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Biological and chemical characterizations of three new species of *Dysidea* (Porifera: Demospongiae) from the Pacific Mexican coast

José Luis Carballo [a,*], Eva Zubía [b], María J. Ortega [b]

[a] *Instituto de Ciencias del Mar y Limnología, UNAM (Estación Mazatlán), Avenida Joel Montes Camarena s/n, PO Box 811, Mazatlán (SIN) 82000, Mexico*
[b] *Departamento de Química Orgánica, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Apdo. 40, 11510 Puerto Real (Cádiz), Spain*

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Abstract

The integration of biological and chemical data has provided patterns to identify three new *Dysidea* species from the Gulf of California (Pacific Ocean, Mexico). These three species are differentiated by distinctive morphological characters, such as the prominent subdermal fibers running along the surface in *Dysidea reformensis* n. sp., the granular appearance of the surface in conjunction with the consistent habit in *Dysidea cachui* n. sp., and the skeletal details such as tertiary network between the secondary fibers in *Dysidea uriae* n. sp. Differences are also observed in the natural products content of these species. Sesquiterpene-hydroquinone derivatives are the main secondary metabolites of *D. reformensis* and *D. cachui*, with each species displaying a different chemical profile, while the furanosesquiterpene dendrolasin has been isolated from *D. uriae*. The distinctive morphological characters and the natural products contained by each species are also compared with those of related *Dysidea* species.
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Keywords: *Dysidea*; Natural products; Chemotaxonomy; Sesquiterpenes; Sesquiterpene-hydroquinones

1. Introduction

Taxonomy based on morphological characters of Porifera is relatively difficult compared with other invertebrate phyla. Although several classes of chemical compounds such as fatty acids (Lawson et al., 1984), sterols (Bergquist et al., 1980; Fromont et al., 1994), brominated derivatives, and terpenes (Bergquist and Wells, 1983) have been used to distinguish among different groups of sponges (Bergquist and Wells, 1983; Rovirosa et al., 1990), the degree of inconsistency found prevents the use of chemical data as a single tool to solve taxonomic classification problems, or to erect new taxa (Soest and Braekman, 1999). However, multidisciplinary approaches that combine histological,

* Corresponding author. Fax: +52 669 985 2845.
E-mail address: jlcarballo@ola.icmyl.unam.mx (J.L. Carballo).

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ecological, and/or chemical data with sponge morphology have proven useful to differentiate between closely related species (Braekman et al., 1992; Kelly-Borges et al., 1994).

The sponges of the family Dysideidae Gray, 1867 are characterized by possessing a distinctive structure of detritus-cored fibers, a reticulate skeleton, and eurypylous choanocyte chambers (Cook and Bergquist, 2002). The most diverse genus within this family is *Dysidea* Johnston, 1842 which encompasses more than 40 identified species, distributed mainly along tropical and temperate waters of all oceans (Cook and Bergquist, 2002).

Species of the genus *Dysidea* are frequently difficult to differentiate due to the plasticity of their morphological characteristics (Bergquist, 1965, 1980, 1995; Bergquist et al., 1990). Thus, the use of additional criteria may provide valuable clues for taxonomic classification. From a chemical point of view, the species of the genus *Dysidea* have been the source of an array of new bioactive natural products, which include terpenes, meroterpenes and sterols (Faulkner, 2002; Blunt et al., 2005). In addition, certain Dysideidae species, exemplified by *Lamellodysidea herbacea* (Keller, 1889) and *Lamellodysidea chlorea* (de Laubenfels, 1954) (originally described as *Dysidea* species), have given rise to brominated diphenyl ethers and chlorinated amino acid derivatives (Faulkner, 2002; Blunt et al., 2005), although there is evidence to support the likely symbiont origin of these metabolites (Faulkner et al., 1993, 1994; Unson and Faulkner, 1993; Unson et al., 1994; Flowers et al., 1998).

In this paper, we report the characterization of three new species of *Dysidea* sponges from the Pacific coast of Mexico by means of the combined analysis of morphological characters and natural products content. These three new species have been named *Dysidea reformensis*, *Dysidea cachui*, and *Dysidea uriae*.

2. Materials and methods

2.1. Biological material

Sponges were collected by SCUBA diving. The samples were stored at −20 °C for chemical analysis, and in 70% ethanol for morphological description. The standard methods were used to prepare and examine the material skeleton for light and electron microscopies (SEM). The type material has been deposited in the Museo Nacional de Ciencias Naturales in Madrid (Spain) (MNCN), in the British Museum of Natural History (BMNH) (London), and in the Colección de Esponjas del Pacífico (LEB-ICML-UNAM), of the Instituto de Ciencias del Mar y Limnología, UNAM (Mazatlán, Mexico). Sponge-specific terms are used according to Boury-Esnault and Rützler (1997).

