Title: New shallow water species of Caribbean *Ircinia* Nardo, 1833 (Porifera: Irciniidae)

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Abstract

Seven *Ircinia* growth forms were collected from three sites in the Caribbean (Bocas del Toro, Panama; the Mesoamerican Barrier Reef, Belize; and the Florida Keys, United States of America). Previous research used an integrative taxonomic framework to delimit species boundaries among these growth forms. Here, we present descriptions for these species, six of which are new to science (*Ircinia lowi* sp. nov., *Ircinia bocatorensis* sp. nov., *Ircinia radix* sp. nov., *Ircinia laeviconulosa* sp. nov., *Ircinia vansoesti* sp. nov., *Ircinia rutzleri* sp. nov.) in addition to one species conferre (*Ircinia cf. reteplana* Topsent, 1923).

Introduction

*Ircinia* Nardo, 1833 is a genus of sponges diagnosable from other genera in the family Irciniidae Gray, 1867 by the possession of cored fascicular primary fibers and the lack of cortical armoring (Hooper & van Soest, 2002). The study of *Ircinia* taxonomy has been historically
problematic as *Ircinia*, like other members of the order Dictyoceratida Minichin, 1900, are aspiculate and present few anatomical features that can be used to infer relatedness and taxonomic boundaries among species (Erpenbeck et al., 2020). *Ircinia* also display a considerable degree of morphological plasticity that confounds the identification of features that are representative of a given species (de C. Cook & Bergquist, 1999). Single locus genetic barcoding has likewise seen limited success, as the loci that are typically used to provide species- and population-level phylogenetic resolution in metazoans, which include the cytochrome oxidase c subunit 1 (CO1) and the internal transcribed spacer set (ITS), typically are either incompletely sorted or largely invariant among nominal species of *Ircinia* (Pöppe et al., 2010; Riesgo et al., 2016; Kelly & Thacker 2020, in review). The pitfalls that arise from the use of morphological data alone or by restricting genetic data to a single locus or a few loci, which is also a questionable practice on the basis of the high probability of gene tree and species tree discordance (Degnan & Rosenberg, 2006), necessitate the implementation of integrative taxonomic research frameworks within *Ircinia* that include genome-wide evidence of species boundaries.

Shallow water environments of the Caribbean are commonly inhabited by three nominal species of *Ircinia*: *I. campana*; *I. strobilina* Lamarck, 1816; and *I. felix* Duchassaing & Michelotti, 1864. Alongside these three species can be found several *Ircinia* growth forms that are recognizable in the field based on the overall shape of their bodies, the spacing and height of their conules, and the position and size of their oscula (Diaz, 2005; Erwin & Thacker, 2007; Rützler et al., 2000; van Soest, 1978; Wulff, 1994, 2013). The growth forms are also generally regarded as being ecologically distinct in that they contain different tissue densities of chlorophyll *a* (Erwin & Thacker, 2007), can exhibit habitat preference, and have distinct
microbiome compositions (Kelly & Thacker 2020, in review; Kelly et al. 2020, in review).

Recently, genetic species boundaries were delimited among several of these growth forms using Bayesian species delimitation (BFD*) with genome-wide SNP data (Leaché et al., 2014, Kelly & Thacker 2020, in review; Kelly et al. 2020, in review). On the basis that these growth forms are genetically and morphologically distinct and appear ecologically divergent as evidenced by their possession of unique microbiomes, we designate the growth forms as new species to science and provide taxonomic descriptions.

Methods

Ircinia specimens were collected from three sites in the Caribbean: four growth forms were collected from Bocas del Toro, Panama; three from the Mesoamerican Barrier Reef, Belize; and one from the Florida Keys, United States of America (Figure 1, Table 1). Each of the growth forms are specific to a given habitat type and are found in either coral reefs, seagrass beds, or on mangrove prop roots. Tissue samples were fixed in 4% paraformaldehyde (PFA) that was prepared by diluting 32% PFA stock in filtered seawater. The 4% PFA solution was replaced at the 24- and 48-hour marks to ensure complete irrigation of the tissue. Histological sections were inspected using compound light microscopy.
<table>
<thead>
<tr>
<th>Site</th>
<th>Geographic coordinates</th>
<th>L. affinis</th>
<th>L. andina</th>
<th>L. axillaris</th>
<th>L. flavus</th>
<th>L. jonesi</th>
<th>L. nitida</th>
<th>L. harrisi</th>
<th>L. varicena</th>
<th>L. c. elegans</th>
<th>L. c. nigripes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panama Canal (Spill Point)</td>
<td>9.5178, -82.3021</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Isla Holandes (mangrove prop roots)</td>
<td>9.5968, -82.1722</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Puerto Cortes (seagrass bed)</td>
<td>6.8042, -88.0766</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Blue Ground (mangrove prop roots)</td>
<td>16.8063, -88.1496</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summerland Key (seagrass bed)</td>
<td>24.6090, -81.4563</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Sampling locations of *Ircinia* spp.
Results

