Supplementary Information

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Supplementary Text S1

Full taxonomic placement and authorities for the ascidian species investigated in this study are presented below, together with the reference works used to identify each species. Unless otherwise stated in comments, our specimens were in agreement with the referenced descriptions. For specimens that could not be identified to species level, taxonomic remarks are given to help future recovery of these species, along with *in situ* pictures and genetic barcodes.

Order Aplousobranchia Lahille, 1886

Family Clavelinidae Berrill, 1950 (*sensu* Pérez-Portela and Turon 2008)

Clavelina arafurensis Tokioka, 1952

- Kott (1990), p. 38; Monniot (1997), p. 197; Kott (2005), p. 54.

Clavelina meridionalis (Herdman, 1891) (Fig. S1A)

- Kott (1990), p. 48; Monniot and Monniot (1996), p. 205; Monniot (1997), p. 206.

Pycnoclavella diminuta (Kott, 1957) (Fig. S1B)

- Kott (1990), p. 73; Monniot (1997), p. 195; Kott (2002), p. 22.
- Remarks: This is a species with high chromatic polymorphism. The colonies found have a bright blue circle surrounding the oral siphons, interrupted dorsally and ventrally, and a blue crescent posterior to the atrial siphons. Some colonies have also yellow rims in the siphons. This pigmentation pattern has not been reported before.

Pycnoclavella sp. (Fig. S1C)

- Remarks: Unfortunately, the sample for taxonomic observation of this specimen was lost. The picture shows a small colony, with whitish thoraces and, apparently, three stigmata rows, which is found in some species of the genus *Pycnoclavella*. The closest matches of the COI sequence are also *Pycnoclavella* species.

Family Didemnidae Giard, 1872

Didemnum cf. *albopunctatum* Sluiter, 1909 (Figs. S1D, S3A)

- Kott (2001), p. 148; Kott (2004a), p. 2488, Kott (2005), p. 81.
- Remarks: The colony has whitish, elevated ridges of tunic separating zones with zooids that are of a greenish-brownish color. In fixative, these areas appear dark brown due to pigment granules in the tunic. The common cavity is thoracic, with abdmina embedded in the basal layer of tunic. The spicules are very small (up to 20 μm), made up of numerous, needle-like rays (Fig. S3A), and sparse in the superficial layer of the colony. The aspect of the colony and the structure of the tunic and spicules are consistent with *D. albopunctatum*, but the absence of gonads or larvae precludes a definite identification.

Didemnum cf. *granulatum* Tokioka, 1954 (Figs. S1E, S3B)

- Kott (2001), p. 188; Kott (2004a), p. 2493.
- Remarks: The colony is an extensive sheet of brown color with clear areas in green tinges surrounding the cloacal apertures. The most conspicuous character is the presence on the surface of minute papillae filled with spicules. The common cavity is thoracic and there is a thick basal tunic embedding abdomina and embryos. The spicules are abundant, up to 30 μm in diameter (Fig. S3B), and with ca. 7 rays in optical section (Kott 2001). There are numerous filamentous cyanobacteria in the tunic. The zooids are very small and immature

in general. The incubating embryos are not developed. The characteristics of the colony are in agreement with *D. granulatum*, but this seems to be a polymorphic species poorly described in general, so it may be in fact a group of species. Without the observation of mature larvae the identification should be left pending confirmation.

Didemnum multispirale Kott, 2001 (Figs. S1F, S3C)

- Kott (2001), p. 213.

Didemnum sp. 1 (Figs. S1G, S3D)

- Remarks: An encrusting colony of yellow-orange color. The common cavity is at the thoracic level and abdomina are embedded in the basal layer of tunic. The spicules are densely distributed, up to 40 μm in diameter, and feature low numbers (5-6 in optical section) of long and blunt rays (Fig. S3D). The whitish zooids are contracted and some of them are in the budding process. There are no gonads or larvae. Although the spicules are similar to those of *D. candidum*, the colonies of the latter are reddish and the zooids are pigmented (Kott 2001). It is preferable to leave the specific assignment pending the observation of mature individuals.

Didemnum sp. 2 (Figs. S1H, S3E)

- Remarks: The colony is encrusting and of yellowish color, but has a network of darker areas where the branchial apertures open. In these darker zones the surface has minute warts filled with spicules that were absent from the rest of the colony. The common cavity is large and surrounds both the thoracic and the abdominal region. The spicules are up to 70 μm in diameter, with scarce (ca. 7 in optical section) and pointed rays (Fig. S3E). There are no gonads or larvae and their absence precludes a specific identification.

Leptoclinides madara Tokioka, 1953 (Figs. S1I, S3F)

- Kott (2001), p. 86 (as *L. variegatus*); Kott (2004a), p. 2475 (as *L. variegatus*); Monniot and Monniot (2001), p. 287.
- Remarks: The characters of the specimens agree well with those of *Leptoclinides variegatus*, a species described by Kott (2001). In particular the spicular types, with some chisel-like rays. However, the characters described for *L. variegatus* look identical to *L. madara*, a species widely distributed in the tropical western Pacific (but not reported in Australia), as described in Monniot and Monniot (2001). Our 18S sequence is identical with that of *L. madara* of Yokobori *et al.* (2006). We therefore believe that *L. variegatus* is a synonym of *L. madara*, which has precedence.

Lissoclinum badium Monniot and Monniot, 1996 (Figs. S1J, S3G)

- Monniot and Monniot (1996), p. 170; Kott (2001), p. 296; Kott (2004b), p. 64.

Lissoclinum cf. *capsulatum* Kott, 2007 (Figs. S2A, S3H)

- Kott (2007), p. 1205.
- Remarks: The colony is conical, with a cloacal opening at the top. The color is whitish with a yellow reticule marking the apertures of the zooids in double rows. The tunic is soft and the abdomina are surrounded by a capsule of tunic with spicules that makes the extraction of intact zooids very difficult. Spicules are up to 50 µm in diameter, with long and irregular rays (Fig. S3H). In some cases these rays are numerous, but other spicules have only a few long rays protruding from a central mass. Circular inclusions (cellular components or symbionts) are abundant between spicules (Fig. S3H). The capsules around zooids, the presence of these inclusions and the spicule types are characteristic

of *L. capsulatum* described by Kott (2007), but it is necessary to examine gonads and larvae, absent in the studied specimen, to confirm the identification.

Lissoclinum patella (Gottschaldt, 1898) (Fig. S2B)

- Kott (2001), p. 315; Kott (2004c), p. 769.