2.2. Natural products isolation

The chemical study of *Dysidea reformensis* (freeze-dried specimens, 16.2 g) to yield compounds **1**–**3** (Fig. 2) and the chemical study of *Dysidea cachui* (freeze-dried specimens, 257 g) to yield compounds **1** and **3**–**9**, have been previously described by us (Pérez-García et al., 2005). The chemical analysis of *Dysidea uriae* was performed as follows: 31.2 g of freeze-dried specimens of the sponge were extracted with 1.25 L of acetone/methanol (1:1) at room temperature. After filtration, the solution was evaporated under reduced pressure to obtain a residue that was partitioned between H_2O and Et_2O. The organic layer was taken to dryness to give a brown oil (760 mg) that was chromatographed on a SiO_2 column using solvents of increasing polarities from hexane to Et_2O, then $CHCl_3$/MeOH (8:2), and finally MeOH. The fraction eluted with hexane/Et_2O (95:5) was further purified on a SiO_2 column using hexane as eluent to yield dendrolasin (**10**) that was identified by comparison of its spectroscopic data with those reported in literature (Fontana et al., 1993).

3. Results and discussion

3.1. Dysidea reformensis n. sp.

3.1.1. Material examined

Holotype: MNCN 1.01/354. Paratypes: LEB-ICML-UNAM-82, Isla Talchichitle (Estero del Lanchón, La Reforma, Sinaloa, México), 05/10/2000, 24°54′51″N–108°02′33″W, 1 m depth, on mangrove roots. LEB-ICML-UNAM-188, Isla Altamura (Estero la Pocita La Reforma, Sinaloa, México), 05/11/2000, 24°53′20″N–108°06′45″W, 1 m depth, on mangrove roots.

3.1.2. Morphological and chemical descriptions

Encrusting to cushion-shaped sponge, with multiple flattened lobate projections, which frequently anastomose (Fig. 1A). The species spreads on mangrove roots where the sponge can cover extensions up to 15 cm in diameter and 1.8 cm high, with lobes up to 1 cm in length. The texture is soft and flexible, and the sponge is easily compressible. It has a coarsely conulose surface, with conules 4–7 mm high ending in pointed tips, each of which is elevated by a singular primary tip; sometimes the primary fibers bifurcate at the tip of a single conule. The surface of the sponge is very characteristic because the primary fibers that run along the sub-surface are externally visible, and give an irregular web-like appearance to the surface. The dermal membrane is not charged with detritus. Oscules are 1.5–2 mm in diameter, some with slightly elevated rims. The color in life is light brown to light grey. In some places of the body, the skeleton is almost dendritic, but in most of the body the skeleton is a loose irregular network of fibers formed mainly by primary fibers with few secondary connections (Fig. 1B–D). However, it is not always possible to distinguish between primary and secondary elements on the basis of size or orientation except in the immediate sub-surface area. Moreover, inside some specimens the primary fibers are anchored in a root-like stolon formed by the hydrorhiza of hydrozoans colonies, which are used by the sponge as a substrate (Fig. 1B). Primary fibers are from 50 to 100 μm in diameter (70 μm average), and they are always cored with debris and sand grains. Very little clear spongin remains in the fibers. The secondary fibers are 20–40 μm in diameter (30 μm average) and they are mostly free of debris. Debris only occurs at the point of connection with the primary fibers.

The specimens of *D. reformensis* n. sp. contained the sesquiterpene-hydroquinones avarol (**1**), *ent*-isozonarol (**2**) and 20-*O*-acetyl-21-hydroxy-*ent*-isozonarol (**3**) (Fig. 2). The three metabolites were obtained in similar amounts (**1**: 0.19% dry weight; **2**: 0.25% dry weight; **3**: 0.25% dry weight) (Pérez-García et al., 2005).

3.1.3. Etymology

The specific epithet refers to the locality (La Reforma) where the sponge was found.

