

RESEARCH ARTICLE

Open Access



Boring life: early colony formation and growth in the endolithic bryozoan genus *Penetrantia* Silén, 1946

Sebastian H. Decker^{1*} , Sarah Lemer² , Simone Decker¹ , Masato Hirose³ , Mildred J. Johnson¹  and Thomas Schwaha¹ 

Abstract

As in most colonial and sessile marine invertebrates, bryozoan life history is characterized by asexual propagation of zooids for colonial growth and by sexual production of larvae for dispersal. However, comprehensive life histories, particularly in cryptic species such as endolithic (boring) bryozoans, remain poorly understood. The ctenostome family Penetrantiidae is widespread from temperate to tropical waters and often found in molluscan shells, offering an opportunity to study the boring lifestyle and its potential impact on bioerosion through growth and settlement experiments. Our research focused on *Penetrantia clionoides* from Guam in the Pacific Ocean, *Penetrantia japonica* from Japan, and a *Penetrantia* species from France in the Atlantic Ocean. We found distinct life histories and reproductive patterns potentially influenced by environmental factors such as temperature and food availability. The tropical *P. clionoides* displayed higher rates of larval production and growth compared to its temperate counterpart. For instance, the mean stolon extension was 335.2 $\mu\text{m}/\text{week}$ in *P. clionoides* versus 232.1 $\mu\text{m}/\text{week}$ in *Penetrantia* sp. Autozooid development took 13 days in *P. clionoides* and 31 days in *Penetrantia* sp. Anatomical features like apertural rims aided in species identification and in understanding larval settlement preferences, suggesting a tendency for philopatric settlement behavior. The bioerosional impact of penetrantiids remains little understood, but we generated first projections of bioerosion rates and a protocol for keeping *Penetrantia* under laboratory conditions, laying a foundation for further research in this field.

Keywords Boring bryozoans, Stolonate ctenostomes, Growth rates, Brooding, Bioerosion

Background

The life history of colonial and sessile animals is often complex and characterized by asexual, clonal reproduction for colony growth and habitat colonization, and sexual production of mobile larvae for dispersal [1–4]. That also applies to the colonial lophotrochozoan phylum Bryozoa, which comprises about 6,000 extant species [3, 5]. The majority of all bryozoans live in marine environments, with more than 5,500 species belonging to the clade Gymnolaemata [5]. This group is further subdivided into the calcifying Cheilostomata and the uncalcified and paraphyletic “Ctenostomata” [6, 7], with the

*Correspondence:

Sebastian H. Decker

sebastian.decker@univie.ac.at

¹Department of Evolutionary Biology, University of Vienna, Schlachthausgasse 43, Vienna 1030, Austria

²Marine Laboratory, UOG Station, Mangilao Guam 96923, USA

³School of Marine Biosciences, Kitasato University, Kitasato, Minami-ku, Sagami-hara City, Kanagawa 252-0373, Japan



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

latter group displaying a high morphological plasticity including many cryptic members with highly specialized lifestyles, e.g., endolithic forms [8]. Endolithic bryozoans are often referred to as boring bryozoans, since they are capable to penetrate into hard substrates made of biogenic carbonate. All endolithic bryozoans apply a chemical mechanism to dissolve calcium carbonate, lacking specialized gnawing organs for a mechanical excavation method [9, 10]. Four Recent families of ctenostome bryozoans (Terebriporidae, Spathiporidae, Immergentiidae, and Penetrantiidae) have evolved such a boring lifestyle [8, 9]. The family Penetrantiidae is monogeneric, including 11 extant species, which occur in temperate or tropical waters and most commonly found in the shells of mollusks [9, 11].

Similarly, to other bryozoans, boring bryozoan colonies are a mosaic of genetically identical units called zooids. Individual zooids are compartmentalized into two main structural parts, the cystid and the polypide [7, 12]. The cystid largely represents the body wall while the polypide is placed inside the protective cystid and includes major organs like the digestive system and the lophophore [7, 12]. The lophophore, shaped like a crown of tentacles, is the filter-feeding apparatus in bryozoans and the only part protruding into the water column in boring bryozoans, while the rest of the zooid is completely immersed within calcareous substrates [7, 8, 12–14]. Bryozoan colony growth results from asexual budding of new zooids, creating encrusting or erect colonies on predominantly hard substrates [3]. Many bryozoans are known to produce non-feeding polymorphic zooids with different functions in a colony and are referred to as heterozooids, while feeding zooids are called autozooids [12, 15]. In Penetrantiidae, the stolonial network is composed of polymorphic kenozooids, which play a crucial role in colony growth by extending the colony horizontally to the surface of the substrate and producing autozooids or additional stolons at regular intervals, effectively interconnecting the entire colony [8, 11]. The stolons in endolithic bryozoans feature unique tubulets, which are small tubes that extend towards the substrate surface and create small pores (about 2 μm) within it. They might act as spacers to ensure the colony is placed at a constant depth within the substrate, but their exact function remains unclear [9, 11]. In Penetrantiids, each zooid is connected by a short peduncle to its stolonial network, which is part of the zooid until a septum marks the transition into the corresponding stolon [11, 13]. Penetrantiid gonozooids are specialized polymorphs for reproduction with unique brood chambers for embryo incubation [11, 14]. Since the gonozooids in most species lack a functional polypide including a digestive tract, they are probably not capable of feeding and thereby considered true heterozooids [9, 11, 14]. Penetrantiids are considered colonial

hermaphrodites, in which some autozooids function as males and gonozooids as females [11, 16]. After fertilization, a single zygote is transferred from the zooidal tube of a gonozooid into its brood chamber and develops into a short-lived lecithotrophic larva of the coronate type [11]. During their short pelagic stage, bryozoan larvae scan substrates for a suitable place to settle and metamorphose into an ancestrula, which is the founder zooid of each colony and marks the onset of asexual zooid propagation for colony growth [3, 17].

Since boring bryozoans have to dissolve their substrate during settlement and colony growth, they contribute to bioerosion and are considered internal microborers [18, 19]. The role of boring bryozoans in bioerosion processes remains a mystery, much like the lack of information regarding their general life history, including growth rates, reproduction, and larval behavior [19].

To date, the complete life history of only a few bryozoan species has been investigated in detail and they showed highly diverse reproductive patterns, colony growth forms and larval preferences, often adapted to specific substrate types [3, 17, 20–24].

Observations of live penetrantiids are essential for filling the knowledge gap regarding this peculiar lifestyle in bryozoans. We conducted one of the first growth and settlement experiments on endolithic bryozoans, comparing two different penetrantiid species from distinct geographic and bathymetric regimes. To test whether different environmental parameters affect reproduction and colony growth rates, we selected the tropical *Penetrantia clionoides* from Guam in the Pacific Ocean and the temperate *Penetrantia* sp. from Roscoff, France, in the North Atlantic Ocean as study organisms. Additionally, we gained new insights to colony formation and larval recruitment patterns of the recently described *Penetrantia japonica* from Japan.

Methods

Sample collection

Three different penetrantiid species were investigated for this study. Live specimens of *Penetrantia clionoides* were collected by hand in the intertidal zone of Pago Bay, Guam (13°25.655'N 144°47.890'E) in November and December 2022. Live specimens of *Penetrantia* sp. were collected by dredging around Stolvezen close to Roscoff, France (48°42.847'N 3°53.5'W) in August to September 2021 and additional specimens in December 2022 and March 2023 for further investigations in Vienna. Specimens of *Penetrantia japonica* were only used for histological investigation and were collected by hand in the intertidal zone of Sagami Bay, Japan (35°13.336'N 139°36.152'E) in August 2023. Specimens were fixed either in 96% ethanol or 2% glutaraldehyde and stored at 4 °C until further investigations.

Experimental setup

Colony growth and settlement experiments were carried out only with *P. clionoides* from Guam and *Penetrantia* sp. from Roscoff, France. Experiments on live *P. clionoides* were conducted at the Marine Laboratory of the University of Guam in Mangilao, Guam.

Experiments on live *Penetrantia* sp. were conducted at the Station Biologique de Roscoff in Roscoff, France. To document growth rates of mature colonies, shells with live colonies were selected and kept in large seawater tanks (225 L in Guam, 25 L in Roscoff) with constant exchange of unfiltered seawater for four weeks. Colonies of *P. clionoides* were in alive shells of the gastropod *Drupa morum*. The colony of *Penetrantia* sp. was in a dead shell of the bivalve *Anomia ephippium*. Translucent shell parts were reduced in size into smaller fragments to facilitate better documentation. In total, two colonies of *P. clionoides* and one colony of *Penetrantia* sp. were considered suitable for documentation and the corresponding shell fragments were placed in Petri dishes (diameter 3 cm) and fixed with the Coralscaper gel MICROBE-LIFT (Ecological Laboratories, Inc., Cape Coral, Florida, USA) to ensure daily photo documentation was performed consistently (Fig. S1). Colonies of *Penetrantia* sp. from France were fed daily with a 10 ml mixture of the microalgae *Tisochrysis lutea* and the diatom *Chaetoceros calcitrans*, since the station in Roscoff had a suitable culture of microalgae available. Water temperature was monitored constantly and fluctuated between 28 and 30 °C in the Guam setup (November and December 2022) and between 14 and 18 °C in the Roscoff setup (September - October 2021).

For settlement experiments, 10 pristine and translucent shell fragments (*A. ephippium* or *D. morum*) with no boring traces were placed in small containers within the sea water tanks. Ten shell fragments with mature colonies were selected and placed among the pristine shell fragments for four weeks. Every second day, the pristine shell fragments were checked for any signs of settled larvae. If a shell fragment was colonized by a larva it was glued into a Petri dish (diameter 3 cm) and its growth documented daily.

Documentation and imaging

Stereomicroscopic images of live colonies were obtained with a Nikon SMZ stereomicroscope (Nikon, Tokyo, Japan) combined with a Nikon Z6 mirrorless camera. Images of fixed specimens were taken either with a Nikon SMZ25 stereomicroscope using a DsRi2 microscope camera, or with a Hirox RH-2000 3D digital microscope (Hirox Co., Ltd., Tokyo, Japan).