Family Polycitoridae Michaelsen, 1904

Eudistoma amplum (Sluiter, 1909) (Fig. S2C)

- Kott (1990), p. 194; Monniot and Monniot (1996), p. 185; Kott (2003), p. 1624.
- Remarks: The specimens collected have a high density of very small sand grains (generally over 100 μm, but sometimes smaller), which give the colony the appearance of having spicules. There are characteristic green granular bodies (up to 250 μm) in the tunic that confer a greenish color to the colonies.

Polycitor giganteus (Herdman, 1899) (Fig. S2D)

- Kott (1990), p. 171; Monniot and Monniot (2001), p. 249.

Family Polyclinidae Milne-Edwards, 1841

Aplidium protectans (Herdman, 1899) (Fig. S2E)

- Kott (1992), p. 579.
- Remarks: It is possible that *Aplidium longithorax* Monniot, 1987 is a synonym of this species (Monniot 1987; Monniot and Monniot 2001)

Aplidium sp. (Fig. S2F)

- Remarks: The colony is cushion-like and white in colour. The systems of zooids are small and open into tunic depressions. The tunic is consistent and without incrustations. The zooids are strongly contracted, 7-8 mm long. The thorax has 6 lobes in the oral siphon and a simple and long atrial languet. The number of stigmata rows is difficult to count, but it is between 20 and 25. The abdomen has a stomach with numerous longitudinal, slightly marked, ridges (up to 10 in the

visible sector). Postabdomen with gonads. There are 3 (occasionally 4) larvae incubating in the posterior-right part of the peribranchial cavity. The larvae reach up to 1.1 mm in length; they have three adhesive papillae with conical outgrowths between them, and double rows of small vesicules arching posteriorly in the dorsal and ventral zones. Although the external aspect is reminiscent of *A. crateriferum* (Sluiter, 1909), the combination of >20 stigmata rows and >15 stomach folds is not reported and this specimen may well be an undescribed species.

Synoicum castellatum Kott, 1992 (Fig. S2G)

- Kott (1992), p. 483; Monniot and Monniot (2001), p. 228.

Order Phlebobranchia Lahille, 1886

Family Ascidiidae Herdman, 1882

Phallusia arabica Savigny, 1816 (Fig. S2H)

- Kott (1985) p. 61; Monniot and Monniot (1996), p. 233.

Phallusia julinea Sluiter, 1915 (Fig. S2I)

- Kott (1985) p. 64; Monniot and Monniot (1996), p. 235.

Phallusia philippinensis Millar, 1975

- Millar (1975), p. 273; Monniot and Monniot (2001), p. 310.
- Remarks: This species has not been reported previously from Australia. It was found in abundance in a marina of Magnetic Island, so it is probably an introduced species. The specimens agree well with the description of Monniot and Monniot (2001), with a characteristic dark (deep brown to almost black) outer tunic, becoming more translucent internally, and with minute papillae on the anterior lip of the prepharyngeal groove.

Family Perophoridae Giard, 1872

Ecteinascidia diaphanis Sluiter, 1885

- Kott (1985), p. 90; Monniot and Monniot (1996), p. 227; Kott (2003), p. 1635.

Perophora aff. *modificata* Kott, 1985 (Fig. S2J)

- Kott (1985), p. 104; Monniot (1987), p. 23; Kott (2004b) p. 40.
- Remarks: The colony consists of a handful of zooids united by stolons. The zooids reach 3 mm and the tunic is translucent and whitish, with yellow rims in the siphons. The atrial siphon is distal and there is a short postero-ventral stalk that connects with the stolon. There are four rows of stigmata with ca. 35 stigmata per side in the middle rows. There are incipient testes in some zooids in the form of small and abundant follicles. These characters agree well with *P. modificata* as described by Kott (1985, 2004b). However, *P. modificata* features a characteristic vascular process in the stalks of zooids that we couldn't observe in our specimens. *P. modificata* is also described as being of yellow color, although Monniot and Monniot (2001) report the species from Palau Islands with a color similar to our specimens. Furthermore, the zooids of *P. modificata* are bigger than those we have measured, reaching 1 cm (although Kott 2004b figures zooids of ca. 3 mm). We decided to identify our specimen as a *species affinis* to *P. modificata*, but it is likely that it proves to be a different species.

Order Stolidobranchia Lahille, 1886

Family Styelidae Sluiter, 1895

Polycarpa argentata (Sluiter, 1890)

- Kott (1985), p. 148; Monniot and Monniot (1996), p. 247; Monniot and Monniot (2001), p. 322.

Polycarpa aurata (Quoy and Gaimard, 1834)

- Kott (1985), p. 149.

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Supplementary Text S2

Host Ascidian Phylogeny Methods

Ascidian 18S rRNA gene sequences recovered from the non-target, eukaryotic data component of the pyrosequencing run were processed with Geneious v5.6.3 (Drummond *et al*., 2012) and aligned using Clustal X (Thompson *et al*., 1997) with additional sequences retrieved from GenBank (accession numbers in Figure S4). Neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) analyses were conducted in MEGA v5.05 (Tamura *et al*., 2011). For NJ analyses, the Jukes-Cantor model of nucleotide substitution was used and data were re-sampled using 10,000 bootstrap replicates (Felsenstein, 1985). The MP tree was inferred by close-neighbor interchange and the ML based on the GTR+I+G (Tavaré, 1986) model with substitution rates varying among sites according to an invariant and gamma distribution. Data for MP and ML analyses were re-sampled using 1,000 bootstrap replicates.

Host Ascidian Phylogeny Results

Phylogenetic analyses using 18S rRNA gene sequences retrieved the three major ascidian orders: Aplousobrabchia, Phlebobranchia and Stolydobranchia (Figure S4). The split observed for the Aplousobranchia corresponded to the fast-evolving aplousobranch group that formed a long branch separated from the other sequences (Tsagkogeorga et al. 2009). In contrast, the slower evolving Aplousobranchia families (Clavelinidae in our tree) formed a non-supported clade with the Phlebobranchia. Within the Phlebobrabchia, the *Phallusia* species formed a well-supported clade, while the Stolidobranchia clade was not well resolved. Finally, within the fast evolving

Aplousobranchia, several genera were found to be monophyletic, namely *Lissoclinum*, *Diplosoma*, *Didemnum, Leptoclinides*, and *Eudistoma*.

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Table S1. Ascidian and seawater sample collection details.

