

Supplementary Information

Ecology of endolithic bryozoans: colony development, growth rates and interactions of species in the genus *Immergentia*

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Table S1. Reports of recent immergentiids on substrate from literature and examined in this study.

<i>Immergentia</i> species	Class	Substrate	Substrate condition	Reference	Location (s)
<i>angulata</i>	<i>Gastropoda</i>	<i>Pisania tritonoides</i> (Reeve, 1846)	Possibly living and/or dead gastropods	Soule & Soule, 1969	Collected 15 July 1967, depth 45 Feet (13.7 m), water temperature 81 °F
<i>californica</i>	<i>Gastropoda</i>	<i>Acanthinucella spirata</i> (Blainville, 1832)	dead	Soule, 1950	California, Cabrillo Beach, San Pedro, February 11, 1949, tide pools
	<i>Gastropoda</i>	<i>Tegula brunnea</i> (R. A. Philippi, 1849)	alive	Silén, 1946	Tidepools, Pacific Grove, California, USA
	<i>Gastropoda</i>	<i>Tegula ligulata</i> (Menke, 1850)	alive and dead	Soule, 1950	California, Whites Point, San Pedro, January 27, 1949, tide pools. Portuguese Bend, March 11, 1949; tide pools
	<i>Gastropoda</i>	<i>Haliotis cracherodii</i> (Leach, 1814)	dead	Soule, 1950	Whites Point, San Pedro, January 27, 1949; tide pools
	<i>Gastropoda</i>	<i>Littorina keenae</i> (Rosewater, 1978)	dead	Soule, 1950	Whites Point, San Pedro, January 27, 1949; tide pools
	<i>Gastropoda</i>	<i>Littorina scutulata</i> (Gould, 1849)	dead	Soule, 1950	Whites Point, San Pedro, January 27, 1949; tide pools
<i>cheongpodensis</i>		Mollusc shells; oysters and clams	dead	Seo et al., 2018	Low intertidal zone. Cheongpodae, Taean Coast National Park (36.6334° N, 126.2997° E). 16 June 2017.
<i>orbignyana</i>	<i>Gastropoda</i>	<i>Conus ventricosus</i> (Gmelin, 1791)	-	Fischer, 1866	Arcachon basin (Gironde), France
	<i>Gastropoda</i>	<i>Charonia lampas</i> (Linnaeus, 1758)	-	Fischer, 1866	Arcachon basin (Gironde), France
	<i>Bivalvia</i>	<i>Ostrea edulis</i> (Linnaeus, 1758)	-	Fischer, 1866	Arcachon basin (Gironde), France
	<i>Bivalvia</i>	<i>Unidentified bivalve</i>	dried	Pohowsky, 1978	Arcachon basin (Gironde), France; Fischer material
<i>I. patagoniana</i>	<i>Gastropoda</i>	<i>Buccinanops cochlidium</i> (Dillwyn, 1817)	dried	Pohowsky, 1978	Patagonia, Argentina
	<i>Gastropoda</i>	<i>Argeneuthria cerealis</i> (Rochebrune & Mabile, 1885)	dead	López-Gappa & Zelaya (2021), Johnson et al.	Burdwood Bank -30 Mar 2016, 54°25.144' S, 59°12.892' W depth 120 -8 May 2017 54°00.240' S, 61°04.762' W, depth 139 -10 Apr 2016, 54°30.390' S, 59°48.654' W, depth 105 -31 Mar 2016, 54°31.679' S, 61°27.979' W, depth 137
	<i>Gastropoda</i>	<i>Cerithiopsis caelata</i> (Gould, 1849)	dead	López-Gappa & Zelaya (2021)	
	<i>Gastropoda</i>	<i>Fuegotrophon pallidus</i> (Broderip, 1833)	dead	López-Gappa & Zelaya, 2021	
	<i>Gastropoda</i>	<i>Pareuthria atrata</i> (E. A. Smith, 1881)	dead	López-Gappa & Zelaya (2021), Johnson et al.	
	<i>Gastropoda</i>	<i>Pareuthria fuscata</i> (Bruguière, 1789)	alive, dead	López Gappa, 1981, López-Gappa & Zelaya (2021), Johnson et al., this study	
	<i>Gastropoda</i>	<i>Savatiera chordata</i> (Castellanos, Rolán & Bartolotta, 1987)	dead	Johnson et al.	
	<i>Gastropoda</i>	<i>Trophon ohlini</i> (Strebel, 1904)	dead	Lopez-Gappa & Zelaya (2021), Johnson et al.	