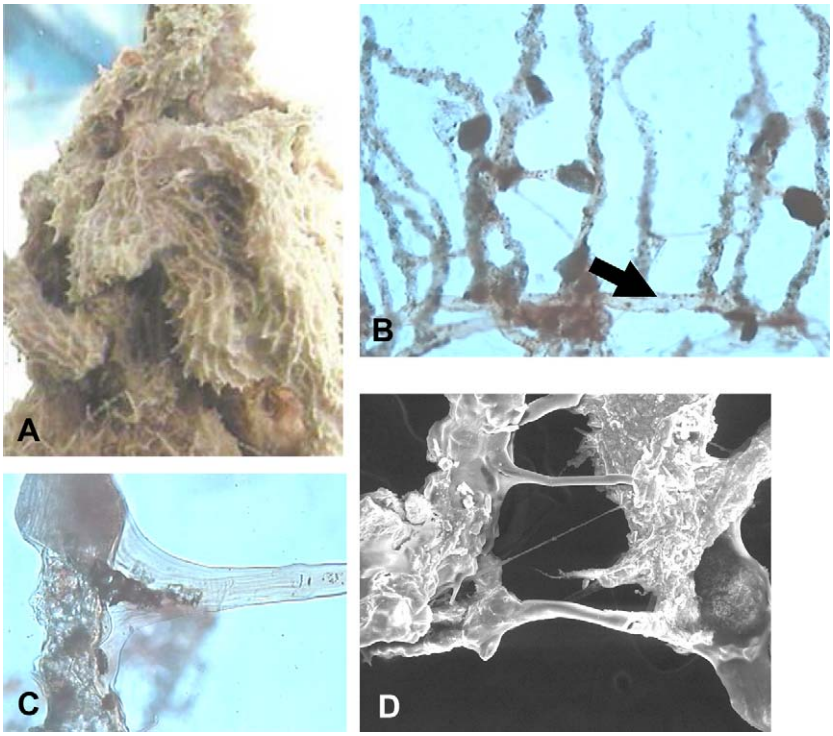

Fig. 1. (A) *Dysidea reformensis* n. sp. on mangrove root. Holotype MNCN 1.01/354. (B) Photomicrograph of the skeleton showing primary fibres arising from the hydrorhiza of a hydrozoan colony (arrow). (C) Photomicrograph of a primary and secondary fibre. (D) SEM detail of primary and secondary fibres.

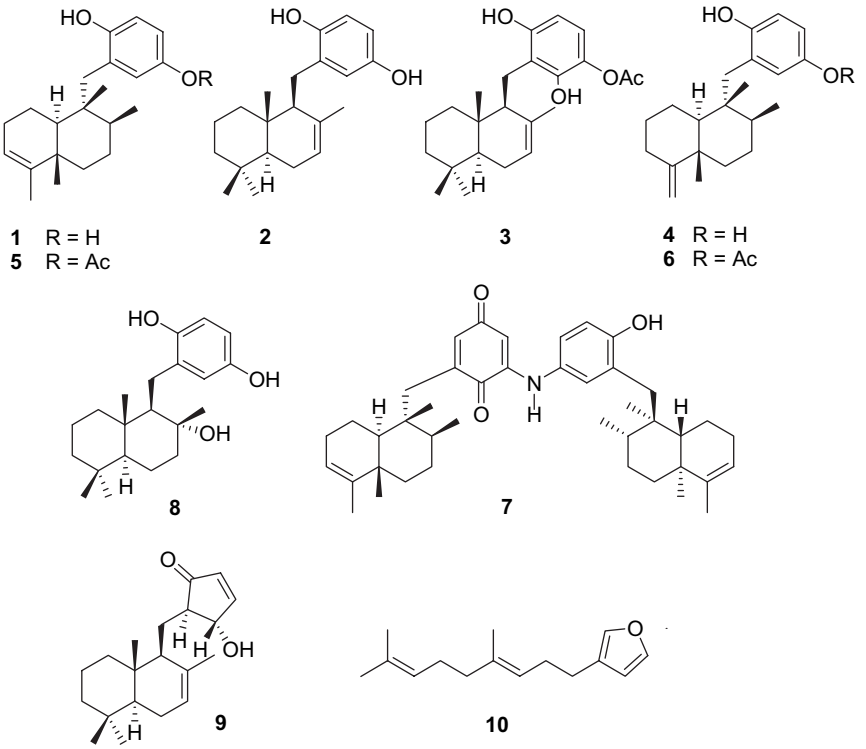

Fig. 2. Natural products from *D. reformensis* n. sp. (**1**–**3**), *D. cachui* n. sp. (**1**, **3**–**9**) and *D. uriae* n. sp. (**10**).

3.1.4. Remarks

The sponge is irregularly ramose, with branches up to 1 cm in length, and with conules irregularly distributed. This species can be differentiated from other *Dysidea* species mainly because there is no a clear distinction between primary and secondary fibers, and by the visible subdermal fibers running along the plane of the surface. *Dysidea arenaria* Bergquist, 1965 is a common irregularly ramose species from New Caledonian, living on sandy substrate (Bergquist, 1965), clearly different from *D. reformensis* n. sp. because of its extremely irregular, coarsely conulose surface, deeply pitted between the conules. Moreover, the presence of a distinct sand cortex also separates it from the new species. *Dysidea frondosa* Bergquist, 1995 is a pink-purple sponge with several features in common with the new species such as form, diameter of the fibers and no distinction between primary and secondary fibers (Bergquist, 1995). However, the tracery of subdermal fibers connects adjacent conules in some areas but not over the whole surface, which gives this sponge a very different appearance from *D. reformensis* n. sp.