General remarks

The characters that distinguish *Ircinia* from other irciniid genera hold for the *Ircinia* spp. nov., in that the current *Ircinia* have cored fascicular primary fibers and lack cortical armoring. However, we note that the cortices of the *Ircinia* spp. nov. sometimes incorporate foreign spicules and sand. Foreign spicules, when present in the mesohyl, fibers, or cortex, are predominantly oxea and style fragments.

The dimensions of the skeletal fibers and conule heights of the *Ircinia* spp. nov. below are dissimilar from *I. campana* (Lamarck, 1814), *I. strobilina* (Lamarck, 1816), and *I. felix* (Duchassaing & Michelotti, 1864) (Table 2). Additionally, all lack the characteristic dermal reticulation of *I. felix*; their conules instead arise from smooth dermal surfaces. The body shape of *I. reteplana* is distinct from those of the aforementioned *Ircinia* in that it is composed of flattened, interconnecting branches, with the exception of the Floridian growth form. Because the Floridian *Ircinia* growth form (called ‘Ramose’ in Kelly & Thacker 2020, *in review* and Kelly et al. 2020, *in review*) often displays a flattened branching morphology, we designate this growth form as *Ircinia* cf. *reteplana* Topsent, 1923. However, *Ircinia* cf. *reteplana* can also possess a rounded branching morphology and the branches of *Ircinia* cf. *reteplana* seldom interconnect. This growth form represents either a new species of *Ircinia* or it represents an extension of the documented range of *I. reteplana* to the Florida Keys. For reference, the range of *I. reteplana* encompasses the entirety of the Antilles and spans the Caribbean to the coast of Venezuela, and extends to the tip of the Yucatan Peninsula (Van Soest et al., 2019). The collections of *I. strobilina*, *I. felix*, and *I. campana* (Kelly & Thacker 2020, *in review*; Kelly et al. 2020, *in review*) were made within the documented ranges of these species (Table 1).
Systematics

Phylum Porifera Grant, 1836
Class Demospongiae Sollas, 1885
Subclass Keratosa Grant, 1861
Order Dictyoceratida Minchin, 1900
Family Irciniidae Gray, 1867
Genus Ircinia Nardo, 1833

Ircinia lowi sp. nov.

Holotype: P16x42 (USNM 1582268).
Paratypes: P16x41 (USNM 1582267), P16x43 (USNM 1582269), P16x44 (USNM 1582270),
P16x52 (USNM 1582278).

Type locality: Bocas del Toro, Panama

Diagnosis. Ircinia that form a thickly encrusting growth habit, with a forest green external surface color. Sometimes possesses digitate projections of the body (Figure 2).

External morphology. Conules small (<2mm in height), sometimes of lighter color than the rest of the body, similar to I. laeviconulosa sp. nov. and I. radix sp. nov., although slightly sharper. The body is dotted with black oscula that are uniform in size and either sit at a slight relief or are flush, usually 0.5 cm or less in diameter.

**Ecology.** All specimens were collected from shallow depths (0.4 – 0.5 m) on patch reefs that occur in association with small seagrass beds.

**Etymology.** This species is named for the immunologist Jun Siong Low.

**Remarks.** Tissue takes on a slightly crisper consistency when preserved in ethanol for several days. Referred to as the ‘Encrusting’ growth form in Kelly & Thacker (2020) and Kelly *et al.* (2020).

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**Ircinia bocatorensis** sp. nov.

**Holotype:** P16x58 (USNM 1582284).

**Paratypes:** P16x56 (USNM 1582282), P16x61 (USNM 1582287), P16x63 (USNM 1582289), P16x65 (USNM 1582291), P16x69 (USNM 1582295).