Species	ID	Depth (m)	Site	Latitude	Longitude	Date
Clavelina arafurensis	42	10	Fantome Island	18º 40.226' S	146º 31.385' E	19-May-2011
Clavelina meridionalis	204	14	Pioneer Bay	18º 36.120' S	146º 29.306' E	12-Nov-2011
Clavelina meridionalis	205	14	Pioneer Bay	18º 36.120' S	146º 29.306' E	12-Nov-2011
Clavelina meridionalis	206	14	Pioneer Bay	18º 36.120' S	146º 29.306' E	12-Nov-2011
Pycnoclavella sp.	207	14	Pioneer Bay	18º 36.120' S	146º 29.306' E	12-Nov-2011
Pycnoclavella sp.	208	14	Pioneer Bay	18º 36.120' S	146º 29.306' E	12-Nov-2011
Pycnoclavella diminuta	6	9-10	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Pycnoclavella diminuta	8	7-8	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Pycnoclavella diminuta	26	9-10	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Didemnum cf. *albopunctatum*	18	7.7	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Didemnum cf. *granulatum*	122	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Didemnum multispirale	47	5	Orpheus Island	18º 34.835' S	146º 28.858' E	18-May-2011
Didemnum multispirale	48	2	Orpheus Island	18º 34.835' S	146º 28.858' E	18-May-2011
Didemnum multispirale	53	14	Pelorus Island	18º 32.710' S	146º 29.273' E	19-May-2011
Didemnum sp.1	104	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Didemnum sp.2	20	7.7	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Leptoclinides madara	72	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Leptoclinides madara	111	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Lissoclinum badium	71	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Lissoclinum badium	74	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Lissoclinum cf. *capsulatum*	114	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Lissoclinum patella	70	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Eudistoma amplum	100	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Eudistoma amplum	110	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Eudistoma amplum	113	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Polycitor giganteus	127	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Aplidium protectans	28	7.5	Orpheus Island	18º 34.835' S	146º 28.858' E	18-May-2011
Aplidium sp.	52	14	Pelorus Island	18º 32.710' S	146º 29.273' E	19-May-2011
Synoicum castellatum	2	9-10	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Synoicum castellatum	13	9-10	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Synoicum castellatum	24	9-10	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Phallusia arabica	4	9-10	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Phallusia arabica	30	8	Orpheus Island	18º 34.835' S	146º 28.858' E	18-May-2011
Phallusia arabica	46	14	Pelorus Island	18º 32.710' S	146º 29.273' E	19-May-2011
Phallusia julinea	213	4-6	Magnetic Island	19º 9.242' S	146º 52.180' E	18-Nov-2011
Phallusia philippinensis	210	2	Magnetic Island	19º 9.530' S	146º 51.100' E	18-Nov-2011
Ecteinascidia diaphanis	7	7-8	Pioneer Bay	18º 36.120' S	146º 29.306' E	17-May-2011
Perophora aff. *modificata*	49	6.5	Orpheus Island	18º 34.835' S	146º 28.858' E	18-May-2011
Polycarpa argentata	31	7	Orpheus Island	18º 34.835' S	146º 28.858' E	18-May-2011
Polycarpa aurata	99	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Polycarpa aurata	101	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Polycarpa aurata	126	4-6	Davies Reef	18º 49.024' S	147º 37.939' E	03-Jun-2011
Filtered Seawater	SW1	5	Pioneer Bay	18º 36.120' S	146º 29.306' E	11-Oct-2011
Filtered Seawater	SW2	5	Pioneer Bay	18º 36.120' S	146º 29.306' E	11-Oct-2011
Filtered Seawater	SW3	5	Pioneer Bay	18º 36.120' S	146º 29.306' E	11-Oct-2011

Table S2. Multiplex identifier barcode sequence for each ascidian and seawater sample.

MID Sequence	Sample	MID Sequence	Sample	MID Sequence	Sample
ACAGC	*Pycnoclavella diminuta* (6)	TAGAC	Seawater (SW2)	ACTGAT	*Leptoclinides madara* (72)
ACGAC	*Pycnoclavella diminuta* (8)	TATGT	Seawater (SW3)	ATGTGT	*Leptoclinides madara* (111)
ACTAT	*Pycnoclavella diminuta* (26)	TCAGT	*Eudistoma amplum* (110)	CACAGT	*Lissoclinum badium* (71)
AGAGT	*Synoicum castellatum* (2)	TCGAT	*Eudistoma amplum* (100)	CACGAT	*Lissoclinum badium* (74)
AGCAC	*Didemnum multispirale* (53)	TCTAC	*Synoicum castellatum* (13)	CACTAC	*Didemnum* sp. 1 (104)
AGTGC	*Synoicum castellatum* (24)	ACACAT	*Eudistoma amplum* (113)	CAGAGC	*Polycitor giganteus* (127)
ATCAT	*Phallusia arabica* (4)	ACACGC	*Clavelina meridionalis* (204)	CAGCAT	*Clavelina arafurensis* (42)
ATCGC	*Phallusia arabica* (30)	ACAGAC	*Clavelina meridionalis* (205)	CTGCGC	*Perophora* aff. *modificata* (49)
CACGT	*Phallusia philippinensis* (210)	ACATGT	*Clavelina meridionalis* (206)	CTGTAC	*Lissoclinum patella* (70)
CAGAT	*Didemnum multispirale* (48)	ACGAGT	*Pycnoclavella* sp. (207)	TACTGC	*Didemnum cf. granulatum* (122)
CATGC	*Aplidium* sp. (52)	ACGCAC	*Pycnoclavella* sp. (208)	TAGCGC	*Lissoclinum cf. capsulatum* (114)
CGCAT	*Phallusia arabica* (46)	ACGTAT	*Ecteinascidia diaphanis* (7)	TAGTAC	*Phallusia julinea* (213)
CGTAC	*Didemnum* sp. 2 (20)	ACGTGC	*Polycarpa aurata* (99)	TATAGC	*Polycarpa argentata* (31)
TACAT	*Didemnum* cf. *albopunctatum* (18)	ACTAGC	*Polycarpa aurata* (101)	TATGAC	*Aplidium protectans* (28)
TACGC	Seawater (SW1)	ACTCGT	*Polycarpa aurata* (126)	TCATAC	*Didemnum multispirale* (47)

Table S3. Diversity indices of ascidian and seawater associated microbial communities, showing expected OTU richness (Chao1) and the common diversity indices Shannon (H') and Simpson's Inverse (1/D).

