tamaulipas, México, and a review of the phylogeny and biogeography of gopher tortoises. Journal of Vertebrate Paleontology 24: 822–837. doi:10.1671/0272-4634(2004)024[0822:ANGTOT]2.0.CO;2

Riedle JD, Averill-Murray RC, Lutz CL, Bolen DK (2008) Habitat use by desert tortoises (*Gopherus agassizii*) on alluvial fans in the Sonoran Desert, south-central Arizona. Copeia 2008: 414–420. doi:10.1643/CH-06-010

Riedle JD, Averill-Murray RC, Grandmaison DD (2010) Seasonal variation in survivorship and mortality of desert tortoises in the Sonoran Desert, Arizona. Journal of Herpetology 44: 164–167. doi:10.1670/08-055.1

Saccheri I, Kuussaari M, Kankare M, Vikman P (1998) Inbreeding and extinction in a butterfly metapopulation. Nature 392: 491–494. doi:10.1038/33136

Schneider JS, Everson GD (1989) The desert tortoise (*Xerobates agassizii*) in the prehistory of the southwestern Great Basin and adjacent areas. Journal of California & Great Basin Anthropology 11: 175–202.

Simpson GG (1961) Principles of Animal Taxonomy. Columbia University Press, New York.

Stejneger L (1893) Annotated list of the reptiles and batrachians collected by the Death Valley Expedition in 1891, with descriptions of new species. North American Fauna 7: 159–228. doi:10.3996/nafa.7.0002

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680. doi:10.1093/nar/22.22.4673

True FW (1881) On the North American land tortoises of the genus *Xerobates*. Proceedings of the U.S. National Museum 4: 434–449.

Turner FB, Medica PA, Lyons CL (1984) Reproduction and survival of the desert tortoise (*Scaptochelys agassizii*) in Ivanpah Valley, California. Copeia 1984: 811–820. doi:10.2307/1445322

Turner FB, Hayden P, Burge BL, Roberson JB (1986) Egg production by the desert tortoise (*Gopherus agassizii*) in California. Herpetologica 42: 93–104.

Turner FB, Berry KH, Randall DC, White GC (1987) Population ecology of the desert tortoise at Goffs, California. Report to the Southern California Edison Company, Research and Development Series 87-RD-81.

U.S. Dept. of the Interior (1990). Endangered and threatened wildlife and plants: determination of threatened status for the Mojave population of the desert tortoise. Federal Register 55: 12178–12191.

U.S. Dept. of the Interior, Fish and Wildlife Service (1994) Desert tortoise (Mojave population) Recovery Plan. Portland, Oregon, U.S. Fish and Wildlife Service.

U.S. Dept. of the Interior, Fish and Wildlife Service (2010a) Mojave Population of the Desert Tortoise (Gopherus agassizii). 5-Year Review: Summary and Evaluation. Reno, Nevada, Desert Tortoise Recovery Office, U.S. Fish and Wildlife Service, September 30, 2010.

U.S. Dept. of the Interior, Fish and Wildlife Service (2010b) Endangered and threatened wildlife and plants; 12-month finding on a petition to list the Sonoran population of the desert tortoise as endangered or threatened. Federal Register 75(239): 78094–78146. December 14, 2010.

Van Denburgh J (1897) The reptiles of the Pacific Coast and Great Basin: An account of the species known to inhabit California, and Oregon, Washington, Idaho and Nevada. Occasional Papers of the California Academy of Sciences 5: 1–236.

Van Devender TR (2002) Natural History of the Sonoran Tortoise in Arizona. In: Van Devender TR (Ed) The Sonoran Desert Tortoise: Natural History, Biology, and Conservation. University of Arizona Press and Arizona-Sonora Desert Museum, Tucson, 3–28.

Wallis IR, Henen BT, Nagy KA (1999) Egg size and annual egg production by female desert tortoises (*Gopherus agassizii*): the importance of food abundance, body size, and date of egg shelling. Journal of Herpetology 33: 394–408. doi:10.2307/1565636

Weinstein MN, Berry KH (1989) Morphometric analysis of desert tortoise populations. Riverside, California, U.S. Dept. of the Interior, Bureau of Land Management.

Wermuth H, Mertens R (1961) Schildkröten, Krokodile, Brückenechsen. Gestav Fischer Verlag, Jena.

Wiley EO (1978) The evolutionary species concept reconsidered. Systematic Biology 27: 17–26. doi: 10.2307/2412809.

Yarrow HC (1883 [1882]) Check list of North American Reptilia and Batrachia with catalogue of specimens in U.S. National Museum. United States National Museum Bulletin 24: ii, 1–249.