The sponge *D. reformensis* n. sp. contains three sesquiterpene-hydroquinones compounds **1**–**3** (Fig. 2), a typical class of *Dysidea* metabolites. Avarol (**1**), which possesses a rearranged drimane sesquiterpenoid moiety, was first isolated as a major metabolite of the sponge *Dysidea avara* (Schmidt, 1862) collected in the Mediterranean Sea (Minale et al., 1974; De Rosa et al., 1976). After this initial report, all accounts of avarol (**1**) have been associated to *Dysidea* sponges, mainly to species from west-Pacific regions (Baker, 1976; Iguchi et al., 1990; Shubina et al., 1990; Alvi et al., 1992). The two remaining metabolites of *D. reformensis* n. sp., *ent*-isozonarol (**2**) and 20-*O*-acetyl-20-hydroxy-*ent*-isozonarol (**3**), are characterized by possessing a drimane sesquiterpenoid residue. This structural feature is uncommon among the sesquiterpene-hydroquinones from *Dysidea* sponges. Thus, in addition to *D. reformensis* n. sp., only the Mediterranean *Dysidea pallescens* Schmidt, 1868 has been reported to contain a drimane-hydroquinone metabolite: *ent*-cromazonarol (**11**) (Cimino et al., 1975a) (Fig. 3). However, compound **11** was a minor constituent of the extract of *D. pallescens* (Schmidt, 1862) (0.03% dry weight), which in turn contained a series of sesquiterpene furans (Cimino et al., 1975b,c,d) and a sesterterpene hydroquinone as major constituents (Cimino et al., 1975e). Thus, *D. reformensis* n. sp. displays a distinctive chemical pattern among *Dysidea* species characterized by the presence of sesquiterpene-hydroquinones with both drimane and rearranged drimane sesquiterpenoid portions. The natural

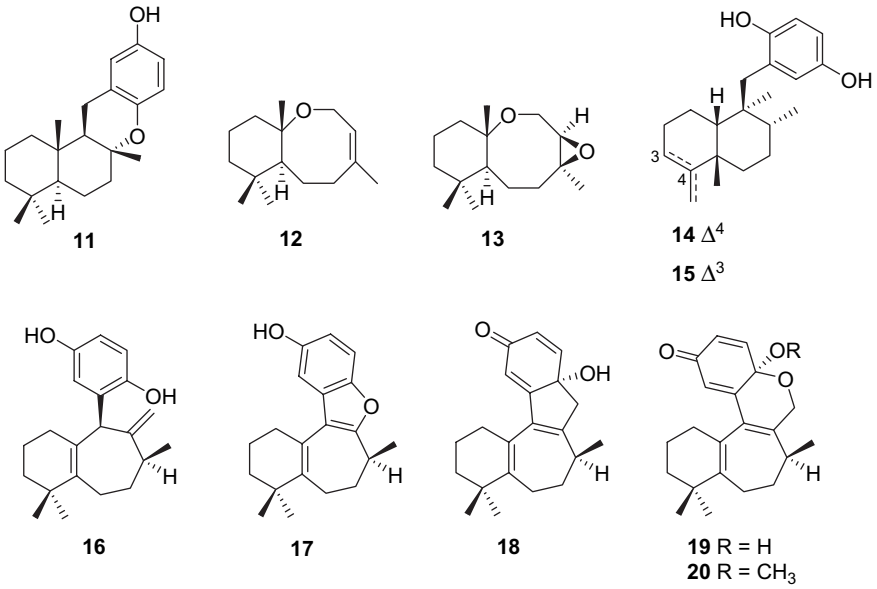

Fig. 3. Sesquiterpenes and sesquiterpene-hydroquinone derivatives from *D. pallescens* (**11**), *D. arenaria* (**12**–**15**), and *D. frondosa* (**16**–**20**).

product content disclosed for *D. reformensis* n. sp. shows divergence from those reported for the morphologically related species *D. arenaria* and *D. frondosa*. Different collections of *D. arenaria* from the Pacific have yielded the sesquiterpenes arenarans A (**12**) and B (**13**) (Horton and Crews, 1995), the merosesquiterpenes arenarol (**14**) and its corresponding quinone (Schmitz et al., 1984), and isoarenarol (**15**) (Yoo et al., 2003) (Fig. 3). The arenarol derivatives **14** and **15** are related to avarol (**1**) but possess a distinctive *cis*-fused decalin system instead of the *trans*-stereochemistry of avarol (**1**). In addition, no derivative containing a drimane residue related to the one present in compounds **2** and **3** of *D. reformensis* n. sp. has been reported from *D. arenaria*. On the other hand, specimens of *D. arenaria* from Okinawa were the source of the potent cytotoxic depsipeptide arenastatin A (Kobayashi et al., 1994; Kobayashi, 2000). However, since this compound was obtained from the sponge in very small amounts and it was later isolated from a terrestrial cyanobacterium (Golakoti et al., 1995), a microorganism origin for arenastatin A may be suspected. With regard to *D. frondosa*, this species has been shown to contain a series of unique merosesquiterpenes, frondosins A–E (**16**–**20**), which possess a 6,7-bicyclic rearranged sesquiterpene moiety (Patil et al., 1997) (Fig. 3).