Species	Order	Family	Chao1	H'	1/D
Clavelina arafurensis	Aplousobranchia	Clavelinidae	479	4.55	44.03
Clavelina meridionalis			303	3.70	16.42
Clavelina meridionalis			897	3.67	7.75
Clavelina meridionalis			1631	4.91	30.83
Pycnoclavella sp.			1024	3.16	4.77
Pycnoclavella sp.			121	2.72	4.92
Pycnoclavella diminuta			676	4.25	19.92
Pycnoclavella diminuta			730	3.92	8.15
Pycnoclavella diminuta			650	4.13	17.72
Didemnum cf. *albopunctatum*		Didemnidae	420	2.17	4.95
Didemnum cf. *granulatum*			52	1.21	2.30
Didemnum multispirale			210	1.85	3.08
Didemnum multispirale			288	2.39	4.64
Didemnum multispirale			398	2.14	3.52
Didemnum sp.1			797	3.72	9.08
Didemnum sp.2			1071	3.31	6.12
Leptoclinides madara			189	2.66	9.13
Leptoclinides madara			23	1.65	3.36
Lissoclinum badium			62	0.32	1.12
Lissoclinum badium			42	0.27	1.10
Lissoclinum cf. *capsulatum*			75	1.40	2.28
Lissoclinum patella			184	1.84	3.35
Eudistoma amplum		Polycitoridae	567	3.80	13.22
Eudistoma amplum			517	4.28	27.93
Eudistoma amplum			719	4.77	46.32
Polycitor giganteus			170	2.27	4.48
Aplidium protectans		Polyclinidae	356	0.95	1.40
Aplidium sp.			449	2.96	7.08
Synoicum castellatum			746	2.87	3.43
Synoicum castellatum			528	3.00	7.83
Synoicum castellatum			145	3.09	11.57
Phallusia arabica	Phlebobranchia	Ascidiidae	28	2.36	5.70
Phallusia arabica			334	2.75	8.09
Phallusia arabica			31	2.10	5.24
Phallusia julinea			132	3.70	20.30
Phallusia philippinensis			13	1.51	3.67
Ecteinascidia diaphanis		Perophoridae	779	4.92	42.83
Perophora aff. *modificata*			504	2.67	5.00
Polycarpa argentata	Stolidobranchia	Styelidae	144	1.41	1.67
Polycarpa aurata			32	0.78	1.47
Polycarpa aurata			44	1.34	1.89
Polycarpa aurata			23	1.32	2.59
Filtered Seawater	n.a.	n.a.	416	1.99	3.29
Filtered Seawater	n.a.	n.a.	502	1.61	2.73
Filtered Seawater	n.a.	n.a.	312	2.04	3.70

Table S4. Phylum-level composition of the ascidian microbiota, including the number and percentage of 97% OTUs ($OTU_{0.03}$), sequence reads and host ascidians for each phylum. Minimum and maximum relative abundance per host individual is also shown.

		$OTU_{0.03}$		**Seq. Reads**		**Host Ascidians**		**Rel. Abund.**
Domain	**Phylum**	No.	%	No.	%	No.	%	(per host)
Archaea	Thaumarchaeota	53	1.60	11993	17.68	39	92.86	0 - 95.57
	Crenarchaeota	6	0.18	11	0.02	4	9.52	0 – 1.13
	Euryarchaeota	34	1.02	110	0.16	17	40.48	0 - 5.33
	Unclassified	11	0.33	14	0.02	8	19.05	0 - 2.01
Bacteria	Proteobacteria	1251	37.67	23717	34.97	42	100	0.67 - 94.82
	Bacteroidetes	496	14.94	3778	5.57	40	95.24	0 - 33.39
	Planctomycetes	486	14.63	2876	4.24	37	88.1	0 - 18.83
	Cyanobacteria	172	5.18	14227	20.98	41	97.62	0 - 97.78
	Chloroflexi	103	3.10	1029	1.52	27	64.29	0 - 15.78
	Acidobacteria	87	2.62	335	0.49	29	69.05	0 - 2.65
	Actinobacteria	62	1.87	424	0.63	31	73.81	0 - 7.35
	Verrucomicrobia	51	1.54	121	0.18	21	50	0 - 1.11
	Firmicutes	45	1.36	285	0.42	25	59.52	0 - 13.33
	SBR1093	23	0.69	643	0.95	30	71.43	0 - 11.27
	Lentisphaerae	21	0.63	43	0.06	9	21.43	0 - 0.98
	Chlamydiae	20	0.60	39	0.06	6	14.29	0 - 0.52
	Tenericutes	18	0.54	193	0.28	11	26.19	0 - 1.78
	TM7	15	0.45	51	0.08	6	14.29	0 - 0.65
	WS3	10	0.30	13	0.02	7	16.67	0 - 0.86
	Spirochaetes	8	0.24	16	0.02	5	11.9	0 - 17.86
	Nitrospirae	6	0.18	16	0.02	5	11.9	0 - 1.74
	OP3	6	0.18	6	0.01	5	11.9	0 - 0.58
	TM6	6	0.18	6	0.01	6	14.29	0 - 0.09
	Thermi	5	0.15	23	0.03	9	21.43	0 - 0.40
	Chlorobi	5	0.15	20	0.03	8	19.05	0 - 0.51
	OP11	5	0.15	7	0.01	3	7.14	0 - 0.07
	Fusobacteria	3	0.09	27	0.04	6	14.29	0 - 1.86
	Armatimonadetes	3	0.09	5	0.01	3	7.14	0 - 0.26
	NKB19	3	0.09	3	0.00	2	4.76	0 - 0.17
	Caldithrix	2	0.06	4	0.01	2	4.76	0 - 0.15
	OP8	2	0.06	4	0.01	2	4.76	0 - 0.05
	PAUC34f	2	0.06	4	0.01	2	4.76	0 - 0.18
	BRC1	1	0.03	1	0.00	1	2.38	0 - 0.09
	Elusimicrobia	1	0.03	1	0.00	1	2.38	0 - 0.06
	GN04	1	0.03	1	0.00	1	2.38	0 - 0.05
	KSB1	1	0.03	1	0.00	1	2.38	0 - 0.19
	SM2F11	1	0.03	1	0.00	1	2.38	0 - 0.03
	Unclassified	296	8.91	7778	11.47	36	85.71	0 - 64.91