	<i>Gastropoda</i>	<i>Typhlodaphne</i> sp. (Powell, 1951)	dead	Johnson et al.	
<i>I. stephanieae</i>	<i>Gastropoda</i>	<i>Littorina littorea</i> (Linnaeus, 1758)	alive	Johnson et al.	Intertidal zone around the Roscoff Marine Station 48°43.698'N, 3°59.721'W and Santec 48°42.787' N, 4°1.315' W -intertidal Îll Callot, intertidal
	<i>Gastropoda</i>	<i>Nucella lapillus</i> (Linnaeus, 1758)	alive	Johnson et al.	Intertidal zone around the Roscoff Marine Station
	<i>Polyplacophora</i>	<i>Acanthochitona</i> sp. (J. E. Gray, 1821)	alive	This study	Intertidal zone around the Roscoff Marine Station
<i>I. suecica</i>	<i>Bivalvia</i>	<i>Pseudamussium peslutrae</i> (Linnaeus, 1771)	alive, dead	Silén (1947)	Gullmar Fjord, West Coast of Sweden, north of Flatholmen, depth 45 m
<i>I. cf. suecica</i> France	<i>Bivalvia</i>	<i>Aequipecten opercularis</i> (Linnaeus, 1758)	dead	This study	Stolvezen, Roscoff 48°42.847' N, 3°53.500' W; 48°42.846' N, 3°53.5' W; 48°40.000' N, 3°52.999' W, depth 15 – 25
	<i>Bivalvia</i>	<i>Acanthocardia echinata</i> (Linnaeus, 1758)	dead	This study	Térenéz, Roscoff 48°41.532' N, 03°52.075' W, depth ≈10
	<i>Bivalvia</i>	<i>Anomia ephippium</i> (Linnaeus, 1758)	dead	This study	Chateaux du Taureau, Roscoff 48°40.2' N, 3°53.12' W; 48°40.200' N, 3°52.999' W, depth 10 – 15 m
	<i>Gastropoda</i>	<i>Buccinum undatum</i> (Linnaeus, 1758)	dead	This study	Primel, Roscoff 48°43.467'N, 03°50.55'W
	<i>Gastropoda</i>	<i>Crepidula fornicata</i> (Linnaeus, 1758)	dead	This study	Pleine du Vezeal, Roscoff 48°42.48'N, 003°55.33'W
	<i>Bivalvia</i>	<i>Ensis</i> sp. (Schumacher, 1817)	dead	Johnson et al., This study	Near L'Île Louët, and the Roscoff Harbour
	<i>Bivalvia</i>	<i>Glycymeris</i> (Linnaeus, 1758)	dead	Johnson et al., This study	
	<i>Bivalvia</i>	<i>Lutraria lutraria</i> (Linnaeus, 1758)	dead	Johnson et al., This study	
	<i>Bivalvia</i>	<i>Mimachlamys varia</i> (Linnaeus, 1758)	dead	This study	
	<i>Bivalvia</i>	<i>Pecten maximus</i> (Linnaeus, 1758)	dead	Johnson et al., This study	
	<i>Bivalvia</i>	<i>Polititapes aureus</i> (Gmelin, 1791)	dead	This study	
	<i>Gastropoda</i>	<i>Tritia reticulata</i> (Linnaeus, 1758)	dead	This study	
<i>I. cf. suecica</i> Norway	<i>Gastropoda</i>	<i>Buccinum undatum</i> (Linnaeus, 1758)	dead, hermit	Johnson et al., This study	
<i>I. zelandica</i>	<i>Gastropoda</i>	<i>Buccinulum littorinoides</i> (Reeve, 1846)	alive	Silén (1946)	Slipper Island, New Zealand, intertidal
<i>I. cf. zelandica</i>	<i>Gastropoda</i>	Buccinoidea Rafinesque, 1815, Molluscan shells,	dead	Johnson et al.	Otago inner shelf, New Zealand (45° 45.87' S, 170° 49.50' E). Collected 5 November 2021, depth 40 m
<i>I. zelandica</i> var <i>minuta</i>	<i>Gastropoda</i>	<i>Conus striatus</i> (Linnaeus, 1758)	-	Soule & Soule (1969)	Hawaiian Island, Haena Bay, Kauai; collected 1 July 1967, depth 20 feet (6.1 m), water temperature 80°F
	<i>Gastropoda</i>	<i>Cypraea</i> sp.? (Linnaeus, 1758)	-	Soule & Soule (1969)	
	<i>Gastropoda</i>	<i>Stomatella planulata</i> (Lamarck, 1816)	alive	Soule 1950	Zamboanga, Philippine Islands, tide pools
<i>Immergentia</i> spp. Guam	<i>Gastropoda</i>	<i>Conus</i> sp. (Linnaeus, 1758)	dead, hermit	This study	Pago Bay (13°25'36.9"N, 144°47'41.8"E), area behind University of Guam Marine Lab, intertidal, 13 & 15 November 2022
	<i>Gastropoda</i>	<i>Morula uva</i> (Röding, 1798)	alive	This study	