Interestingly, sesquiterpene-hydroquinone/quinone derivatives containing a drimane residue have been more commonly found in species of the families Thorectidae and Spongiidae. For example, puupehenone (**21**) and a number of congeners have been described from *Hyrtios* species (Thorectidae) (Amade et al., 1983; Nasu et al., 1995; Bourguet-Kondracki et al., 1999; Piña et al., 2003) while certain *Spongia* and *Euryspongia* species (Spongiidae) contain spongiaquinone (**22**) or analogues (Capon et al., 1993; Urban and Capon, 1996) (Fig. 4). These data could suggest an affinity of *D. reformensis* n. sp. with certain members of Thorectidae and Spongiidae families.

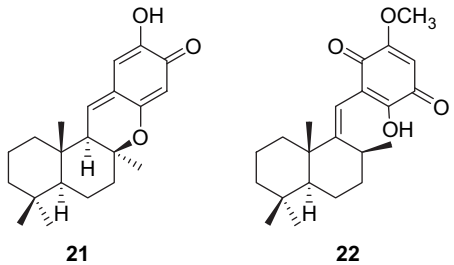

Fig. 4. Puupehenone (**21**) from *Hyrtios* species and spongiaquinone (**22**) from *Spongia* sp.

3.2. Dysidea cachui n. sp.

3.2.1. Material examined

Holotype: MNCN 1.01/355. Paratypes: BMNH: 2005.4.21.1, Islas Verdes (Topolobampo, Sinaloa), 25°31′47″N–109°05′27″W, 2 m depth, 11/13/2002. LEB-ICML-UNAM-207, Cerro San Carlos (Topolobampo, Sinaloa), 25°35′33″N–109°02′39″W, 4 m depth, 06/22/2000. LEB-ICML-UNAM-235, Estero Zacate (Topolobampo, Sinaloa), 25°36′25″N–109°04′33″W, 2 m depth, 06/21/2000. LEB-ICML-UNAM-666, Cerro San Carlos (Topolobampo, Sinaloa), 25°35′33″N–109°02′39″W, 4 m depth, 11/12/2002. LEB-ICML-UNAM-668, Muelle del Contenedor (Topolobampo, Sinaloa), 25°34′55″N–109°03′33″W, 5 m depth, 11/12/2002. LEB-ICML-UNAM-683, Islas Verdes (Topolobampo, Sinaloa), 25°31′47″N–109°05′27″W, 2 m depth, 11/13/2002. LEB-ICML-UNAM-694, Estero El Bichi (Topolobampo, Sinaloa), 25°32′27″N–109°05′29″W, 1 m depth, 11/13/2002. LEB-ICML-UNAM-716, Estero Zacate (Topolobampo, Sinaloa), 25°36′25″N–109°04′33″W, 2 m depth, 11/14/2002. LEB-ICML-UNAM-729, Puente Maviri (Los Mochis, Sinaloa), 25°34′55″N–109°06′52″W, 2 m depth, 11/14/2002. LEB-ICML-UNAM-828, Islas Verdes (Topolobampo, Sinaloa), 25°31′47″N–109°05′27″W, 2 m depth, 11/13/2002.

3.2.2. Description

It is an encrusting to massive sponge, with rounded lobes provided most of them with a central oscule (Fig. 5A). The color varies from light grey to almost white, and the individuals can cover up to 12 cm in diameter, but are most commonly around 7–11 cm long and wide and 3–5 cm high. The sponge has a very characteristic lobate appearance, with simple lobes, although two or more lobes can frequently fuse. The lobes vary in diameter from 0.3 cm in the simple form to 1.5 cm when fused, and can be up to 0.8 mm high. The surface is evenly covered with rounded conules each of which is elevated by a single primary fiber, giving a granular appearance to the surface (Fig. 5B). Oscules are usually circular, 1.5–3.5 mm in diameter, scattered over the top of the lobes and flush with the surface. The texture is soft and friable and the sponge is easily torn. The ectosomal region has no debris. The skeleton is arranged following a rectangular plan, typical of the genus *Dysidea* (Fig. 5C–E). The primary fibers are simple, and they are spaced approximately 400–500 mm apart. They vary from 150 to 200 μm in diameter, and they are completely cored with sand