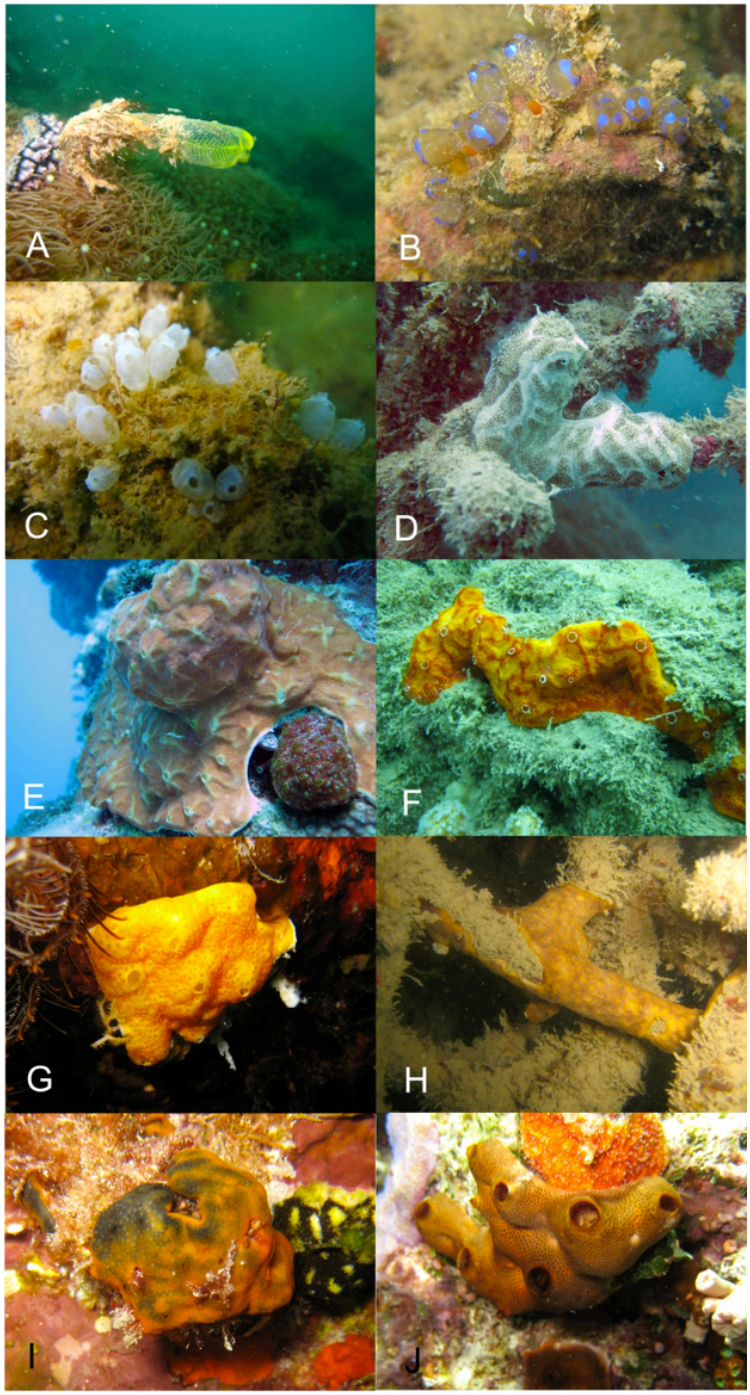

Figure S1. Underwater images of ascidian species studied. (A) *Clavelina meridionalis*; (B) *Pycnoclavella diminuta*; (C) *Pycnoclavella* sp.; (D) *Didemnum* cf. *albopunctatum*; (E) *Didemnum* cf. *granulatum*; (F) *Didemnum multispirale*; (G) *Didemnum* sp. 1; (H) *Didemnum* sp. 2; (I) *Leptoclinides madara*; (J) *Lissoclinum badium.*

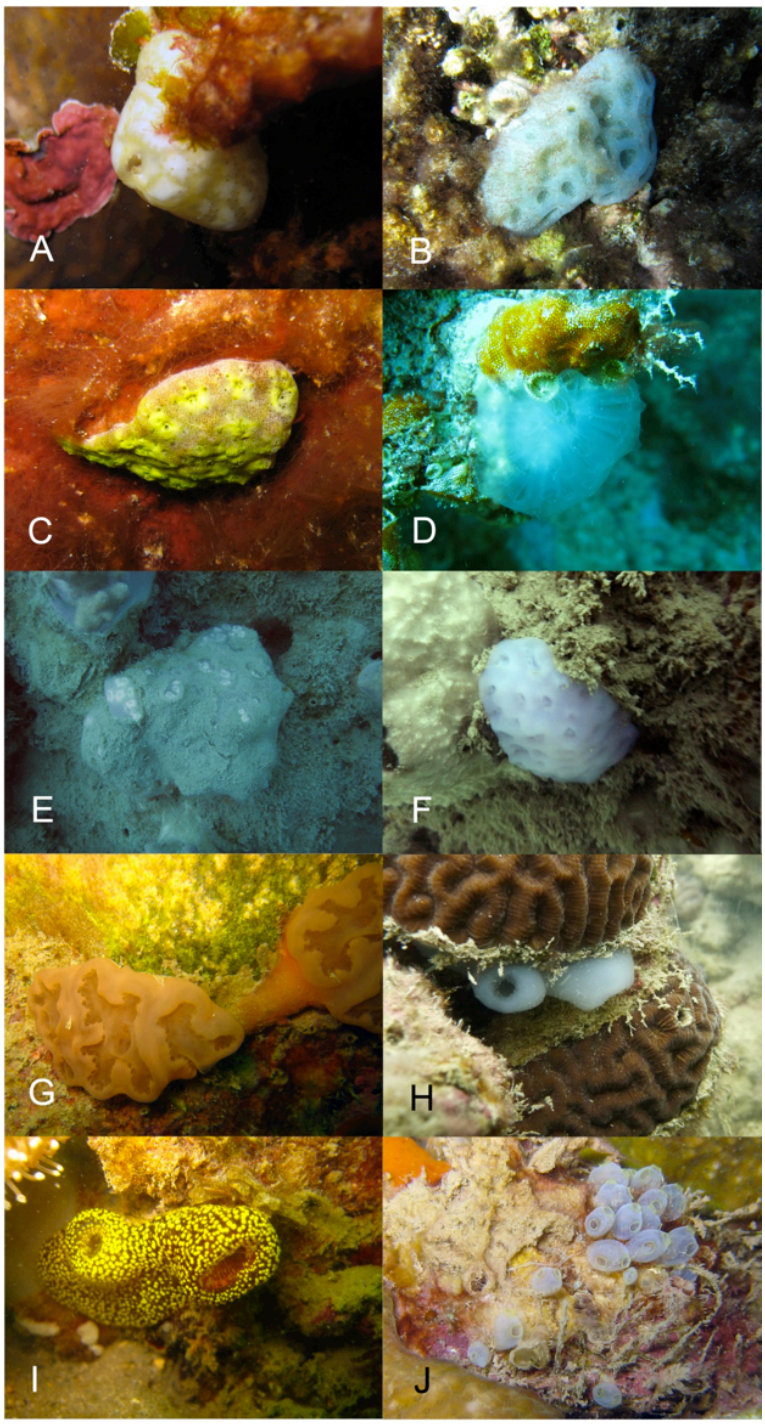

Figure S2. Underwater images of ascidian species studied. (A) *Lissoclinum* cf. *capsulatum*; (B) *Lissoclinum patella*; (C) *Eudistoma amplum*; (D) *Polycitor giganteus*; (E) *Aplidium protectans*; (F) *Aplidium* sp.; (G): *Synoicum castellatum*; (H) *Phallusia arabica*; (I) *Phallusia julinea*; (J) *Perophora* aff. *modificata*.