	<i>Gastropoda</i>	<i>Fusinus colus</i> (Linnaeus, 1758)	dead	This study	Family Beach (13°27'41.7"N, 144°38'51.1"E), Guam, depth 7 m
<i>Immergentia</i> spp. Japan	<i>Gastropoda</i>	Shell fragment	dead	This study	Sagami Bay west, Manazuru, Japan (35°8'29.03"N, 139°9'39.74"E), collected 19 August 2023, intertidal
					Ise Bay area, Japan Dredge: Enshu Sea (34°17'60.00"N, 137°5'45.00"E-34°18'4.20"N, 137°6'24.60"E), depth 153-154 m Beam Trawl: Enshu Sea (34°17'51.60"N, 137°7'16.80"E-34°17'45.00"N, 137°8'7.20"E), depth 165-180 m Both collected 23 August 2023
					Sagami Bay, east Tenjin-jima, Japan (35°13'16.82"N, 139°36'10.56"E), collected 29 August 2023, intertidal
<i>Immergentia</i> spp.	<i>Gastropoda</i>	<i>Buccinulum corneum</i> (Linnaeus, 1758)	dried	This study*	Syracuse, Sicily, Italy Depth not reported (sample no. G860.40.10.1)
	<i>Gastropoda</i>	<i>Bursa thomae</i> (d'Orbigny, 1842)	dried	This study*	Tenerife, Canary Islands (South of Teno Point) 20 - 25m depth (sample G790.10.40.1; G790.10.40.2)
	<i>Bivalvia</i>	<i>Chlamys islandica</i> (Müller, 1776)	dried	This study*	Ramfjorden, Tromsø, Norway (N69.308-E10.567) 119m depth
	<i>Bivalvia</i>	<i>Chlamys islandica</i> (Müller, 1776)	dried	This study*	Bay of Biscay-Penmarc'h, France (N46.9043-W5.4432) 610m depth (sample G790.10.40.1; B190.130.40.9, SaM77947)
	<i>Gastropoda</i>	<i>Ocenebra brevirobusta</i> (Houart, 2000)	dried	This study*	Moroccan Coast 15km North of Agadir. Intertidal (sample G860.90.5.1)
	<i>Gastropoda</i>	<i>Tritia reticulata</i> (Linnaeus, 1758)	dead	This study	Roscoff, intertidal zone
	<i>Bivalvia</i>	<i>Polititapes aureus</i> (Gmelin, 1791)	dead	This study	See locations for <i>I. cf. suecica</i> France
	<i>Bivalvia</i>	<i>Pseudamussium clavatum</i> (Poli, 1795)	dried	This study*	Bay of Biscay-Penmarc'h, France (N46.9043-W5.4432) 610m depth (sample G790.10.40.1; B190.80.10.5, SaM70699)
	<i>Gastropoda</i>	Unidentified gastropod	dead	This study*	Guadeloupe; between the south of Basse-Terre and Marie-Galante, Caribbean Sea (15°58'00.0"N, 61°25'50.0"W), 55m depth, Poseidon cruise 316, SAM ID-15820