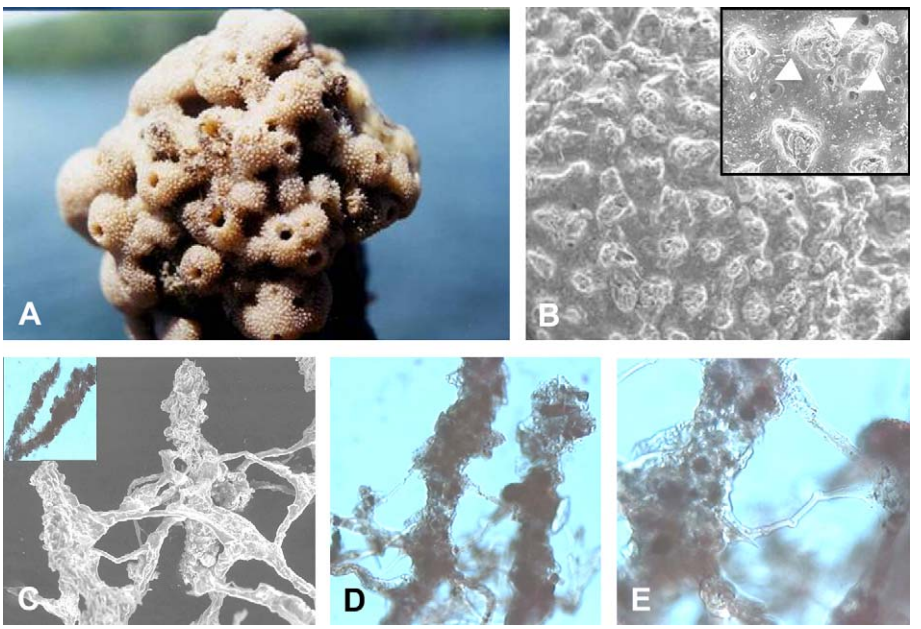

Fig. 5. (A) *Dysidea cachui* n. sp. Holotype MNCN 1.01/355. (B) SEM image showing the typical rounded conules on the surface of the new species. In the small upper box the ostia are shown (arrows). (C) SEM image showing primary and secondary fibres. In the small upper box, a bifurcated primary fibre is shown. (D) Photomicrograph of the skeleton showing primary and secondary fibres. (E) Detail of the secondary network.

grains. Secondary fibers are 40–100 μm in diameter, and also incorporate debris, although the spongin is always visible around the material depending on the nature and extent of the debris.

The specimens of *D. cachui* n. sp. contained the secondary metabolites avarol (**1**), 20-*O*-acetyl-21-hydroxy-*ent*-isozonarol (**3**), neoavarol (**4**), 20-*O*-acetylavarol (**5**), 20-*O*-acetylneoavarol (**6**), popolohuanone C (**7**), *ent*-yahazunol (**8**) and dysienone (**9**) (Fig. 2). The mayor metabolites were the sesquiterpene-hydroquinones **1** and **4** (inseparable mixture, 0.47% dry weight), while compounds **3** (8.8×10^{-3} dry weight) and **5–9** (% dry weight: 9.3×10^{-3}, 1.6×10^{-3}, 1.1×10^{-3}, 1.0×10^{-3}, and 8.5×10^{-4}, respectively) were minor components of the extract (Pérez-García et al., 2005).

3.2.3. Etymology

The species is named after Mr. Dimas Cachua, for his help with our work at the Bahías of Ohuira and Topolobampo where the new species was found.

3.2.4. Remarks

This sponge can be differentiated from other species of *Dysidea* by the granular appearance of the surface, in conjunction with its consistent habit. The closest species is *Dysidea nigrescens* Bergquist, 1995, which has a regular skeletal arrangement and a surface with similar rounded conules (Bergquist, 1995). However, the surface of this species has a characteristic regularly spotted appearance, because the apex of each conule is cream colored where the sandy fiber content is exposed on the dark surface (Bergquist, 1995).

The major metabolites of *D. cachui* n. sp. have been identified as the sesquiterpene-hydroquinones, avarol (**1**) and neovarol (isoavarol) (**4**) (Fig. 2). These two compounds have also been reported as being the major constituents of two unidentified *Dysidea* species from Okinawa (Iguchi et al., 1990) and Australia (Shubina et al., 1990), respectively. The minor constituents isolated from the sponge, compounds **3** and **5–9** (Fig. 2) were not considered for comparison purposes. However, it is worth noting that the sesquiterpene-hydroquinone derivatives **5–7** are typical *Dysidea* metabolites. Thus, compound **5** was firstly isolated as a minor constituent of *D. avara* (Crispino et al., 1989), while popolohuanone C (**7**) belongs to a series of dimeric derivatives previously described from *Dysidea* species (Rodriguez et al., 1990; Alvi et al., 1992; Carney and Scheuer, 1993). A comparison with the chemistry of the morphologically related *D. nigrescens* is precluded since no chemical accounts on this species have been found.