**Type locality:** Bocas del Toro, Panama

**Diagnosis.** *Ircinia* with a massive, sometime cone-like growth morphology and a tan exterior color (Figure 3).

**Exterior morphology.** Conules 3-5 mm in height, dully sharp to knobby, typically darker or lighter in color than the rest of the sponge body. Oscula usually black, either flush or positioned at the ends of cone-like outgrowths, of varying diameters but seldom wider than 1.5 cm.

**Internal morphology.** Massive fascicular fibers 200-520 μm wide, sparsely cored. Interconnecting fibers 15-65 μm wide, uncored. Irciniid filaments 6-10 μm wide.

**Ecology.** All specimens were collected from 0.4 – 1 m depth on a *Thalassia* bed interspersed with small coral colonies.

**Etymology.** The species is named for the Panamanian province Bocas del Toro.
Remarks. One specimen was observed growing in physical contact with *I. strobilina*.

Referred to as the ‘Massive B’ growth form in Kelly & Thacker (2020) and Kelly *et al.* (2020).

*Ircinia radix* sp. nov.

**Holotype:** P16x32 (USNM 1582258).

**Paratypes:** P16x31 (USNM 1582257), P16x33 (USNM 1582259), P16x34 (USNM 1582260).

**Type locality:** Bocas del Toro, Panama

**Diagnosis.** *Ircinia* with a massive growth form and light pink pinacoderm (Figure 4).

**External morphology.** Surface is relatively smooth with low, rounded conules (2 mm). Oscula flush or slightly recessed, occasionally lighter than the exterior of the sponge, usually no larger than 1.2 cm in diameter.

**Interior morphology.** Massive fascicular fibers 110-250 μm wide, cored. Interconnecting fibers 10-50 μm wide, sparsely cored. Irciniid filaments 6-8 μm wide. Mesohyl and pinacoderm contain haphazardly scattered spicule fragments, with concentrations of foreign inclusions higher in the pinacoderm.

**Ecology.** This species inhabits shaded entanglements of mangrove roots. Specimens were collected from depths of 0.5-0.75 m.

**Etymology.** The name refers to the mangrove roots that this species lives on.

Remarks. Growth morphology can range from a round ball (p16x32-34) to an elongated massive form (p16x31). Referred to as the ‘Massive A pink’ growth form in Kelly & Thacker (2020) and Kelly *et al.* (2020).

*Ircinia laeviconulosa* sp. nov.
Holotype: P16x57 (USNM 1582283).

Paratypes: P16x59 (USNM 1582285), P16x60 (USNM 1582286), P16x62 (USNM 1582288).

Type locality: Bocas del Toro, Panama

Diagnosis. *Ircinia* with a massive growth form and dark green pinacoderm (Figure 5).

External morphology. Surface texture is similar to *I. radix* sp. nov., although is smoother with lower conules (1.5-1.75 mm). Oscula flush, sometimes slightly darker than the exterior of the sponge, usually no larger than 1.2 cm in diameter.

Interior morphology. Massive fascicular fibers 110-160 μm wide, cored. Interconnecting fibers 20-50 μm wide, sparsely cored. Irciniid filaments 4-6 μm wide. Mesohyl and pinacoderm contain inclusions that resemble those of *I. radix* sp. nov.

Ecology. This species is found among *Thalassia* spp. and coral patches in shallow depths. Specimens were collected from depths of 1-1.5 m.

Etymology. The name refers to its smooth surface.

Remarks. All specimens collected had a globose growth morphology. Referred to as the ‘Massive A green’ growth form in Kelly & Thacker (2020) and Kelly et al. (2020).

*Ircinia vансoesti* sp. nov.

Holotype: JK18x20 (USNM ####### pending)

Paratypes: JK18x18 (USNM ####### pending), JK18x28 (USNM ####### pending), JK18x34 (USNM ####### pending), JK18x35 (USNM ####### pending).

Type locality: Mesoamerican Barrier Reef, Belize

Note: lodging of museum vouchers pending.
Diagnosis. *Ircinia* with a massive growth form, occasionally lobate, with pinkish brown or gray pinacoderm (Figure 6).

**External morphology.** Surface texture is similar to *I. radix* sp. nov., although with smaller conules (1-1.5 mm). Oscula typically 0.5-1.2 cm in diameter.

**Interior morphology.** Massive fascicular fibers 90-300 μm wide, sometimes cored, and always more heavily than interconnecting fibers. Interconnecting fibers 25-60 μm wide, usually uncored. Irciniid filaments 7.5-9 μm wide. Cortex usually uncored, sand and foreign spicules occasionally included in mesohyl. Fascicles can be difficult to discern from interconnecting fibers and can be rare.