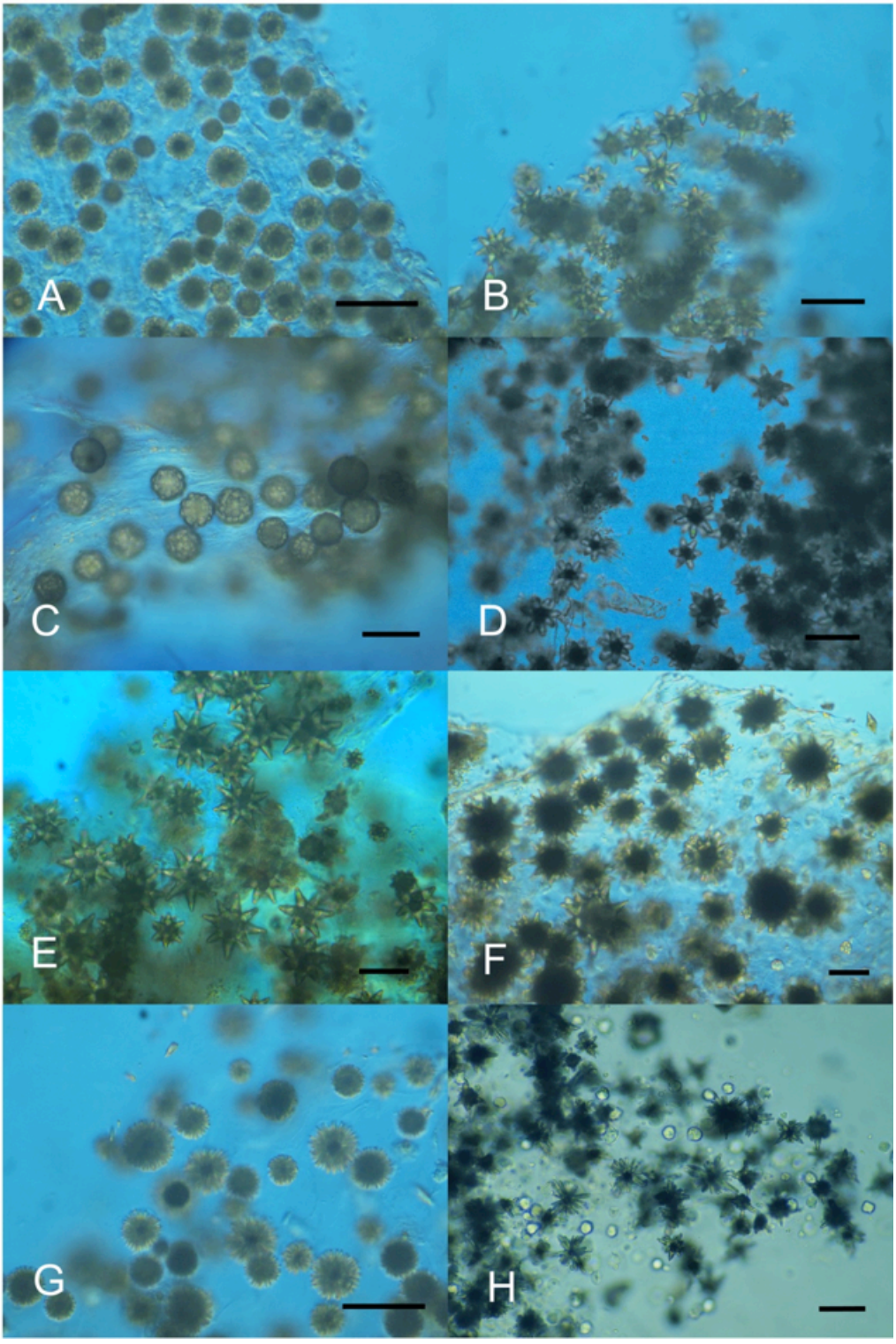

Figure S3. Light microscope images of spicules of didemnid species studied. (A) *Didemnum* cf. *albopunctatum*; (B) *Didemnum* cf. *granulatum*; (C) *Didemnum multispirale*; (D) *Didemnum* sp. 1; (E) *Didemnum* sp. 2; (F) *Leptoclinides madara*; (G) *Lissoclinum badium*; (H) *Lissoclinum* cf. *capsulatum*. Scale bars: 50 µm.