Scanning electron microscopic imaging and element analysis were carried out with dry samples of *P. clionoides* and *P. japonica* using a JEOL IT 300 (JEOL, Akishima,

Tokyo, Japan) scanning electron microscope with either a secondary or backscattered electron detector at 10–25 KeV. For imaging, samples were gold sputtered for 120 s with a JEOL JFC-2300 h sputter coater while samples for element analysis were left uncoated.

The shell piece containing *Colony 1* of *P. clionoides* which was observed during the growth experiments for four weeks, was fixed in 2% glutaraldehyde and used for computer tomographic (CT) scans. First, it was bleached and then dried in an ethanol series. A Bruker SkyScan 1272 (Bruker, Billerica, Massachusetts, USA) was used to obtain micro-CT scans. An overview scan was obtained at a voltage of 100 kV and reconstructed with 11.9 µm voxel size, followed by detailed scans at a voltage of 100 kV and 3.9 µm voxel size. The reconstructed tomographic images were further processed in Amira v. 2020.2 (FEI, Oregon, USA). Shell and boring traces were segmented and visualized as surface renderings. The volume eroded by *Colony 1* of *P. clionoides* within the four weeks of observation was calculated using the label analysis tool within Amira.

Data analyses

For *P. clionoides* and *Penetrantia* sp., stolon growth was measured weekly based on stereomicroscopic images using Photoshop (Adobe Inc., San Jose, CA, USA). The weekly mean was calculated for each individual stolon, for all stolons within one colony, and for all stolons within one species. Daily mean is based on the total length extension of the corresponding stolon within the observed time. Stolon growth of ancestrulae was not included in the overall mean of the corresponding species.

To estimate reproductive rates, 10 shell fragments with mature colonies were collected within a single month and decalcified in 20% ethylenediaminetetraacetic acid (EDTA). In extracted colonies, zooids were counted in a defined area of 0.1 cm² and the ratio between autozooids, gonozooids and brooding gonozooids was calculated.

Results

Reproductive patterns

In 10 colonies of *Penetrantia clionoides* collected in November and December 2022, gonozooids with brooding embryos were always present. For one colony the ratio of brooding gonozooids and autozooids was estimated: in an area of 0.1 cm² the colony contained 108 zooids: 72 autozooids and 36 gonozooids, 30 of 36 gonozooids were brooding an embryo which equals 27% of all zooids in the examined area (Fig. 1A). All autozooids contained several brown bodies, indicating many polypide regeneration cycles, while not a single gonozooid contained a brown body (Fig. 1A–C).

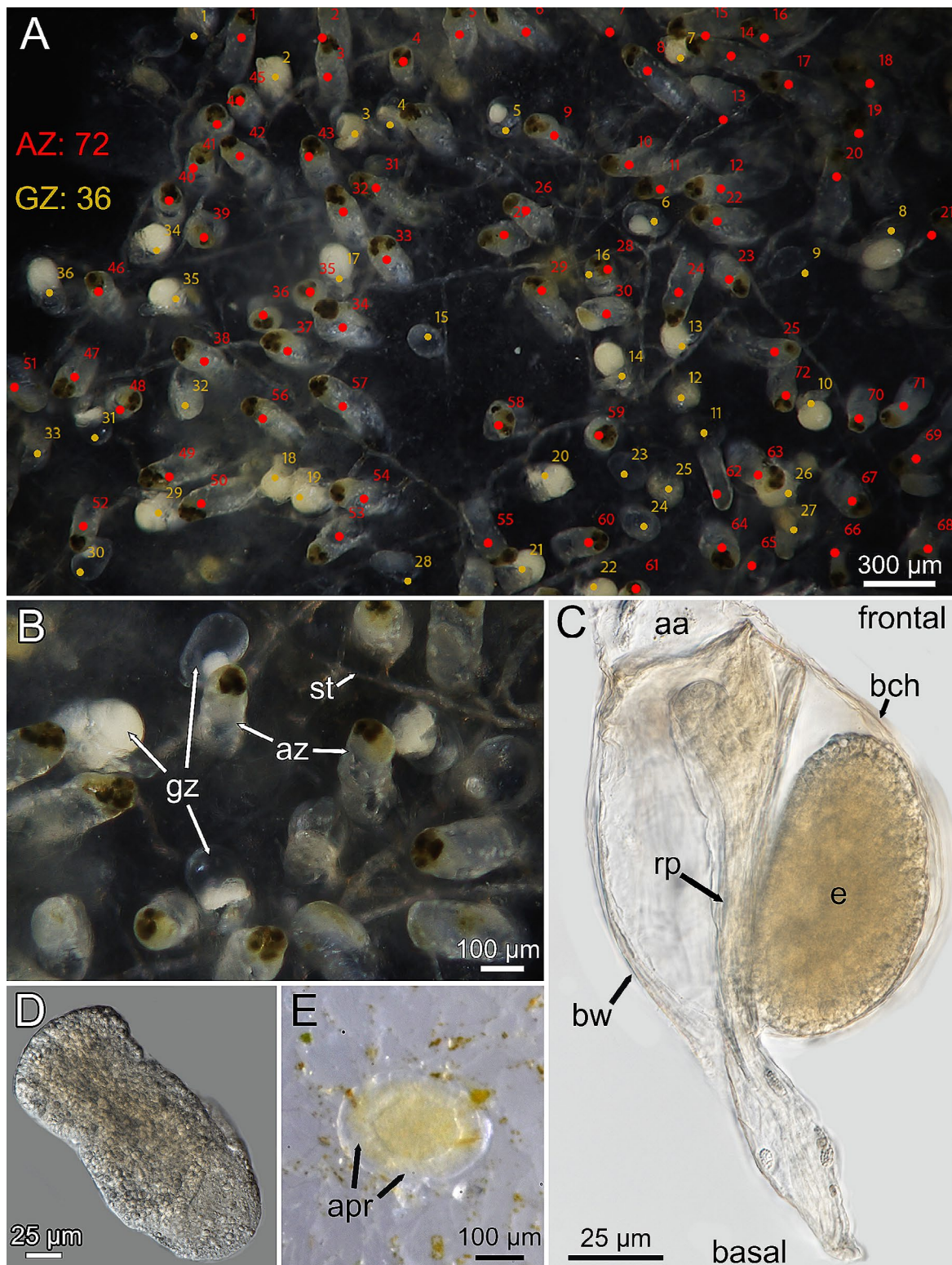


Fig. 1 Reproduction in *Penetrantia clionoides* from Guam. **A** Overview image of a decalcified colony within the area of 0.1 cm², including count of autozooids (AZ) in red and gonozooid (GZ) in yellow. **B** Close-up of autozooids and brooding gonozooids with embryos of same colony shown in A. **C** Microscopic images of a gonozooid from a lateral perspective. **D** Extracted embryo. **E** Preancestrula two days post settling. *Abbreviations* aa – apertural area, apr – apertural rim, az – autozooid, bha – bore hole aperture, bch – brood chamber, bw – body wall, e – embryo, gz – gonozooid, rp – reduced polypide, st – stolon

In contrast, 10 colonies of *Penetrantia* sp. from France collected in August and September 2022, contained no brooding embryos. In March 2023, two of 10 colonies contained one brooding gonozooid. For one colony the ratio of brooding gonozooids and autozooids was estimated: in an area of 0.1 cm² the colony contained 53 zooids: 52 autozooids and one brooding gonozooid, which equals 1.9%.

Larval settlement and preferences

Neither free-swimming larvae nor the actual settlement of larvae were observed in this study and thereby our data corresponds to the recruitment pattern of successfully settled larvae that have already metamorphosed. Based on the appearance of two new preancestrulae after only a day from the start of the settlement experiment with *P. clionoides*, it is clear that the free-swimming phase is short and that larvae settle immediately or shortly after release (Fig. 1D, E). In *P. clionoides* and *Penetrantia japonica* larvae tend to settle in close proximity to each other and to the parent colony if more space is available (Fig. 2A–C). In *P. japonica* most ancestrulae were observed within shell sutures (gap between shell whorls), as in the gastropod *Tegula rugata* (Fig. 2A–D). In *P. clionoides*, the preancestrula is elliptical with flat edges that rise towards the center and measures approximately 250 µm in length, 185 µm in width and 15 µm in height ($n=6$) (Fig. 1E). It has a translucent margin while most internal tissue is accumulated in the center as a yellow mass, which corresponds to the area below the ancestrula where it will start to dissolve the substrate (Fig. 1E).

Ancestrula and early colony formation

All ancestrulae in *P. clionoides* and *P. japonica* had distinct apertural rims surrounding the borehole apertures. The rims were present only in ancestrulae and not in any other zooids (Figs. 2E and 3A–D). These rims were partially composed of calcium carbonate (Files S2, S3).

Actual ancestrula formation and metamorphosis were observed for three ancestrulae of *P. clionoides* only. After establishment of the preancestrula, it took 10 days until the aperture broke through, creating a hole in the center, while the rest of the cuticle around the borehole aperture formed an elliptical donut-shaped apertural rim, about 70 µm wide (Figs. 2E and 3A–D and F). There is a gap between the apertural rim and the substrate in *P. clionoides* and *P. japonica*, which becomes more evident when the apertural rim is eroded in older ancestrulae (Figs. 2D and E and 3A, B and D). After 12 days the operculum started to move and eventually the lophophore was able to protrude (Fig. 3C, Movie 1). Within these 12 days, the preancestrula transformed into a mature ancestrula and bored itself into the substrate approximately 300 µm deep (Fig. 3A–C). Simultaneously, the peduncle and the

initial stolon developed and were already 300 µm long at the time of first lophophore protrusion (Fig. 3C).

Such detailed information about larval settlement and metamorphosis are missing for *Penetrantia* sp. from France as this species was not spawning in August to October 2022 nor in March 2023. However, two established ancestrulae were encountered in March 2023 (Fig. 3E, F). Ancestrulae in *Penetrantia* sp. lack apertural rims and do not differ from other zooids in external characters (Fig. 3E, F, H).