**Ecology.** This species is found growing on *Rhizophora* prop roots at depths of 0.2-1.5 m.

**Etymology.** This species is named for the sponge researcher Rob van Soest.

**Remarks.** Interior morphology can vary somewhat depending on population, as the Twin Cays specimens contained less foreign inclusions relative to the Blue Ground specimens. This species is also polymorphic with regard to pinacoderm coloration, and multiple color morphs (gray, dark red, dark green) can be found within a population. Referred to as the ‘Sp. 1’ growth form in Kelly *et al.* (2020).

*Ircinia rutzleri* sp. nov.

**Holotype:** JK18x23 (USNM ####### pending)

**Paratypes:** JK18x21 (USNM ####### pending), JK18x22 (USNM ####### pending), JK18x25 (USNM ####### pending), JK18x26 (USNM ####### pending)

**Type locality:** Mesoamerican Barrier Reef, Belize

**Note:** lodging of museum vouchers pending.
**Diagnosis.** *Ircinia* with an encrusting growth form and dark gray pinacoderm (Figure 7).

**External morphology.** Conules 1-1.3 mm in height. Oscula flush or slightly raised, always black, 0.7-1 cm in diameter.

**Interior morphology.** Massive fascicular fibers 120-240 μm wide, heavily cored and tightly bound. Interconnecting fibers 30-50 μm wide, lightly cored. Irciniid filaments 7.5-9 μm wide. Cortex contains abundant inclusions of sand grains.

**Ecology.** This species is found on patch reefs co-inhabited by *I. strobilina* in shallow depths (0.5-1 m) adjacent to mangrove hammocks inhabited by sp1.

**Etymology.** This species is named for the sponge researcher Klaus Rützler.

**Remarks.** Referred to as the ‘Sp. 2’ growth form in Kelly et al. (2020).

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*Ircinia cf. reteplana* Topsent, 1923

**Representative specimens:** JK18x6 (USNM ##### pending), JK18x7 (USNM ##### pending), JK18x9 (USNM ##### pending), JK18x10 (USNM ##### pending), JK18x14 (USNM ##### pending)

**Collection locality:** Summerland Key, Florida

**Note:** lodging of museum vouchers pending.

**Diagnosis.** *Ircinia* with a flattened, branching morphology. Branches are usually not interconnecting (Figure 8).

**External morphology.** Surface texture is smooth with 1-1.2 mm-high conules. Most oscula are around 0.5 cm in diameter and are found across the face of the branches, where they sit flush, as well as at the edges of the branches.
**Interior morphology.** Massive fascicular fibers are tightly bound, 90-200 μm wide, and heavily cored. Interconnecting fibers 20-80 μm wide, lightly cored with spicules and sand. Irciniid filaments 7.5-9 μm wide. Cortex routinely incorporates sand and foreign spicule fragments.

**Ecology.** Specimens were collected from a shallow (0.5-1 m) *Thalassia*-dominated seagrass bed and co-occurred next to *I. campana*, sometimes within a meter of each other.

**Remarks.** Eukaryotic commensals are mostly crustaceans and polychaetes. Referred to as the ‘Ramose’ growth form in Kelly *et al.* (2020).
Table 2. A morphological comparison of shallow water Caribbean *Ircinia* (van Soest, 1978).