3.3. Dysidea uriae n. sp.

3.3.1. Material examined

Holotype: MNCN 1.01/356. Paratypes: BMNH: 2005.4.21.2, Antiguo Muelle de Atraque (Mazatlán, Sinaloa), 23°11′57″N–106°25′15″W, 3 m depth, 03/23/2002. LEB-ICML-UNAM-180, Isla lobos (Mazatlán, Sinaloa), 23°13′49″N–106°27′43″W, 5 m depth, 05/02/2000. LEB-ICML-UNAM-328, Isla Lobos (Mazatlán, Sinaloa), 23°13′49″N–106°27′43″W, 6 m depth, 01/26/2001. LEB-ICML-UNAM-451, Antiguo Muelle de Atraque (Mazatlán, Sinaloa), 23°11′57″N–106°25′15″W, 3 m depth, 03/23/2002. LEB-ICML-UNAM-898, Isla el Crestón (Mazatlán, Sinaloa), 23°11′02″N–106°25′37″W, 5 m depth, 09/12/2003. LEB-ICML-UNAM-971, Isla Chivos (Mazatlán, Sinaloa), 23°10′39.9″N–106°24′48.4″W, 7 m depth, 11/26/2003. LEB-ICML-UNAM-992, Isla Cardones (Mazatlán, Sinaloa), 23°11′05″N–106°24′07″W, 6 m depth, 11/26/2003.

3.3.2. Description

Massive to cushion-shaped sponge, up to 13 cm long and 2.5 cm thick (Fig. 6A). The surface varies from finely conulose to smooth in patches, with conules about 1.5 mm high and 2–6 mm apart. Oscules are about 0.5–1.6 mm in diameter, dispersed randomly and flushing with the surface. They have a smooth dermal membrane surrounding them, and with a slightly elevated elastic lip. The texture is spongy and very compressible in life. The color varies from black in the specimens exposed directly to light, to gray and almost white in the specimens found in dark habits. The interior is cream. The skeleton is an irregular network of meshes constituted with primary fibers heavily cored with detritus, and secondary fibers predominantly uncored (Fig. 6B–D). The primary fibers are simple, and vary from 180 to 333 μm in diameter, but the secondary fibers are intertwined in some places. Very little clear spongin remains in the primary fibers. They are 400 mm apart in average. The secondary fibers are from 50 to 100 μm, and the

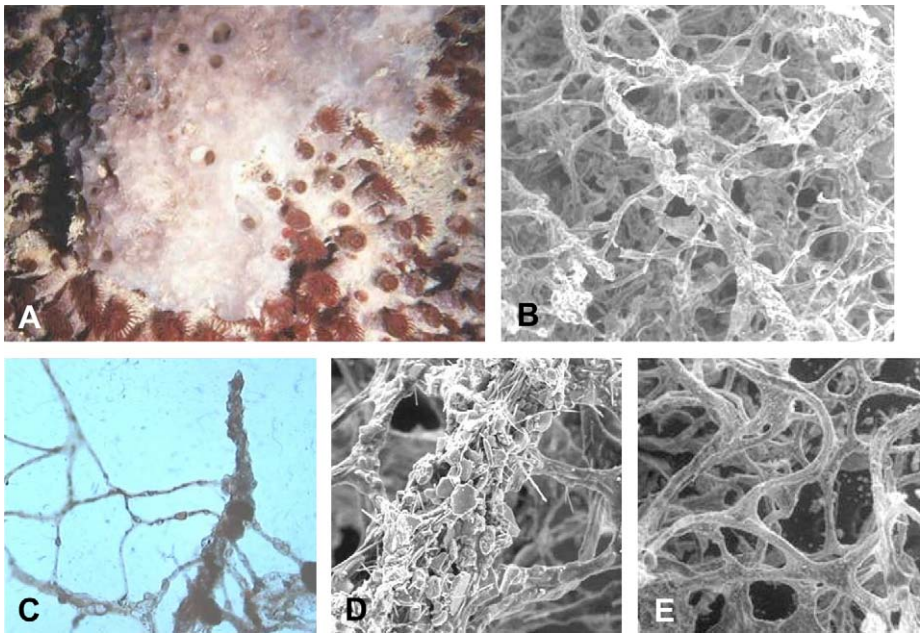

Fig. 6. (A) *Dysidea uriae* n. sp. Holotype MNCN 1.01/356, in situ. (B) SEM image showing arrangement of skeleton. (C and D) Primary fibre heavily cored by detritus. (E) Secondary network to the SEM.

spongin is always visible around the material. This species has been found mainly on artificial substrate, and concrete rocks in the mouth of an estuarine ecosystem.