The orientation of the ancestrula to its initial principal stolon differs in *P. clionoides* and *Penetrantia* sp. (Fig. 3G, H). The peduncle forming the initial stolon developed on the anal side of *P. clionoides* ancestrulae (Fig. 3G) but on the lateral side in *Penetrantia* sp. (Fig. 3H). The initial stolon grew about 300 µm in *P. clionoides* ($n3$) (Fig. 3D, G) and 600 µm in *Penetrantia* sp. ($n2$) (Fig. 3F, H), before two additional stolons emerged on both lateral sides of their initial stolons. These three ancestrular stolons create a triradiate arrangement, radiating in different directions giving the ancestrula complex a distinct appearance (Fig. 3D, F, G, H). The angle between the central initial stolon and the lateral ones was about 120° in *P. clionoides* ($n3$) and 100° in *Penetrantia* sp. ($n2$) (Fig. 3G, H). In the latter species the first bud started to develop on the initial stolon before the additional lateral stolons emerged (Fig. 3E, H), while in *P. clionoides* the first pair of additional stolons developed before the first bud (Fig. 3D, G).

Colonial growth and growth rates

Stolon growth in *P. clionoides* and *Penetrantia* sp. followed the same pattern. While the principal stolon continued to grow in length, it produced either one autozooidal bud or a pair of additional stolon branches on its lateral sides, at consistent intervals of about 200 µm (Figs. 4 and 5C–E). The position of subsequent autozooids mostly alternates between the lateral sides of the principal stolon (left and right) but not regularly (Figs. 4C, E and G and 5C–D). Stolon branches always developed simultaneously on both lateral sides of the principal stolon (Figs. 4D–G and 5C–E). The development of autozooids followed the same pattern in both species and started with a lateral extension of the principal stolon. This extension then further progressed and bent about 90° in the frontal direction until it reached the surface of the substrate (Figs. 4C and D and 5D and E). Once the cystid reached the surface, the bud elongated further downwards in a basal direction until it reached its final size (e.g., bud1 in Figs. 4E and F and 5D and E). At the same time the future borehole aperture began to break through the surface (Figs. 2E, 4E and 5B). The entire developmental sequence into a mature autozooid took 13 days in *P. clionoides* and 31 days in *Penetrantia* sp., from

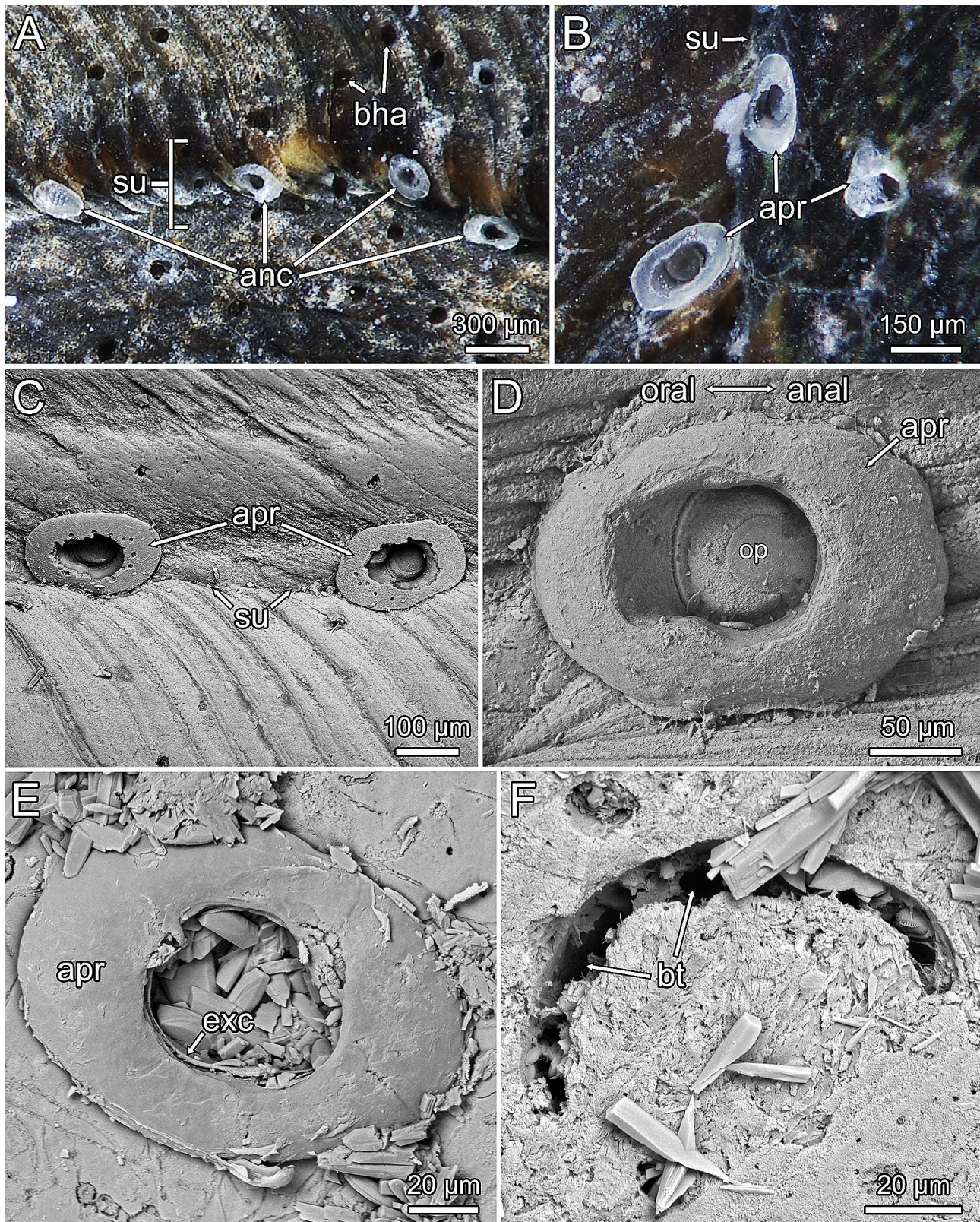


Fig. 2 Borehole apertures with apertural rim in *Penetrantia japonica* from Japan **A–D** and *Penetrantia clionoides* from Guam **E–F**. **A–B** Stereomicroscopic images of *P. japonica* with prominent apertural rims sitting in the sutures of shells of the gastropod *Tegula rugata*. **C–D** Scanning electron microscopic (SEM) images of apertural rims in *P. japonica*. **E** SEM images of the apertural rim in *P. clionoides*. **F** SEM image of developing borehole aperture of an autozoid in *P. clionoides*. Abbreviations anc – ancestrula, apr – apertural rim, bha – bore hole aperture, bt – boring trace, exc – exterior cuticle, op – operculum, su – suture of gastropod shell

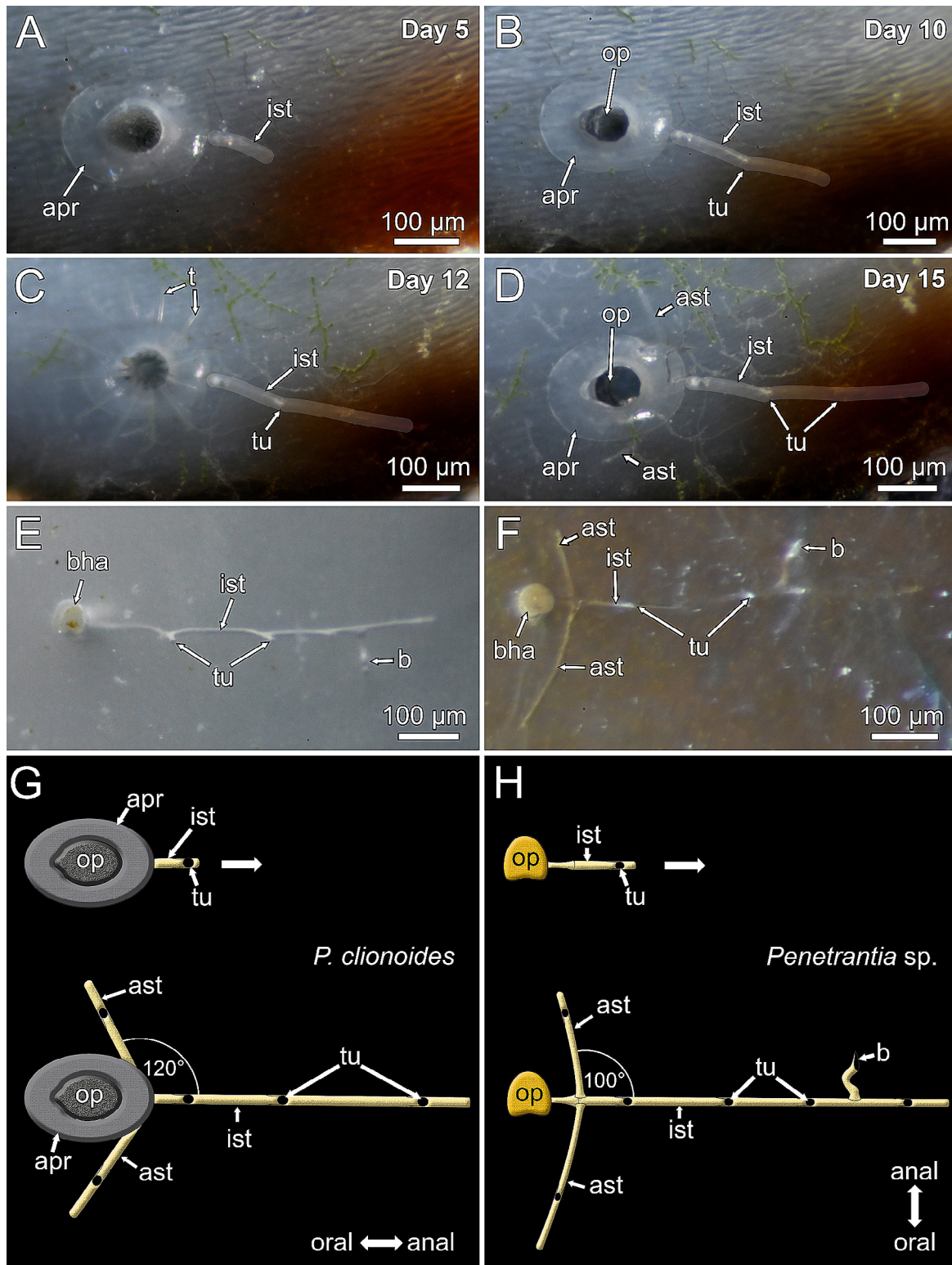


Fig. 3 Stereomicroscopic images and schematic representation of ancestrula formation and colonial growth in *Penetrantia clionoides* from Guam and *Penetrantia* sp. from Roscoff, France. **A–D** Observation of ancestrulae of *P. clionoides* with intervals of days between images indicated. **C** On day 12 ancestrula protruded its lophophore for the first time. **E–F** Two different ancestrulae of *Penetrantia* sp. **G–H** Schematic representation of ancestrula–stolon complexes in *P. clionoides* and *Penetrantia* sp. respectively. Abbreviations apr – apertural rim, ast – additional stolon; b – bud; bha – bore hole aperture, ist – initial stolon, op – operculum, t – tentacle, tu – tubulet