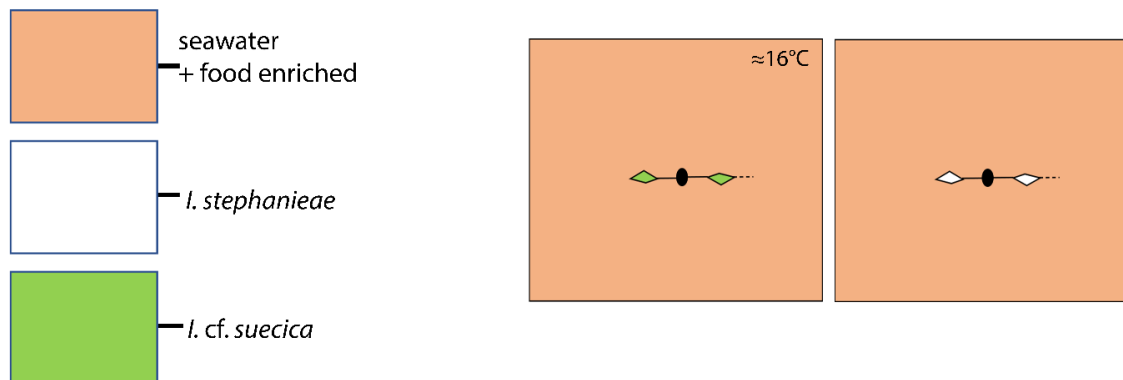
Note: Substrate species names according to WoRMS classification

(*) = Senckenberg am Meer collection

Table S2. Characteristics of immergentiids from new locations examined in this study.

Location <i>Immergentia</i> Species	Mean aperture width (μm)	Borehole aperture shape	Colony + zooidal characteristics
Guam	51.07 $\pm$ SD 12.96 Min - max: 26.37 – 76.95	Oval to spindle-shaped	Only one shell left Densely packed difficult to distinguish colony pattern.
	n = 30		
Helgoland	47.176 $\pm$ SD 9.61 Min - max: 31.98 – 57.66	Circular to oval-shaped	Densely packed difficult to distinguish colony pattern.
	n = 15		
Japan	51.34 $\pm$ SD 7.45 Min - max: 41.95 – 70.56 Ancestrula width 40.49	Circular to oval-shaped	Distinct feather-like pattern of colony. Lateral cystid appendages always extend opposite each other if two are present.
	n = 14		
Guadeloupe	39.67 $\pm$ SD 5.8 Min - max: 30,39 – 48.5	Circular	Colony form not determined (cystid appendages not visible on shell) Zooids typical vase shape with rounded or pointed tip. Sometimes tip tilted in direction of primary cystid appendage.
	n = 11		Zooid length 296.56 $\pm$ SD 30 Min - max: 257.92 – 362.96 Zooid width 64.04 $\pm$ SD 9.55 Min - max: 47.06 – 81.50 n = 10