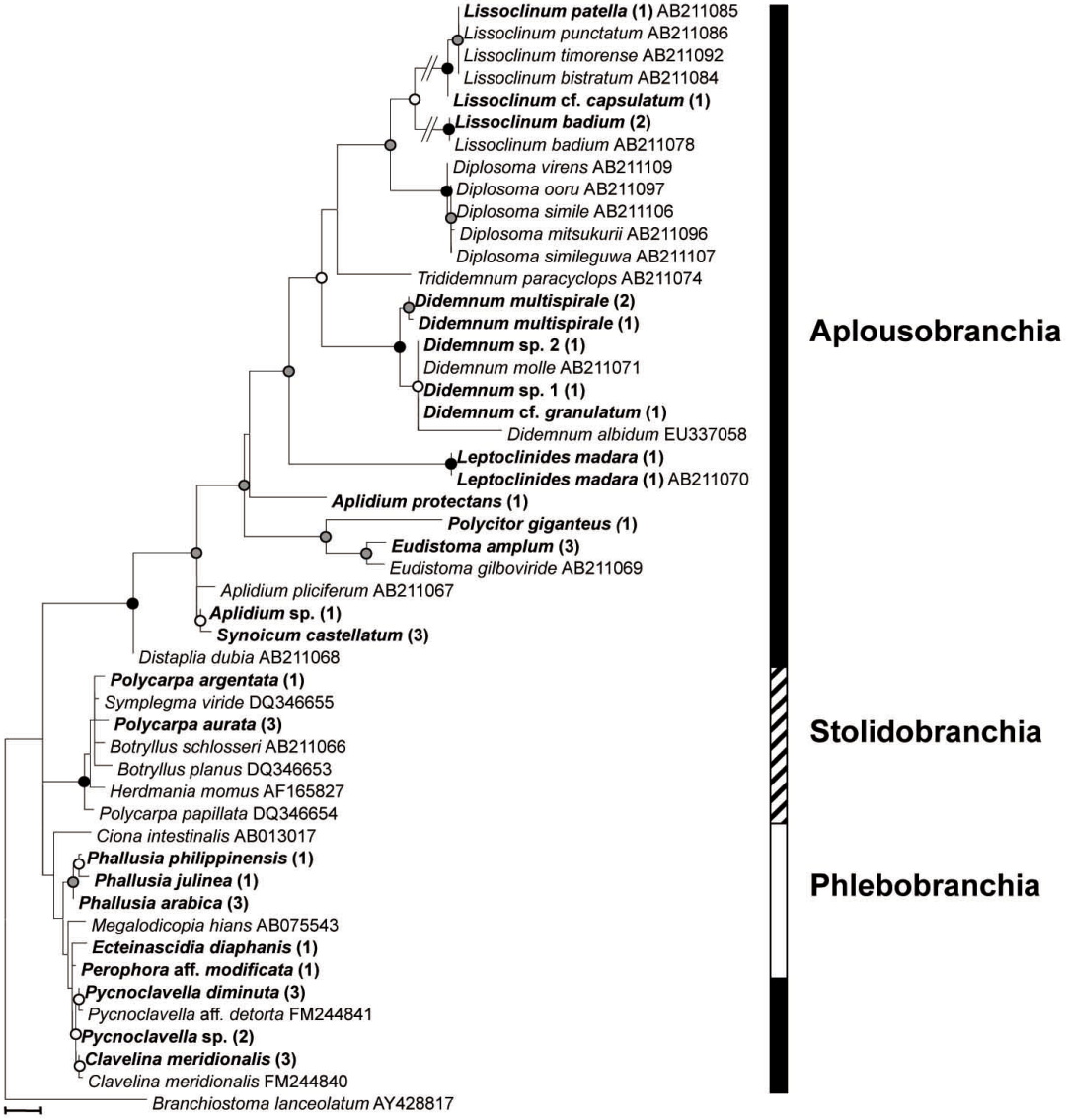

Figure S4. Phylogeny of ascidian hosts based on 18S rRNA gene sequence data. Bars on the far right denote ordinal taxonomy: Aplousobranchia (*black*), Stolidobranchia (*striped*) and Phlebobranchia (*white*). Tree topology was constructed using maximum likelihood (ML) criteria. Bootstrap support values for ML, maximum parsimony and neighbor-joining criteria are indicated with black (>90% in all 3 analyses), gray (>70% in all 3 analysis) and white (>50% in 2 of 3 analyses) circles. Terminal nodes denote the ascidian species, with sequences derived herein shown in bold, followed by the number of individuals or GenBank accession number. Scale bar represents 0.5 substitutions per site.

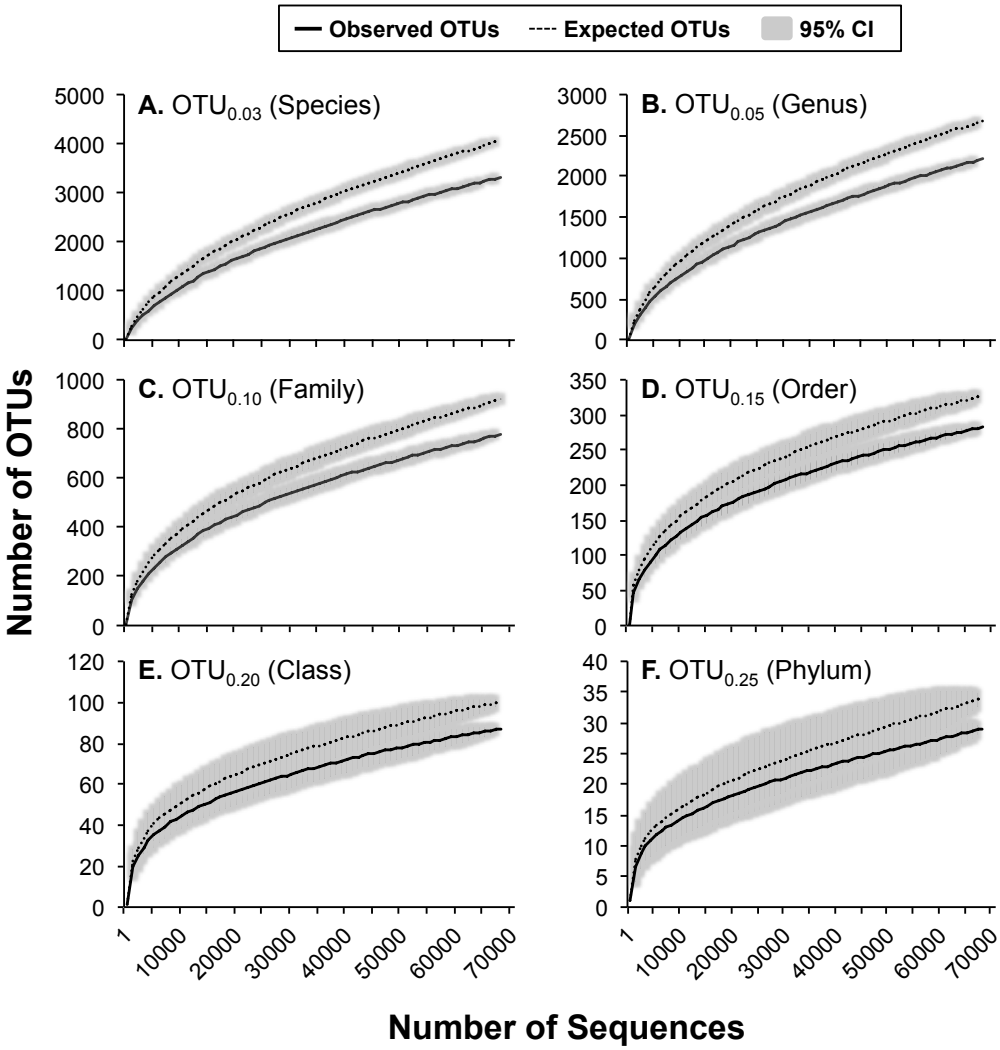

Figure S5. Rarefaction curves for observed (*solid lines*) and expected (*dotted lines*) OTU diversity in the ascidian microbiota. *Gray bars* denote 95% confidence intervals. OTU sequence identity levels shown correspond approximately to the taxonomic levels of (A) species, (B) genus, (C), family, (D) order, (E) class and (F) phylum.