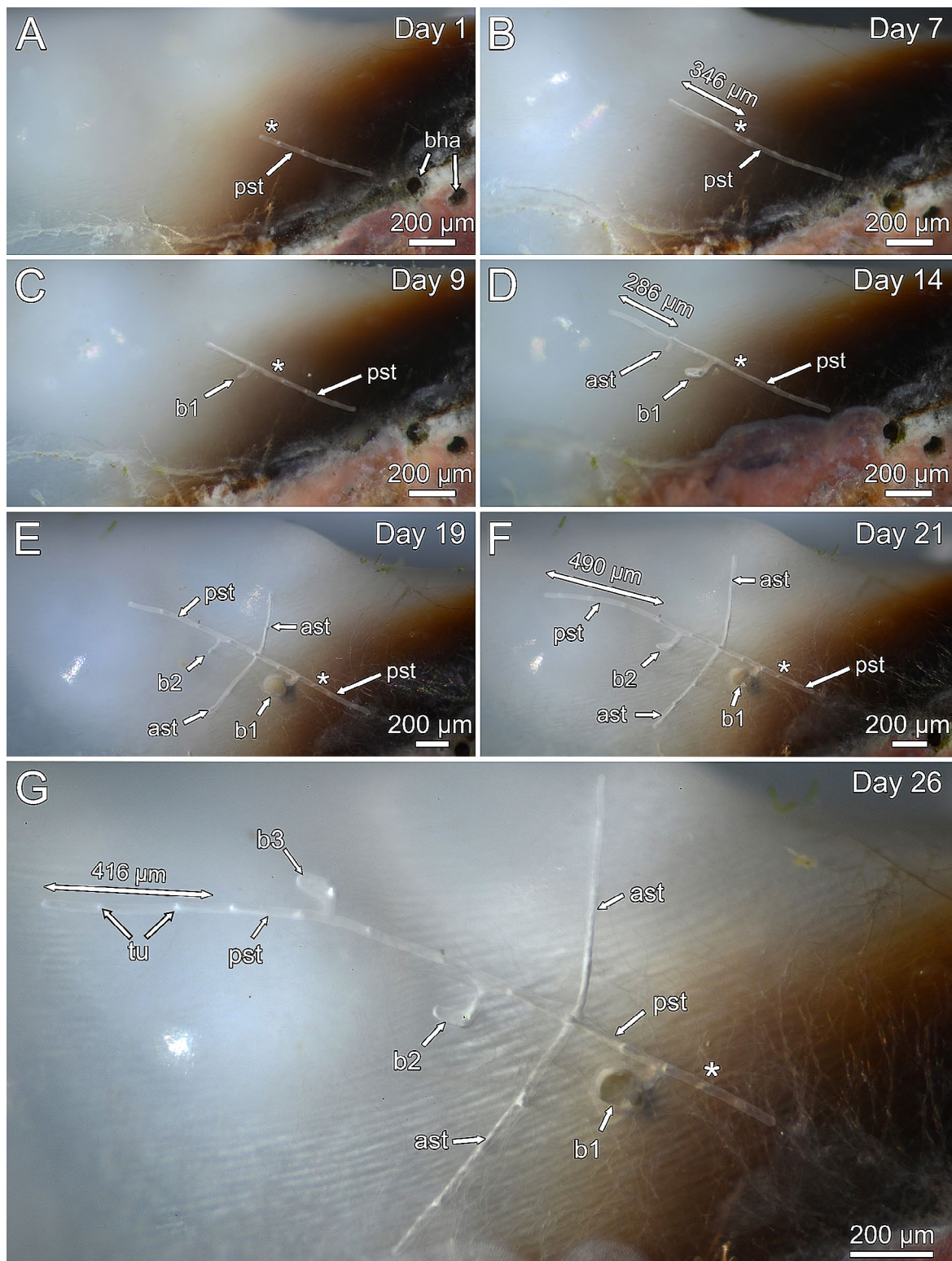


Fig. 4 Colony growth of *Penetrantia clionoides* within 26 days. In its growth, the principal stolon either produces further autozooids or pairs of additional stolons in regular intervals on its lateral sides. **A** Beginning of growth experiment. Asterisk marks the length of principal stolon at the beginning of growth experiment. **B** Stolon extension within week one. **C** First bud emerges. **D** Stolon extension within week two and lateral stolon branch emerges. **E** Second bud emerges. **F** Stolon extension within week three. **G** Final size of colony which is reconstructed in Fig. 5. *Abbreviations* ast – additional stolon, b1 – bud1; b2 – bud2, b3 – bud3, bha – borehole apertures, pst – principal stolon, tu – tubule

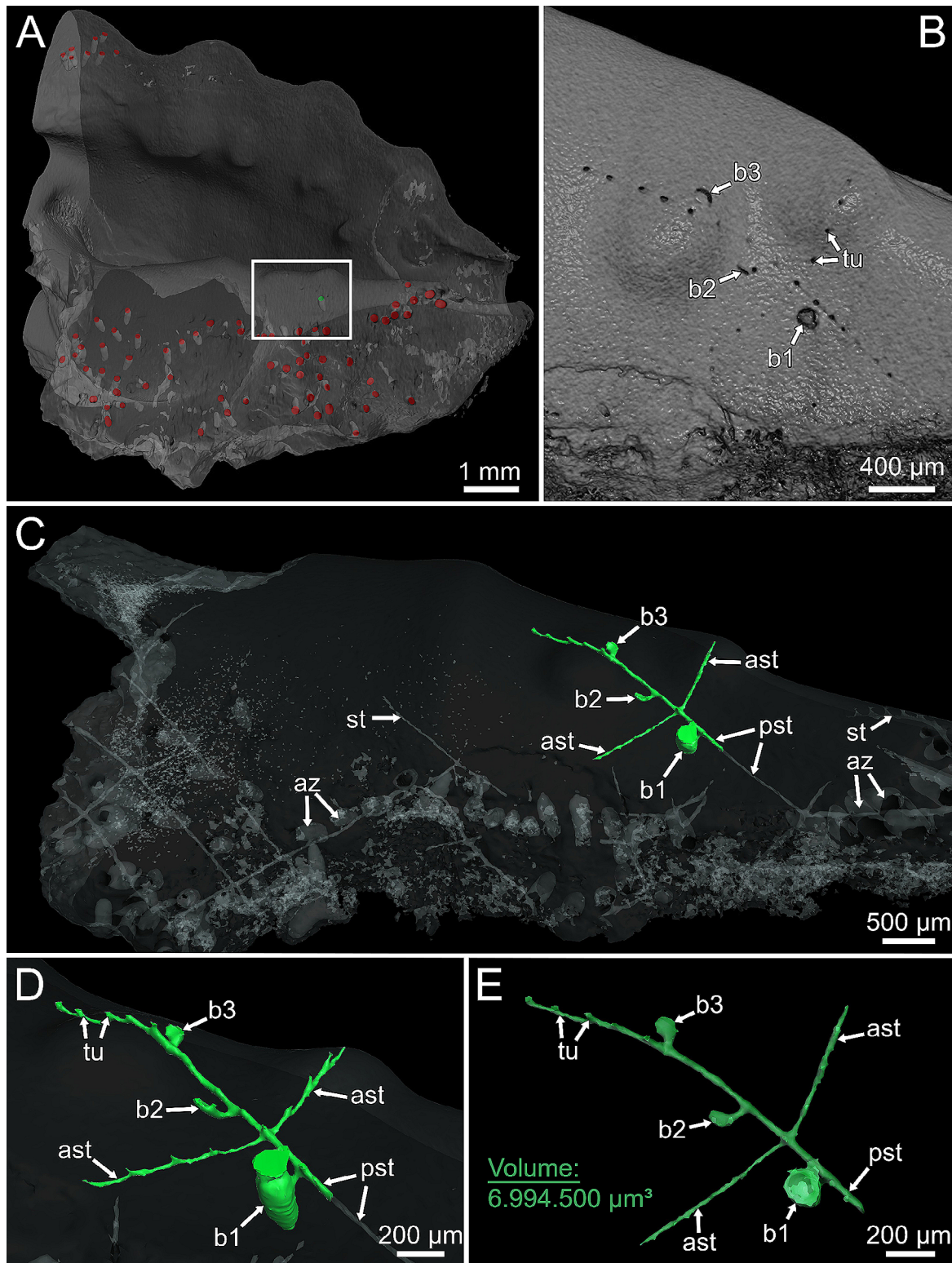


Fig. 5 Micro-CT renderings of shell piece that contains the colony of *P. clionoides* observed during the growth experiment shown in Fig. 4. **A** Overview of bored shell of the gastropod *Drupa morum* with zooids labeled in red and bud1 in green. Rectangle indicates area of detailed scans shown in B–E. **B** Close-up of shell surface with boring traces of tubulets and apertures. **C–E** The colony part that developed during the growth experiment was segmented separately and is marked in green. The volume of the green colony part was calculated with the label analysis tool within the reconstruction software Amira. Abbreviations ast – additional stolon, az – autozooid, b1 – bud1; b2 – bud2, b3 – bud3, pst – principal stolon, st – stolon, tu – tubulet

the first bud anlage to the first protrusion of the lophophore (Figs. 4 and 5C–E; Movie 2, 3).