Growth experiment 1



Growth experiment 2

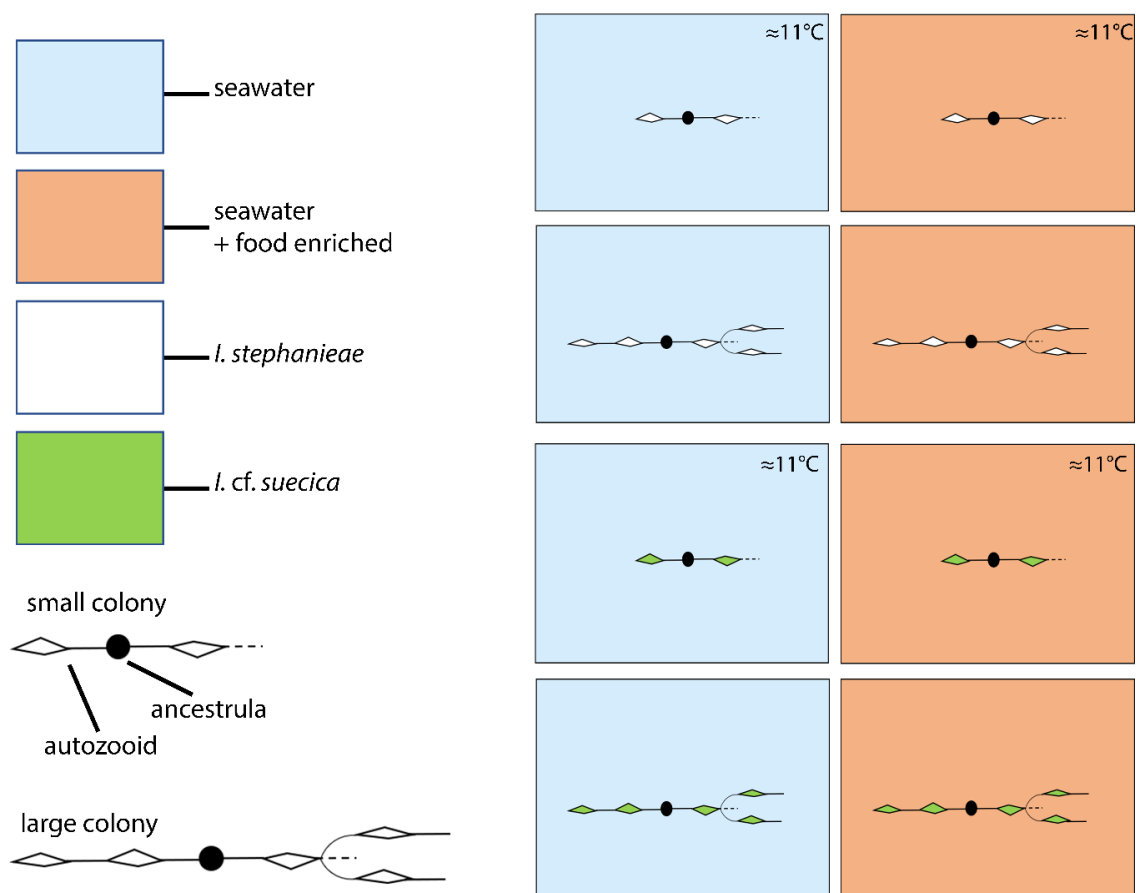


Figure S1. Experimental design of growth experiments 1 occurred in the period between 16 August to 16 October 2021 and growth experiment 2 between 27 February to 22 March 2023.



Figure S2. **a** Experimental set up for growth experiment 1 from 16 August to 16 October 2021. **b** Nunc 6-well multidishes for settlement experiment 1A with shells uncolonized molluscan shells. **c** Gastropod shells with colonies placed on uncolonized molluscan shells in a disk with fine mesh for settlement experiment 