<table>
<thead>
<tr>
<th>Species</th>
<th>Massive fascicular fiber width (μm)</th>
<th>Inter-connecting fiber width (μm)</th>
<th>Irciniid fiber width (μm)</th>
<th>Conule height (mm)</th>
<th>Oscula diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ircinia campana</em> Lamark, 1816</td>
<td>300-700</td>
<td>30-150</td>
<td>3-6</td>
<td>~ 4</td>
<td>0.4-1</td>
</tr>
<tr>
<td><em>Ircinia strobilina</em> Lamark, 1816</td>
<td>200-300</td>
<td>20-60</td>
<td>4-6</td>
<td>2-15</td>
<td>0.4-1</td>
</tr>
<tr>
<td><em>Ircinia felix</em> Duchassaing &amp; Michelotti, 1864</td>
<td>200-550</td>
<td>15-100</td>
<td>2-6</td>
<td>1-2</td>
<td>0.1-0.8</td>
</tr>
<tr>
<td><em>Ircinia reteplana</em> Topsent, 1923</td>
<td>not reported</td>
<td>25-100</td>
<td>~6</td>
<td>not reported</td>
<td>0.5-0.8</td>
</tr>
<tr>
<td><em>Ircinia lowi</em> sp. nov.</td>
<td>80-200</td>
<td>20-60</td>
<td>6-11</td>
<td>&gt; 2</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td><em>Ircinia bocatorensis</em> sp. nov.</td>
<td>200-520</td>
<td>15-65</td>
<td>6-10</td>
<td>3-5</td>
<td>&gt; 1.5</td>
</tr>
<tr>
<td><em>Ircinia laeviconulosa</em> sp. nov.</td>
<td>110-160</td>
<td>20-50</td>
<td>4-6</td>
<td>1.5-1.75</td>
<td>&gt; 1.2</td>
</tr>
<tr>
<td><em>Ircinia radix</em> sp. nov.</td>
<td>110-250</td>
<td>10-50</td>
<td>6-8</td>
<td>~2</td>
<td>&gt; 1.2</td>
</tr>
<tr>
<td><em>Ircinia vansoesti</em> sp. nov.</td>
<td>90-300</td>
<td>25-60</td>
<td>7.5-9</td>
<td>1-1.5</td>
<td>0.5-1.2</td>
</tr>
<tr>
<td><em>Ircinia rutzleri</em> sp. nov.</td>
<td>120-240</td>
<td>30-50</td>
<td>7.5-9</td>
<td>1-1.3</td>
<td>0.7-1</td>
</tr>
<tr>
<td><em>Ircinia cf. reteplana</em> Topsent, 1923</td>
<td>90-200</td>
<td>20-80</td>
<td>7.5-9</td>
<td>1-1.2</td>
<td>~ 0.5</td>
</tr>
</tbody>
</table>

Outlook

Understanding sponge biodiversity is imperative to the protection of tropical marine habitats given the multitude of core ecological functions sponges perform (Bell, 2008; Diaz & Rützler, 2001; Wulff, 2006, 2013). *Ircinia* spp. are among the most abundant and ecologically influential sponges on Caribbean reefs although they are also, unfortunately, among the most susceptible to environmental perturbations (Wulff, 2006, 2013). Here, we have demonstrated that disentangling species boundaries within *Ircinia*, arguably one of the most taxonomically challenging sponge genera, can be accomplished with high confidence using an integrative
taxonomic framework that evaluates morphology, microbiome composition, and genome-wide SNP data (Kelly & Thacker 2020, *in review*; Kelly et al. 2020, *in review*). The adoption of these data criteria in future species delimitation studies could help further describe species richness within this ecologically important genus and ultimately help science document and defend the hidden biodiversity in sponge fauna.

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References


Figure 1. Maps of sampling locations. **A:** Bocas del Toro, Panama; **B:** Mesoamerican Barrier Reef, Belize; **C:** Summerland Key, United States of America. Circles are coral reefs or coral patch reefs, squares are seagrass beds, and triangles are mangroves.
Figure 2. *Ircinia lowi* sp. nov. A: USNM 1582268 (holotype), B: USNM 1582267 (paratype), C: USNM 1582269 (paratype), D: USNM 1582270 (paratype).
Figure 3. *Ircinia bocatorensis* sp. nov. A: USNM 1582284 (holotype), B: USNM 1582289 (paratype), C: USNM 1582287 (paratype), D: USNM 1582295 (paratype), E: USNM 1582291 (paratype) adjacent to *I. strobilina*, F: USNM 1582282 (paratype).
Figure 4. *Ircinia radix* sp. nov. A: USNM 1582258 (holotype), B: USNM 1582259 (paratype), C: USNM 1582260 (paratype), D: USNM 1582257 (paratype).
Figure 5. *Ircinia laeviconulosa* sp. nov. A: USNM 1582283 (holotype), B: USNM 1582285 (paratype), C: USNM 1582288 (paratype), D: USNM 1582286 (paratype).
Figure 6. *Ircinia vansoesti* sp. nov. **A:** JK18x20 (holotype), **B:** JK18x18 (paratype), **C:** JK18x28 (paratype), **D:** JK18x35 (paratype).
Figure 7. *Ircinia rutzleri* sp. nov. A: JK18x23 (holotype), B: JK18x26 (paratype), C: JK18x22 (paratype), D: JK18x25 (paratype).
Figure 8. *Ircinia cf. reteplana* Topsent, 1923. A: JK18x9, B: JK18x7, C: JK18x6, D: JK18x10, E: JK18x14.