Likewise, stolonal growth was generally faster in *P. clionoides* than in *Penetrantia* sp. In two mature colonies of *P. clionoides* the overall mean of stolonal growth within four weeks was $335.2 \pm 64.3 \mu\text{m}/\text{week}$, $n=14$ (Table 1; Figs. 4 and 5C–E). However, in *Colony 1* the mean growth of the principal stolon was more than 100 μm higher than of the additional lateral stolons (Table 1). In *Colony 2* the difference in the mean growth rates between principal and additional stolons is less distinct but in the principal stolon it was 24.7 μm higher per week (Table 1). The growth rates of individual stolons exhibited fluctuations between weeks. Overall, growth of the principal

stolon in *Colony 1* of *P. clionoides* was the highest in week three (490.2 μm) and the lowest in week two (286.5 μm). In *Colony 2* of *P. clionoides* growth of the principal stolon was the highest in week two (371.6 μm) and the lowest in week one (304.3 μm) (Table 1; Fig. 6). The mean growth rate of the initial stolon of three ancestrulae in *P. clionoides* was $201.3 \pm 132.7 \mu\text{m}/\text{week}$, $n=6$. In two ancestrulae the growth of the initial stolon was much lower in the second week than in the first week (Table 1; Fig. 3A–D).

In a single colony of *Penetrantia* sp. the overall mean stolonal growth rate within four weeks was $232.1 \pm 73.8 \mu\text{m}/\text{week}$, $n=8$, and therefore over 100 μm less than in *P. clionoides* (Table 1). Overall, the growth rate of the principal stolon in *Colony 1* of *Penetrantia* sp.

Table 1 Colony growth rates of *Penetrantia clionoides* from Guam and *Penetrantia* sp. from Roscoff, France. Growth rates measured in length (μm) of stolon extension. Growth rates of ancestrulae correspond to the extension of their initial stolon

<i>P. clionoides</i> <i>Colony 1</i>	Week				Total	Mean			
	1	2	3	4		Week	\pm SD	n	Day
pst	346.5	286.5	490.2	416.3	1539.5	384.9	88.0	4	55.0
ast1	-	-	351.2	281.1	632.3	316.2	49.6	2	45.2
ast2	-	-	316.2	222.2	538.4	269.2	66.5	2	38.5
						338.8	83.9	8	46.2
<i>P. clionoides</i> <i>Colony 2</i>	Week				Total	Mean			
	1	2	3	4		Week	\pm SD	n	Day
pst	304.3	371.6	352.6	-	1028.5	342.8	34.7	3	49.0
ast1	339.3	318.9	296.1	-	954.3	318.1	21.6	3	45.4
						330.5	29.2	6	47.2
weekly mean of all stolons of <i>Colony 1</i> and <i>Colony 2</i>						335.2	64.3	14	
<i>P. clionoides</i> Ancestrulae	Week				Total	Mean			
	1	2	3	4		Week	\pm SD	n	Day
a	385.7	81.7	-	-	467.4	233.7	214.9	2	33.4
b	350.5	92.5	-	-	443.0	221.5	182.4	2	31.6
c	155.9	141.8	-	-	297.7	148.9	9.9	2	21.3
						201.3	132.7	6	28.8
<i>Penetrantia</i> sp. <i>Colony 1</i>	Week				Total	Mean			
	1	2	3	4		Week	\pm SD	n	Day
pst	279.0	297.7	262.6	102.3	941.6	235.4	89.9	4	33.6
ast1	266.2	284.9	231.7	132.5	782.8	228.8	67.9	4	32.7
						232.1	73.8	8	33.2

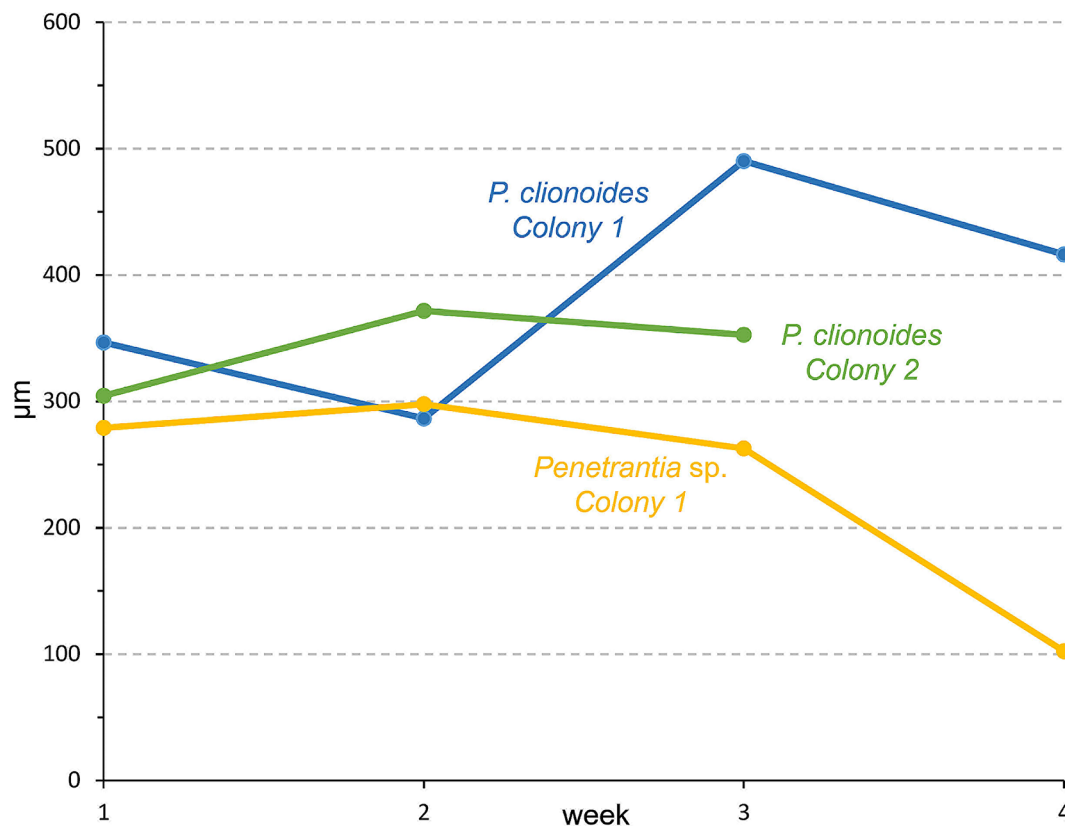


Fig. 6 Weekly growth (length extension) of the principal stolons in three distinct colonies of two different penetrantiid species over a four-week period, based on measurements presented in Table 1. Including Colony 1 (blue) and Colony 2 (green) of *Penetrantia clionoides* from Guam, and Colony 1 (yellow) of *Penetrantia* sp. from France

was the highest in week two (297.7 µm) and about three times lower in week four (102.3 µm) (Table 1; Fig. 6).

In total, the observed colony of *P. clionoides* (Figs. 4 and 5) dissolved a shell volume of 6,994,500 µm³ within 26 days, which equals 0.0197 mg of aragonite or 0.0188 mg of calcite (aragonite density of 2.83 g/cm³, calcite density of 2.71 g/cm³, according to [25]). This translates to 1,883,134 µm³ per week (0.0053 mg aragonite, 0.0051 mg calcite) and 269,019 µm³ per day (0.00076 mg aragonite, 0.00072 mg calcite).

Discussion

Reproductive patterns

Bryozoans demonstrate a large variety of life-histories and reproduction patterns often with seasonal adaptations, correlating with their geographic and bathymetric distribution as well as with their colonial growth and larval type [3, 16, 26, 27]. Penetrantiids are no exception, and the presence of brooding gonozooids with embryos gives valid information about potential seasonal patterns in their reproduction [3, 11]. The number of gonozooids as well as incubating embryos varied drastically among colonies of *Penetrantia clionoides* from Guam and *Penetrantia* sp. from France, with *P. clionoides* demonstrating

a much higher larval production overall. There are many reasons that can potentially alter reproductive patterns often associated with adaptations to seasonal changes in environmental conditions, e.g., temperature, food availability or substrate availability [3, 16, 20, 23]. *Penetrantia clionoides* and *Penetrantia* sp. live in very different environments offering an interesting comparison. While *P. clionoides* thrives in tropical intertidal habitats along the coast of Guam, *Penetrantia* sp. was found in the shallow subtidal zone of the temperate North Atlantic and North Sea [11]. Colonial organisms living in such temperate climate regimes usually show a stronger seasonality of asexual colonial growth and sexual reproduction, which strongly correlates with food availability [3, 20, 24, 26]. The cheilostome *Chartella papyracea*, similar to the penetrantiids, is a non-placental brooder that also occurs in the North Atlantic. Observations demonstrated that *C. papyracea* releases most larvae from autumn to spring, after the summer months when plankton concentration is highest and the colonies have enough nutrition to form gametes and larvae [26]. Since most young ancestrulae of *Penetrantia* sp. were encountered in February, larval release may have occurred in December/January. However, in this case we would expect a higher abundance of

gonozooids in colonies from December. Perhaps, *Penetrantia* sp. is not seasonal and produces larvae, in smaller quantities, but all-year-round instead. In temperate climates, sexual reproduction tends to correlate with colonial growth: species with long-lived colonies (more than one year) tend to produce larvae throughout the year, whereas species with short-lived colonies often show distinct peaks in their reproductive cycle [3, 28]. The latter trend is also present in shallower intertidal species that show more distinct seasonality of growth and reproduction compared to species in deeper waters [3, 20, 28]. *Penetrantia* sp. may follow the reproductive strategy of long-lived colonies with a deeper bathymetric distribution, which is also underlined by lower growth rates than *P. clionoides* (see below).