1B. **d – f** Settlement experiment 2 from 27 February to 22 March 2023. **d** *Littorina littorina* bearing *I. stephanieae* colonies placed with uncolonized shells for settlement experiment 2B. **e** bivalve shells with *I. cf. suecica* shells placed on uncolonized bivalve shells for settlement experiment B. **f** Growth and settlement experiment 2A set up with food enriched and not enriched (control) tanks.

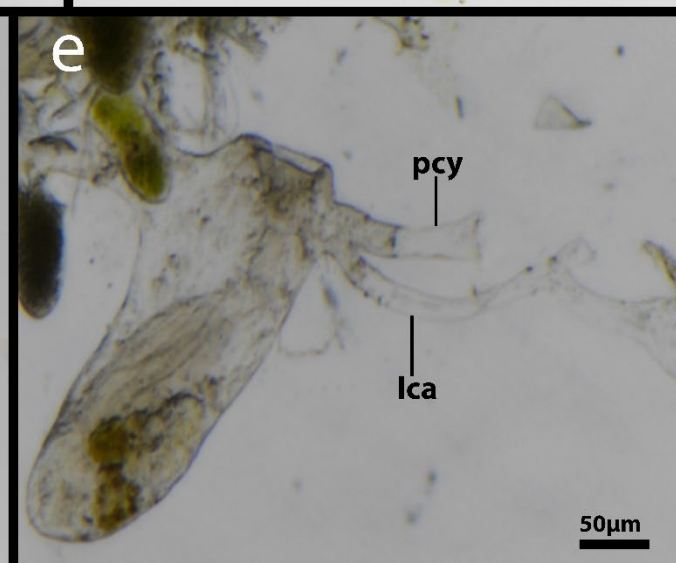
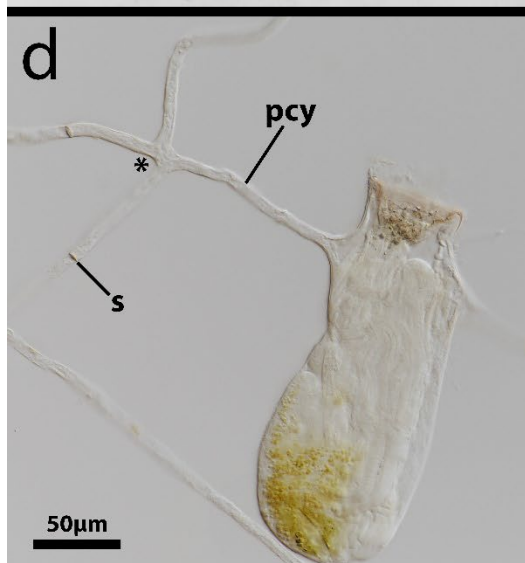
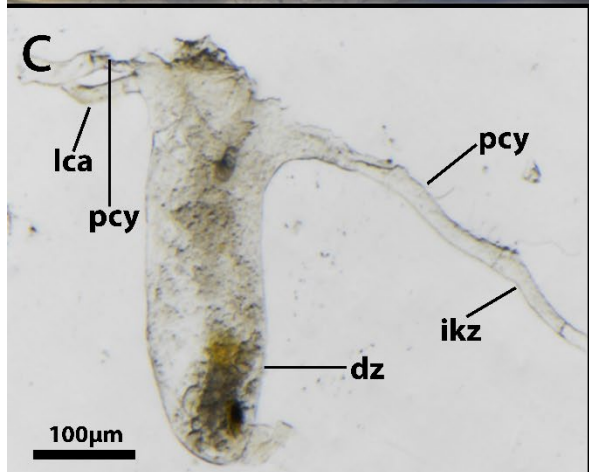
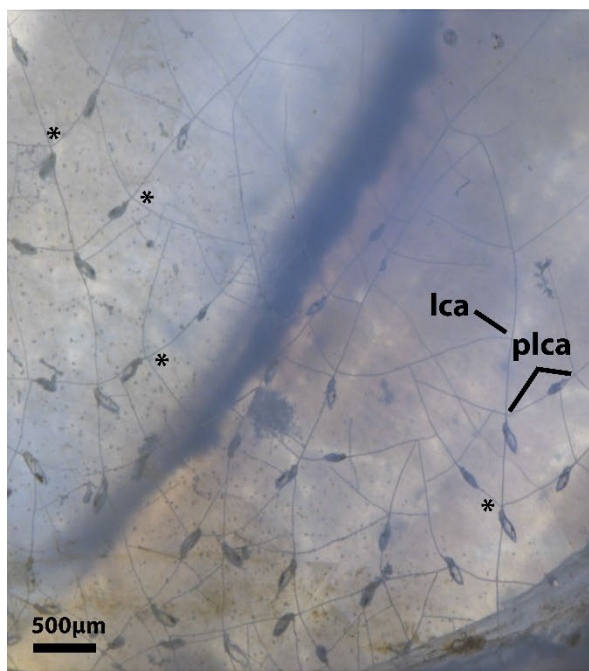