D. uriae n. sp. contained the secondary metabolite dendrolasin (**10**) (0.045% dry weight).

3.3.3. Etymology

The specific epithet refers to the name of the estuary (Urías estuary) where the sponge was found.

3.3.4. Remarks

The closest species is *Dysidea amblia* De Laubenfels, 1930, described in California, USA (De Laubenfels, 1930), and later cited in the northern Sea of Cortes (Dickinson, 1945). This is a digitate and somewhat ramose sponge up to 20–30 cm high, and about 1 cm in diameter, with principal fibers heavily cored 100–200 μm in diameter, and secondary fibers 10–25 μm, usually free from inclusions. The skeletal arrangement is similar in both sponges; however, the new species can be differentiated from *D. amblia* by its consistent habit, in conjunction with the surface evenly distributed with rounded conules, and the presence of a tertiary network between the secondary fibers.

Dendrolasin (**10**) (Fig. 2) is the only secondary metabolite isolated from *D. uriae* n. sp. This result relates *D. uriae* n. sp. with a series of *Dysidea* species which have been shown to contain a variety of furanosesquiterpenes as main metabolites, exemplified by *Dysidea fragilis* (Montagu, 1818) (Schulte et al., 1980; Guella et al., 1983, 1985; Manzini et al., 1990; Avila et al., 1991; Aiello et al., 1996) and *D. pallescens* (Cimino et al., 1975b,c,d). As noted above, *D. amblia* is the species most related to *D. uriae* n. sp. morphologically. Although the natural products of both species belong to the furanoterpenoid class, diagnostic differences are observed, since furanoditerpenes have been identified as the most abundant metabolites in *D. amblia*. Thus, the first chemical study of specimens of *D. amblia* from California, yielded the diterpene furans ambliol A (**23**) and B (**24**) as main metabolites (1.1% and 1.0% dry weight, respectively) together with minor amounts of three related compounds: dehydroambliol A (**25**), ambliofuran (**26**), and ambliolide (**27**) (0.04%, 0.05%, and 0.04% dry weight, respectively) (Walker and Faulkner, 1981) (Fig. 7). The study of separate animals revealed the existence of two chemical varieties of *D. amblia*, one of them containing **23** as a major metabolite, while the other one contained the diterpene **24**. The analysis of a second collection of *D. amblia* (Walker et al., 1984) led to the isolation of the related diterpene ambliol C (**28**) as main metabolite (1% dry weight), together with significant amounts of **26** (0.6% dry weight) and the sesquiterpene pallescensin (**29**) (0.4%), while the

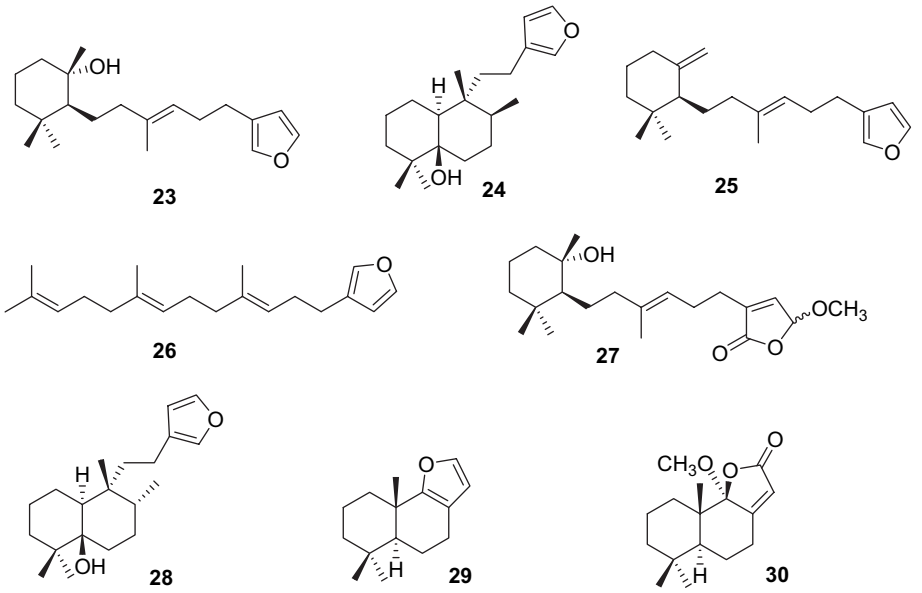

Fig. 7. Furanoditerpenes (**23**–**28**) and sesquiterpenes (**29**, **30**) from *D. amblia*.

sesquiterpene pallescensolide (**30**) was obtained in lower yield (0.03% dry weight) (Fig. 7). These data suggest that diterpenes are the most prominent metabolites of *D. amblia*, despite the intraspecific variations of individual compounds among different collections.

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