Colonies of *P. clionoides* were only investigated in November and December and showed a high abundance of brooding gonozooids. Tropical species tend to have less distinct reproductive periods and lower reproductive rates stretched over the entire year [3, 29]. The exceptionally high number of brooding gonozooids in November could either represent a reproductive peak or *P. clionoides* may retain a high larval production throughout the year. Gonozooids of *P. clionoides* are able to brood larvae sequentially [11], which renders a longer reproductive phase plausible but also implies a high constant food uptake [3]. Guam has minimal seasonal changes in seawater temperature, but a distinct dry and wet season with large differences in rainfall [30]. The rainy season lasts from July to November [30], which places our observations on reproduction of *P. clionoides* at the end of this period. Observations on reproduction in the intertidal oyster *Saccostrea cucullata* from Guam showed a pattern with constant production of larvae throughout the year but with three distinct peaks, including one in November [31]. Such a reproductive pattern seems likely for *P. clionoides*, but its validation would require perennial observations. Nonetheless, the high accumulation of brown bodies in autozooids of colonies investigated herein indicates that they passed through several polypide regeneration cycles and probably were several months old [11, 32]. Brown bodies are encapsulated residuals resulting from polypide degeneration and either accumulate within a zooid, roughly indicating the number of polypide regeneration cycles, or are ejected by the subsequent polypide [32, 33]. Interestingly, no brown bodies have been observed in penetrantiid gonozooids, suggesting either that these gonozooids are younger than autozooids or that they do not generate brown bodies at all. Since brown bodies have never been observed in any penetrantiid gonozooid [11, 14], polypide regeneration might not take place in these heterozooids and as a consequence brown bodies do not accumulate in gonozooids [11]. Penetrantiid gonozooids are not capable of feeding

since they have reduced polypides, lacking a digestive tract and in most cases a lophophore, which renders the production of brown bodies unnecessary [3, 11, 14, 32]. However, some bryozoans are capable of expelling or ejecting brown bodies after a polypide degeneration event, but since such a mechanism has never been observed in any penetrantiid and their autozooids commonly have many brown bodies incorporated, such an ejection mechanism is probably absent in penetrantiids [8, 11, 14].

Questions also remain regarding the formation and development of penetrantiid gonozooids. A complete developmental sequence has still not been documented, and in the current study, all investigated gonozooids were already fully developed. Gonozooids in different developmental stages have only been observed in *Penetrantia densa*, suggesting that the brood chamber develops early in the ontogeny of a gonozooid [14]. Since most gonozooids are also noticeably shorter than their corresponding autozooids, they seem to develop independently from autozooids and thereby represent true polymorphic heterozooids [3, 11]. Unlike many ovicells in cheilostomes, the brood chamber of penetrantiid gonozooids does not represent a kenozooid, but rather an outgrowth of the gonozooidal body wall [11, 16, 34]. The entire gonozooid, including its brood chamber, is lined by one continuous body wall and only the brood chamber plug separates the brood chamber from the gonozooidal tube, with no pore plates between [11]. Consequently, the brood chamber represents an outgrowth of the gonozooid and not an individual kenozooid [11, 15, 16, 34]. Altogether, the brood chamber in penetrantiids is not homologous to cheilostome ovicells and represents another example of convergent evolution in this phylum [11, 16, 34].

Larval settlement in *P. clionoides*

Lecithotrophic bryozoan larvae have a short free-swimming phase of only a few hours to a few days to find a suitable substrate for the future colony [3, 16, 17]. Identification of ancestrulae in *P. clionoides*, *Penetrantia japonica* and *Penetrantia bellardiellae* (see below, apertural rims) is a very useful tool to assess recruitment patterns of penetrantiid larvae. Settlement experiments with *P. clionoides* demonstrated that the free-swimming phase of its larvae is very short, maybe only a few hours or minutes, and might explain why so many ancestrulae are found in such close proximity to each other and their parent colony. Ideally, larvae form ancestrulae on uncolonized substrates, with no or little competition for space [3, 17]. Settling on the same substrate as the parent colony might ensure suitable substrate conditions, but will lead to over-colonization, intergrowth of colonies, and in case of endolithic bryozoans, probably to a faster deterioration of the substrate. Bryozoan larvae are known to

follow environmental cues such as light conditions, gravity, temperature or chemical composition of the substrate [3, 35, 36]. After a free-swimming phase, larvae will perform an exploratory behavior, investigating chemical and mechanical properties of the surface of the substrate and its microbial film [3, 17, 37, 38]. Perhaps the presence of conspecifics triggers settlement in *P. clionoides* and *P. japonica*? Such philopatric settlement behavior is known from many bryozoan species and many other sessile colonial animals with short-lived larvae [1, 2]. This strategy might facilitate finding the same or similar environmental conditions, increase colonization rates and presumably also the chance of potential future breeding events but with the added cost of inbreeding [1, 3]. Aggregated settlement of larvae was also documented in the ctenostome *Alcyonidium hirsutum* [39], and sibling larvae of the cheilostome *Bugula neritina* are also known to settle in clusters close together; however, it is not clear if this is due to true kin-recognition or shared settlement preferences of the larvae [40]. We observed that *P. clionoides* brooded many larvae simultaneously, which could lead to mass-releases of larvae and if the substrate of the parent colony has enough space, larvae may settle immediately after release.

Larvae of *P. japonica* showed a strong tendency to settle on or in the sutures of dead gastropod shells occupied (hermited) by hermit crabs. These sutures are slightly grooved and may offer different hydrodynamic characteristics than the remaining shell, which may promote larval settlement and/or increases the survival rate of larvae settled in the sutures in contrast to other shell areas. Similarly, images of *P. bellardiellae* indicate that most ancestrulae were situated in the sutures of its gastropod substrate (see supporting information S1a and S4 in [41]), suggesting that this preference is common among penetrantiid larvae. Similar larval settlement preferences were observed in the ctenostome *Alcyonidium hirsutum*, which showed a strong tendency to settle in concavities of its algal substrate [39, 42]. In contrast, ancestrulae of *P. clionoides* were only encountered close to the aperture of live *Drupa morum* shells, an area devoid of periostracum in live gastropods, which suggests that larval preferences of penetrantiids may correlate with the presence and/or condition of the periostracum [11]. The periostracum is a protective organic layer that covers most of the outer shell in mollusks [43]. The tendency to preferentially settle on shell areas lacking an intact periostracum was already suggested for most penetrantiids [9, 11, 14]. *Penetrantia clionoides* was reported to predominantly bore into gastropod shells that were already occupied by hermit crabs [44]. In older, dead, and hermit shells, the periostracum is usually more worn and degraded [9, 11], allowing penetrantiid larvae access to the calcareous layer more easily [11]. Likewise, *Penetrantia* sp. from

France was found predominantly in dead bivalve shells, again in areas without or degraded periostracum [11]. Periostracum-free areas probably have different mechanical as well as microbial properties which initiate settlement of larvae. Altogether, penetrantiid larvae seem to have two major settlement preferences (1) older shell parts with little or no periostracum (2) close proximity to conspecifics (philopatry) and/or siblings.

Once larvae find a suitable place for settlement a connection between larva and substrate is established, a process that is similar in most investigated gymnolaemate bryozoans (e.g., *Amathia gracilis*, *Bugula neritina*) [3, 17]. This process starts with eversion of the larval internal sac which incorporates secretory glands and effectively glues the larva to the substrate [3, 45]. During this attachment process the larva flattens and becomes a preancestrula which also marks the onset of the “catastrophic” metamorphosis where all the larval organs are rearranged [3, 17]. The same holds true at least for *P. clionoides* in which the yellow area in the center of the preancestrula resembles the area where the boring process takes place during the expansion of the future cystid. The outer more translucent margin corresponds to the cystid epithelium similar to preancestrulae found in the cheilostome *Watersipora arcuata* [46]. However, further observations of preancestrulae and ancestrulae in different developmental stages are necessary to verify this hypothesis.

Ancestrula and early colony formation

The formation of the preancestrula takes only a few minutes, whereas the development into a mature ancestrula takes several days to weeks depending on the species [17, 46]. In most gymnolaemate ancestrulae a functional polypide develops within 2–4 days [20]. In the epiphytic ctenostome *Pherusella minima*, ancestrula formation takes 7–10 days [47], and in the stolonate ctenostome *Amathia gracilis*, five days [48]. In *P. clionoides*, ancestrula formation took considerably longer (12 days), possibly a result of the boring process of penetrantiid ancestrulae.

The apertural rim is a useful external character to identify the ancestrula of a colony which can give important information about recruitment patterns of larvae and colony growth.

These peculiar rims around some borehole apertures have been reported previously in four species (*Penetrantia densa*, *P. clionoides*, *P. bellardiellae* and *P. japonica*) but have never been associated with ancestrulae [14, 41, 49, 50]. The apertural rim was mentioned in *P. clionoides* as a calcareous margin around some borehole apertures and suggested to pertain to zooids undergoing polypide regeneration, with sealing of their apertures (see Fig. 4 in [49]). This idea cannot be verified, since no autozooid of

P. clionoides was observed to have an apertural rim in the current study, despite evidence of several polypide regeneration cycles, evidenced by the number of brown bodies [32]. A similar apertural rim was also reported in *P. bellardiellae* from Papua New Guinea [41]. Images of the aforementioned species (see supporting information S1, S2 and S3 in [41]) show a few zooids with apertural rims that look strikingly similar to the apertural rims found in *P. clionoides*. They have the same overall size and shape (donut-shaped), are only present around a few zooids, are partially composed of calcium carbonate, and there is a gap between apertural rim and substrate [41]. Consequently, we consider zooids with an apertural rim in *P. bellardiellae* as ancestrulae too. The two species also share other characters such as distinct opercular features: composed of calcium carbonate, and with a crescent-shaped rough patch [11, 41]. Recently, zooids of *P. japonica* were also reported to have apertural rims [50] and this study confirms that they are also an ancestrular feature. The fourth species with calcareous apertural rims is *P. densa* from South Africa. However, these rims represent a narrow margin between the borehole and the surrounding substrate and it is indicated that autozooids also have such rims (see Fig. 56 in [14]). Consequently, the apertural rim in *P. densa* probably is not an ancestrular feature. Nevertheless, reinvestigation of *P. densa* is required to support this notion.