Figure S3. a *Immergentia* cf. *suecica* colony with paired lateral cystid appendages (asterisks) creating a feather-like pattern and lateral cystid appendages. **b** Part of *Immergentia stephanieae* colony with zooids in different developmental stages. Lateral cystid appendages extend from the primary cystid appendage. Intercalary kenozooids with septum separate cystid appendages of neighbouring zooids. **c** Lateral cystid appendage developed near the primary cystid appendage in *Immergentia stephanieae* from Roscoff, France. **d** Primary cystid appendage split, septa develop after and paired lateral cystid appendages developed (asterisk) in *Immergentia* cf. *zelandica* from Otago Inner shelf, New Zealand **e** Lateral cystid appendage derived from cystid wall developed near the primary cystid appendage *Immergentia stephanieae*.

Abbreviations: anc – ancestrula, az – autozooid, b – bud, d – degenerated zooid, ikz – intercalary zooid, lca – lateral cystid appendage, pcy – primary cystid appendage, plca – paired lateral cystid appendage, rz – reproductive zooid, s – septum

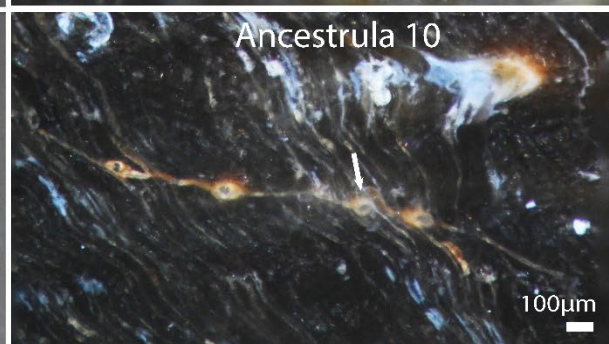
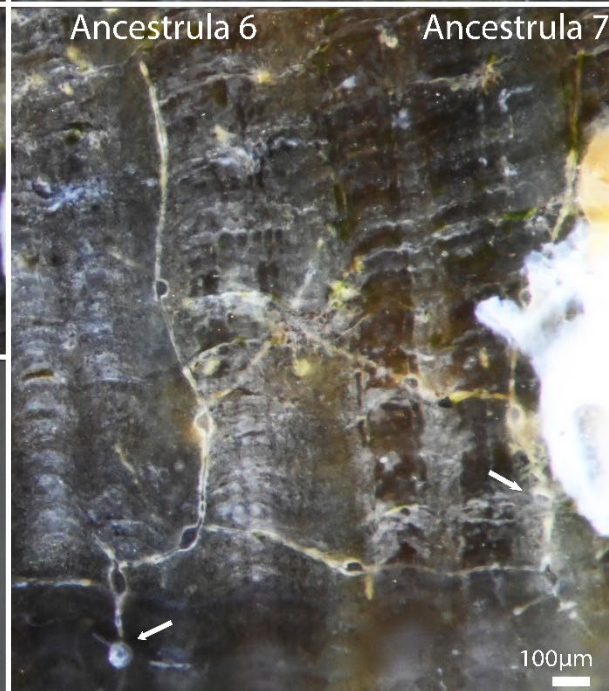
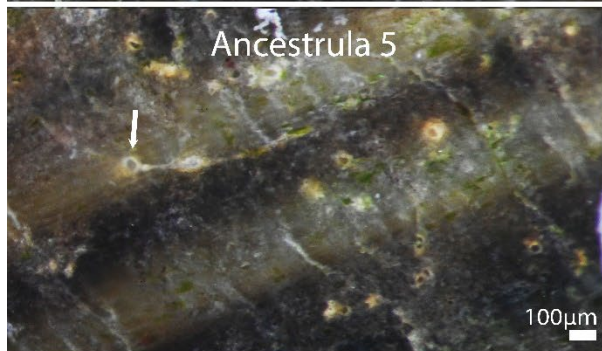
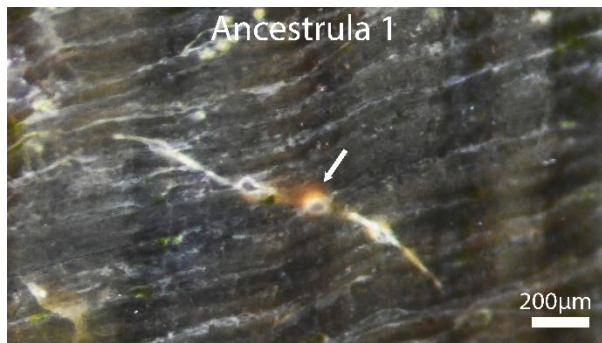


Figure S4. Ancestrulae and small colonies of *I. stephanieae* from Growth experiment 1 corresponding to Table 1 in the main text. Images on final day of observation.