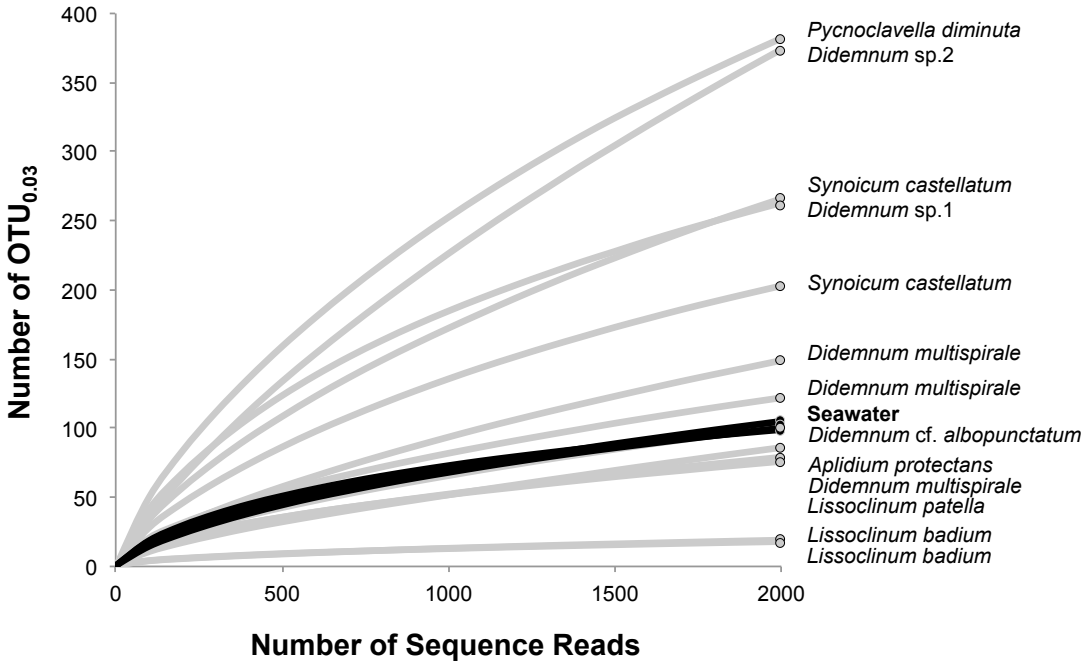

Figure S6. Standardized rarefaction curves for $OTU_{0.03}$ diversity in well-sampled (>2,000 sequence reads) ascidian hosts (*gray lines*) and seawater samples (*black lines*).

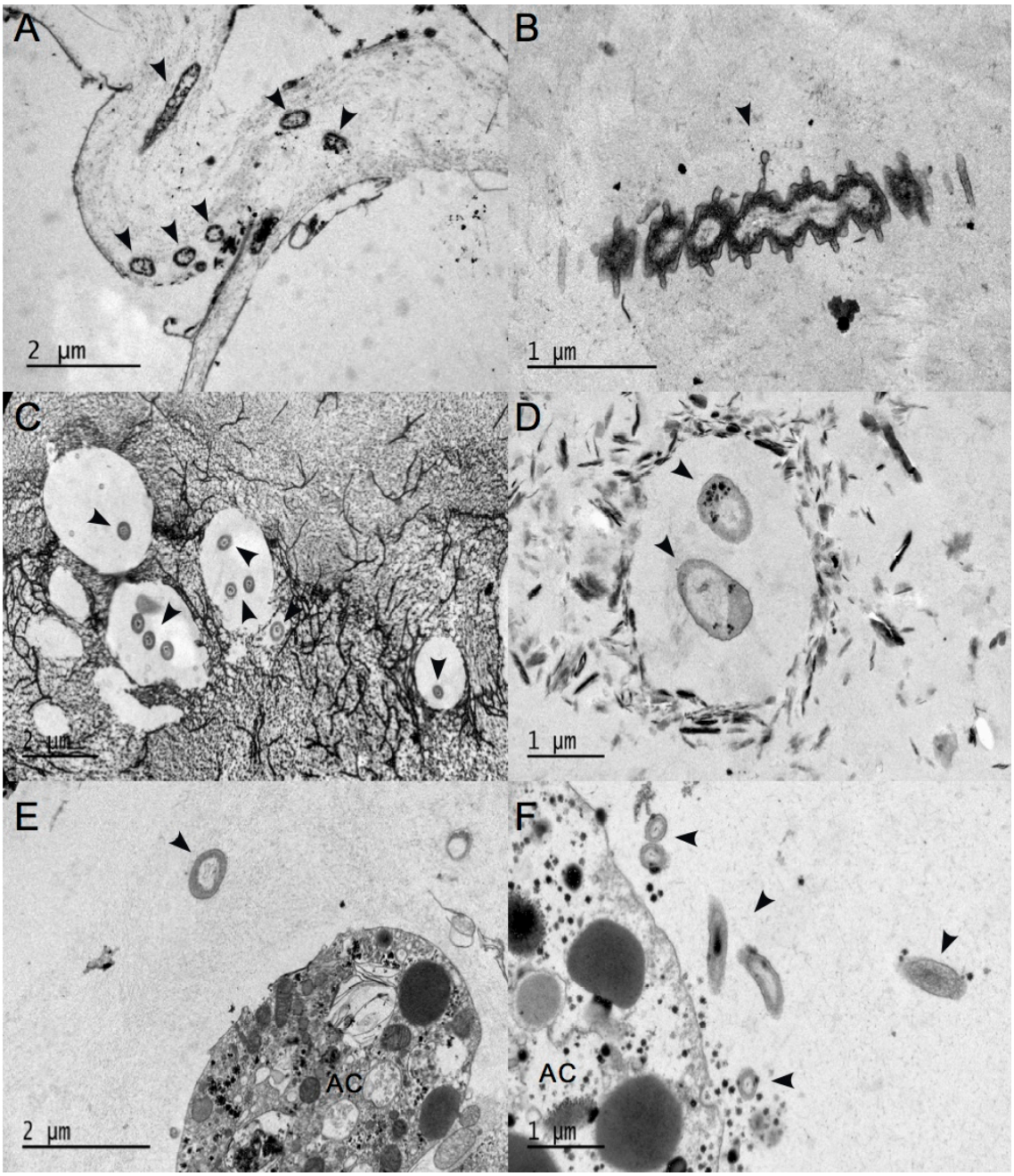

Figure S7. Transmission electron microscopy images of bacteria observed in the inner tunic of the ascidians: (A) *Phallusia julinea*, (B) *Polycarpa aurata*, (C) *Pycnoclavella* sp., (D) *Clavelina meridionalis*, (E) *Lissoclinum badium*, and (F) *Synoicum castellatum*. Arrowheads point to bacterial cells, (AC) ascidian cell.