The apertural rim may represent the former larval cuticle or the pallial epithelium of the preancestrula [46], since the actual ancestrular cystid is formed below the preancestrula. This was evident in a few ancestrulae of *P. clionoides* where the exterior cuticle of the cystid is clearly separated from the apertural rim. Additionally, preancestrulae already have the same size as the future apertural rim, while the formation of the ancestrular cystid is ongoing [3, 47]. The incorporation of calcium carbonate into the body wall is atypical for ctenostome bryozoans, but since, *P. clionoides*, *P. japonica* and *P. bellardiellae* also have calcified opercula, they might have the capability of biomineralization [11, 41]. However, whether true biomineralization or reuse and remolding of calcium carbonate released during the boring process occurs still needs to be validated. Nonetheless, the preancestrula/early ancestrula is already calcified shortly after settlement, indicating independent biomineralization. The reason why only some penetrantiids form apertural rims and others do not remains unknown, but we observed that *P. clionoides*, *P. japonica*, and *P. bellardiellae* occur exclusively in the intertidal zone and the calcification of their opercula might be an adaptation to this environment and potentially helps to better seal the aperture during low tide [41, 49, 50].

Another common feature of all investigated penetrantiid ancestrulae is the early stolonial pattern with three

stolons radiating in different directions to create a triradiate arrangement [9, 11], whereas autozooids are commonly associated with a single stolon [9, 11, 14]. The peduncle and initial stolon develop simultaneously at the ancestrular zooid in all investigated penetrantiids, hence the term ancestrula–stolon complex, as the ancestrula effectively consists of two zooids: the feeding ancestrula and its first kenozooidal stolon. A similar pattern with the stolon forming already in the preancestrula was observed in the ctenostome *Amathia gracilis* [48]. The precise timing of stolon development in *P. clionoides* could not be determined, but the initial stolon was present before first lophophore protrusion. Although the overall outline is similar, there are differences between the ancestrula–stolon complexes in *P. clionoides* and *Penetrantia* sp. from France. Most obvious are the different budding sites of the peduncle and initial stolon (anal in *P. clionoides* and lateral in *Penetrantia* sp.) and the delayed development of the first pair of additional stolons in *Penetrantia* sp. However, in both species the first pair of additional stolons are not part of the ancestrula–stolon complex as they develop after the ancestrula and initial stolon are established. The angle between the central initial stolon and the first pair of additional stolons was consistent in all investigated specimens. Whether this serves as a reliable species character needs detailed investigations of other penetrantiids.

Colonial growth and growth rates

The overall growth pattern of *P. clionoides* and *Penetrantia* sp. corresponds well to typical penetrantiid colony forms, where stolon branches and zooids develop on the lateral sides of a principal stolon, eventually leading to strongly ramified and feather-shaped colonies [9, 11, 14]. However, this pattern becomes complicated as penetrantiid ancestrulae were often observed in close proximity (see above), suggesting that one large ‘colony’ can probably be the result of several intertwined individual colonies.

Autozooid development is similar in *P. clionoides* and *Penetrantia* sp. and follows the same sequence as described for *P. densa* [14]. Consequently, this process seems to be uniform among Penetrantiidae. Gonozooids are considered to develop separately and independently from autozooids (see above) [11, 14].

Data on growth rates of bryozoans are generally rare and even more so for endolithic organisms, including boring bryozoans. Most bryozoan growth rates have been estimated for species with larger colonies and show great variations across different species but also between colonies of the same species from different localities [3, 51–53]. Allowing for the large diversity in growth forms and colony structures, there are many different ways to measure growth, e.g., colony diameter, branch length,

surface area or zooid number, making direct comparisons ambiguous [51, 52, 54, 55]. In the case of encrusting cheilostomes, the linear extension of their colonies varies greatly across different species, with an estimated mean of 3.2 mm/year [52]. Growth data on ctenostome colonies is mostly restricted to a few epiphytic and encrusting species in temperate waters, e.g., *Alcyonidium hirsutum* and *Flustrellidra hispida* [21, 39, 56]. However, these growth rates can hardly be compared to stolonate ctenostomes, where colony growth is mostly reflected in proliferation and extension of their stolons while autozooid density is much lower [8, 57]. Consequently, we measured the change in stolon length in our analysis. The mean growth rate of stolons in *P. clionoides* (335.2 $\mu\text{m}/\text{week}$ which translates to 18.3 mm/year) was much higher than the mean extension of encrusting cheilostome colonies (3.2 mm/year) [52]. However, stolon extension is expected to be faster than colony extension by autozooidal budding, since there is no polypide development involved in stolon growth, highlighting the incompatibility of these growth rates. Although stolon ontogeny and colony growth pattern have been documented for several stolonate ctenostomes, growth rates were not documented [8, 57, 58].

Distinct differences are present in stolon growth and autozooid development between *P. clionoides* and *Penetrantia* sp. The tropical *P. clionoides* displayed much higher growth rates than the temperate *Penetrantia* sp., which most likely correlates to the warmer water temperatures in Guam (Guam: 28–30 °C; France, Roscoff: 14–18 °C). Temperature, food availability, and colony size, are all known to have large effects on bryozoan growth rates, which is also reflected in lower growth rates of most bryozoans from higher latitudes [3, 20, 52, 59–61]. Food supply and availability may also be a major factor in our analysis. In both cases, the unfiltered-seawater was taken from the intertidal zone, but *Penetrantia* sp. inhabits the subtidal and therefore food composition might not have matched its species-specific preferences, though colonies were supplied with a culture mixture. These presumably suboptimal conditions might also be the reason why growth rates in *Penetrantia* sp. further decreased during the experiment, while *P. clionoides* showed more constant growth rates that even increased in the case of two stolon branches. Growth experiments on the cheilostome *Membranipora membranacea* showed significantly lower growth rates of colonies kept under laboratory conditions compared to colonies observed in the field, which indicates that reproducing optimal conditions in the laboratory remains difficult [60]. Growth rates in this species are also affected by colony size, with larger colonies growing exponentially faster [60]. This might explain why stolons of ancestrulae in *P. clionoides* had lower growth rates than mature colonies. Consequently,

colonial growth rates in *P. clionoides* probably correlate with the number of feeding autozooids. However, many species are also known to reach a peak in growth at a certain colony size—or, in some cases, growth even decelerates [62]—often correlating with reproductive phases when colonies invest more energy into larval production [63]. Differences in growth rates may also be influenced by varying decalcification rates, attributed to the nature of substrates inhabited by *P. clionoides* and *Penetrantia* sp. While the tropical *P. clionoides* inhabits live gastropod shells, the temperate *Penetrantia* sp. inhabits deceased bivalve shells. This distinction suggests varying mineralogical compositions (calcite vs. aragonite) and organic content levels between the two substrates. There is a clear latitudinal gradient in molluscan shell mineralogy, with species from higher latitudes generally exhibiting a higher amount of calcite in their shells, while species from lower latitudes tend to have a higher amount of aragonite [64, 65]. Shells with a higher calcite content are typically more robust and less soluble [64], which could potentially explain the lower growth and decalcification rates observed in the temperate *Penetrantia* sp. However, bivalve shells from the Arctic Ocean were recently discovered to be primarily composed of aragonite, contradicting the previously observed latitudinal gradient [66]. Additionally, the bivalve shell was already deceased and more porous than the live tropical gastropod shell, which should have increased the growth rates of *Penetrantia* sp. Consequently, the shell composition of the temperate bivalve *Anomia ephippium*, as well as its older (dead) state, should have favored faster growth of *Penetrantia* sp. from France. However, the opposite was observed, suggesting that other environmental parameters such as temperature and food availability had a larger impact in this case. In general, substrate properties are expected to have a significant impact on the decalcification rates of boring bryozoans, especially between shells composed entirely of calcite versus those composed solely of aragonite.

We observed a similar trend in the duration of autozooid formation by asexual budding as for stolon growth, which was faster in *P. clionoides* than in *Penetrantia* sp. (13 days vs. 31 days, respectively). These differences most likely correlate with the stolon growth rates and are underlined by the same environmental parameters as mentioned before (temperature, food availability, colony size, and shell mineralogy) [39, 56, 60, 61]. In general, asexual autozooid formation in both penetrantiid species seems to be slower than in most epibenthic bryozoans including other ctenostomes [21, 39, 52, 56]. This indicates that the endolithic lifestyle requires a longer developmental period of autozooids, as they have to chemically dissolve the substrate during their growth. Growth rates of other endolithic organisms are little

documented and often restricted to larger macroborers (e.g. sponges, polychaetes) that mostly use a combination of chemical and mechanical mechanisms [19]. Bioerosion rates are about five times higher in the tropical boring sponge *Cliona orientalis* than in its temperate counterpart *Cliona celata*, indicating that bioerosion rates are higher in lower latitudes [67]. This could be another factor for *P. clionoides*' higher growth rates, occurring as it does at lower latitudes.

Impact on bioerosion

The impact of boring bryozoans on bioerosion is unknown, since there are neither published growth experiments nor dedicated bioerosion experiments available. So far, the impact of penetrantiids on their substrate has been considered minimal as they penetrate only the superficial layers (upper 100–500 μm) of the substrate, and live gastropods were assumed to be unaffected by the boring activity [9, 11, 14]. Consequently, the relationship between boring bryozoans and their substrate is considered to be non-parasitic [9, 14].

All boring bryozoans are considered internal microborers according to their size (diameter < 100 μm) and the utilization of a chemical boring mechanism only [18, 19, 68, 69]. Nonetheless, since the true abundance, distribution and growth rates of penetrantiids has historically been largely underestimated, their impact in bioerosion is probably higher than previously estimated especially in tropical waters [11]. We have encountered shells that were completely bored by penetrantiids, which alters substrate properties and facilitates other bioerosion effects such as grazing [70, 71]. Additionally, most microborers play a key part in bioerosion processes and are considered pioneer borers that often start the penetration followed by small macroborers like polychaete worms, enabling subsequent larger macroborers to colonize the substrate [71, 72].

Herein, we provide the first estimations in bioerosion rates of a boring bryozoan species. Assuming the observed colony of *P. clionoides* has a linear growth rate throughout the year, it would dissolve 0.09 mm^3 of its substrate within one year which translates to 0.2778 mg of aragonite or 0.2651 mg of calcite (according to [25]). However, the assumption of a linear growth rate is speculative since most bryozoan species exhibit seasonal fluctuations in their growth rates as their colonies become larger, with more growing edges and more feeding autozooids [60]. Consequently, the actual bioerosion rate of *P. clionoides* is probably higher. Nevertheless, our findings give the first indication of the extent to which penetrantiids contribute to bioerosion. However, it is not easy to compare these values since information on internal bioerosion is scarce, particularly for microborers. The boring sponge *Cliona celata* erodes 1.94–2.55 mg per day [67],

which is much higher than our estimate for *P. clionoides* from Guam (0.0007 mg per day). But the sponges in the aforementioned experiment were larger and lived within even larger oyster shells than our *P. clionoides* colony, making this comparison biased. Future studies should conduct dedicated bioerosion experiments on boring bryozoans, including information on the eroded substrate area, to generate comparable data in $\text{kg m}^{-2} \text{year}^{-1}$ [18, 19, 67].