Video S1. Flickering and caging of tentacles by *Immergentia stephanieae*

figshare private link: <https://figshare.com/s/3b410cfc1ffe4a7b152c>

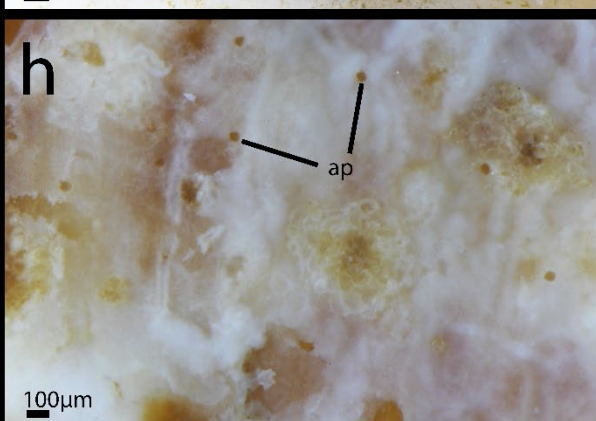
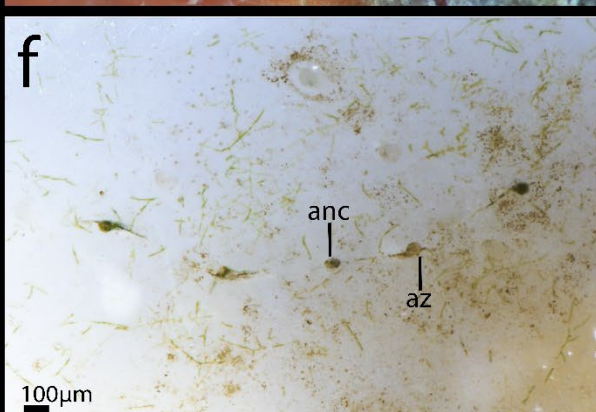
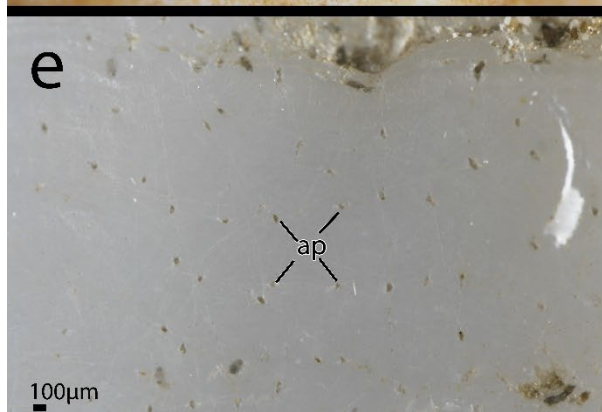
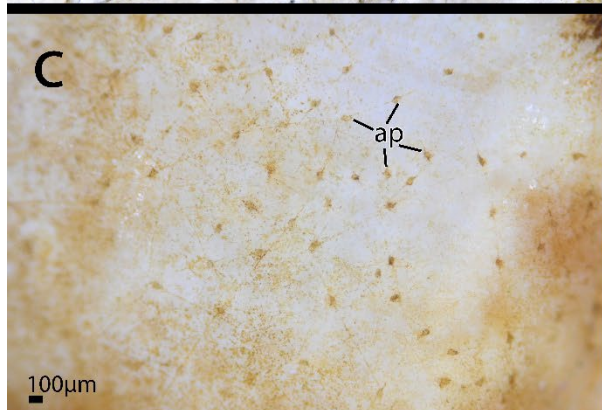
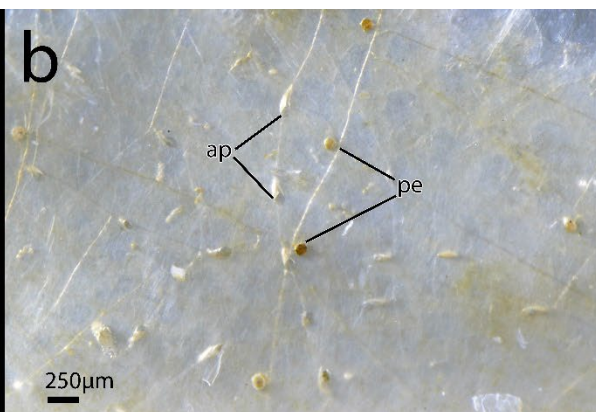
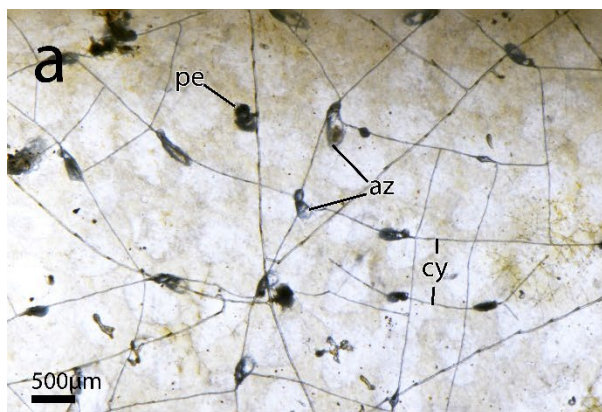


Figure S5. Colonies of immergentiids reported from new locations. **a** Colony of unidentified *Immergentia* sp. from Helgoland in an *Anomia* sp. shell. Autozooids and cystid appendages visible. **b** Same colony. Circular-shaped borehole apertures of immergentiid. Pedunculate *Penetrantia* sp. also visible. **c – e** *Immergentia* sp. from Guam with spindle-shaped borehole apertures. **c** Immergentiid in hermitid *Conus* sp. shell collected from Pago Bay. **d** Immergentiid in gastropod *Morula uva* shell collected from Pago Bay with circular boreholes. **e** Immergentiid in shell of *Fusinus colus* collected from Family Beach. **f** Small immergentiid colony from Sagami area (Japan) with boreholes of ancestrula and two autozooids on either side in gastropod shell. Autozooids directly next to ancestrula extending at 180° angle from ancestrula. **g** Overview of immergentiid colony from Japan. **h** Colony from the Caribbean Sea (Guadeloupe) with circular borehole apertures.

Abbreviations: anc – ancestrula, ap – aperture, az – autozoooid, cy – cystid appendage, pe – *Penetrantia*, tu – tubulets