Conclusion

The life history of penetrantiids appears highly adapted to their endolithic lifestyle and their geographic, as well as bathymetric, distribution. The presence of brooding gonozooids with embryos offers insights into their reproduction patterns, with *P. clionoides* demonstrating notably higher sexual reproductivity. In contrast, *Penetrantia* sp. from temperate waters may exhibit less distinct reproductive peaks. This distinction is further emphasized by different colonial growth rates, with *P. clionoides* exhibiting notably higher growth rates, likely influenced by higher water temperatures and optimal food availability.

Larval recruitment patterns suggest philopatric behavior in both *P. clionoides* and *P. japonica*, potentially driven by conspecific cues or shared settlement preferences. Settlement on or near specific substrate features, such as sutures in gastropod shells, might optimize larval survival and colony establishment, with penetrantiid ancestrulae generally found in areas where the periostracum layer is absent or worn.

Ancestrula formation and early colony development revealed unique anatomical features, such as apertural rims and the triradiate stolon arrangement, providing valuable insights into penetrantiid taxonomy, settlement preferences, and colony formation. Although ancestrulae of all investigated penetrantiid species form star-shaped ancestrula–stolon complexes, there are species-specific differences that provide additional diagnostic characters, such as: (1) varying budding sites of the initial stolon, (2) differences in the timing of development in the first pair of additional lateral stolons, and (3) variations in the angles between initial and additional stolons.

The impact of penetrantiids on bioerosion remains poorly understood, although we have highlighted potential implications for substrate alteration and facilitation of other bioerosion processes. Additionally, we have generated first projections of bioerosion rates for the tropical species *P. clionoides*, along with a protocol for maintaining *Penetrantia* under laboratory conditions.

Overall, this study underscores the importance of understanding the intricate relationships between penetrantiid bryozoans and their environment, contributing to broader insights into marine ecosystem dynamics and bioerosion processes. Further research, including

dedicated bioerosion experiments and long-term ecological monitoring, is essential to fully comprehend the ecological roles and evolutionary adaptations of these intriguing organisms.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40851-024-00234-z>.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7

Acknowledgements

This project was realized thanks to the support of Daniela Gruber, Brian Metscher, Julian Bibermaier, David Combosch, Olivia Barry, Carlos Leiva, Noel Guidal, Gilles Maron and many more helping hands at the UOG Marine Lab in Guam and the Station Biologique de Roscoff. Scanning electron microscopy was performed at the Core Facility Cell Imaging and Ultrastructure Research, University of Vienna - member of the Vienna Life-Science Instruments (VLSI). We also thank three anonymous reviewers who provided helpful comments and suggestions that improved the manuscript. This research was funded in whole or in part by the Austrian Science Fund (FWF) [10.55776/P33585]. For open access purposes, the author has applied a CC BY public copyright license to any author accepted manuscript version arising from this submission.

Authors' contributions

The study's conception was designed by TS and SHD. Sampling was conducted by SL, SD, MH, MJJ, TS, and SHD. Sample fixation and preparation were done by SL, SD, MH, MJJ, TS and SHD. Imaging and data analysis were carried out by SD, MJJ and SHD. The initial draft of the manuscript was written by SHD, with all authors contributing to its final version. The final version of the manuscript was reviewed and approved by all authors.

Funding

This study was supported by Austrian Science Fund (FWF) project nr. P 33585 granted to TS. The research leading to these results received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 730984, ASSEMBLE Plus project application nr. 10715, 237. Open access funding provided by University of Vienna.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study adhered to all relevant international, national, and/or institutional guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 2 February 2024 / Accepted: 23 May 2024

Published online: 14 June 2024

References

1. Jackson JBC. Models of dispersal of clonal benthic invertebrates: consequences for species' distributions and genetic structure of local populations. *Bull Mar Sci*. 1986;39:588–606.
2. Jackson JBC, Coates AG. Life cycles and evolution of clonal (modular) animals. *Phil Trans R Soc Lond B*. 1986;313:7–22.
3. Reed CG. Bryozoa. In: Giese AC, Pearse JS, Pearse VB, editors. *Reproduction of Marine invertebrates VI echinoderms and Lophophorates*. Pacific Grove, California: The Boxwood; 1991. pp. 85–245.
4. Winston JE. Life in the Colonies: Learning the Alien Ways of Colonial Organisms*. *Integr Comp Biol* [Internet]. 2010;50(6):919–33. <https://doi.org/10.1093/icb/icq146>.
5. Bock P, Gordon DP. Phylum Bryozoa Ehrenberg, 1831. *Zootaxa*. 2013;3703:67–74.
6. Waeschenbach A, Taylor PD, Littlewood DTJ. A molecular phylogeny of bryozoans. *Mol Phylogenet Evol* [Internet]. 2012;62(2):718–35. Available from: [:/WOS:000299244400012](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3444001/).
7. Schwaha T. Morphology of bryozoans. In: Schwaha T, editor. *Handbook of Zoology: Bryozoa*. Berlin: DeGruyter; 2020. pp. 57–100.
8. Schwaha T. Ctenostomata. In: Schwaha T, editor. *Handbook of Zoology Bryozoa*. Berlin: de Gruyter; 2020. pp. 269–316.
9. Pohowsky RA. The boring ctenostomate Bryozoa: taxonomy and paleobiology based on cavities in calcareous substrata. *Bulletins Am Paleontology*. 1978;73:1–192.
10. Pohowsky RA. Notes on the study and nomenclature of boring Bryozoa. *J Paleontol*. 1974;48:557–64.
11. Decker SH, Hirose M, Lemer S, Kuklinski P, Spencer HG, Smith AM et al. Boring bryozoans: an investigation into the endolithic bryozoan family Penetrantiidae. *Org Divers Evol* [Internet]. 2023; <https://doi.org/10.1007/s13127-023-00612-z>.
12. Mukai H, Terakado K, Reed CG. Bryozoa. In: Harrison FW, Woollacott RM, editors. *Microscopic anatomy of invertebrates*. New York, Chichester: Wiley-Liss; 1997. pp. 45–206.
13. Silén L. On two new groups of Bryozoa living in the shells of molluscs. *Arkiv för Zoologi*. 1946;38B:1–7.
14. Silén L. On the anatomy and biology of Penetrantiidae and Immergentiidae (Bryozoa). *Arkiv för Zoologi*. 1947;40A(4):1–48.
15. Schack CR, Gordon DP, Ryan KG. Modularity is the mother of invention: a review of polymorphism in bryozoans. *Biological Reviews* [Internet]. 2019;94:773–809. <https://onlinelibrary.wiley.com/doi/abs/10.1111/brv.12478>.
16. Ostrovsky AN. Sexual reproduction in Bryozoa. In: Schwaha T, editor. *Handbook of Zoology Bryozoa*. Berlin: de Gruyter; 2020. pp. 101–22.
17. Gruhl A. Larval structure and metamorphosis. In: Schwaha T, editor. *Handbook of Zoology, Bryozoa*. Berlin: de Gruyter; 2020. pp. 123–42.
18. Wissiak M. Chapter 8 - Microbioerosion. In: Knaust D, Bromley RG, editors. *Developments in Sedimentology* [Internet]. Elsevier; 2012. pp. 213–43. <https://www.sciencedirect.com/science/article/pii/B9780444538130000083>.
19. Schönberg CHL, Fang JKH, Carreiro-Silva M, Tribollet A, Wissiak M. Bioerosion: the other ocean acidification problem. *ICES J Mar Sci* [Internet]. 2017;74(4):895–925. Available from: [:/WOS:000404450100002](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC550100002/).
20. Ryland JS. *Bryozoans*. London: Hutchinson University Library; 1970.
21. Kvach AV, Varfolomeeva MA, Kotenko ON, Sukhotin AA, Kutimov V, Grischenko AV, et al. Life history of the ctenostome bryozoan *Flustrellidra hispida* in the White Sea. *Invertebrate Zool*. 2019;16:343–60.
22. Shevchenko ET, Varfolomeeva MA, Nekliudova UA, Kotenko ON, Usov NV, Granovitch AI et al. *Electra* vs *Callopora*: life histories of two bryozoans with contrasting reproductive strategies in the White Sea. *Invertebr Reprod Dev* [Internet]. 2020;64(2):137–57. <https://doi.org/10.1080/07924259.2020.1729260>.
23. Seed R, Hughes RN. Reproductive strategies of epialgal bryozoans. *Invertebr Reprod Dev*. 1992;22:291–300.
24. Nekliudova UA, Shunkina KV, Grishankov AV, Varfolomeeva MA, Granovitch AI, Ostrovsky AN. Colonies as dynamic systems: reconstructing the life history of *Cribrilina annulata* (Bryozoa) on two algal substrates. *J Mar Biol Association United Kingd*. 2019;99(06):1363–77.
25. Patnaik P. *Handbook of Inorganic chemicals* [Internet]. McGraw-Hill; 2003. <https://books.google.at/books?id=Xqj-TTzkvTEC>.
26. Dyrinda PEJ, Ryland JS. Reproductive strategies and life histories in the cheilostome marine bryozoans *Chartella papyracea* and *Bugula flabellata*. *Mar Biol*. 1982;71:241–56.
27. Ostrovsky AN. Reproductive strategies and patterns in Marine invertebrates: Diversity and Evolution. *Paleontol J*. 2021;55(7):803–10.

28. Eggleston D. Patterns of reproduction in the marine Ectoprocta of the Isle of Man. *J Nat Hist*. 1972;6:31–8.
29. Jackson JBC, Wertheimer SP. Patterns of reproduction in five common species of Jamaican reef-associated bryozoans. In: Nielsen C, Larwood GP, editors. *Bryozoa: Ordovician to recent*. Fredensborg: Olsen & Olsen; 1985. pp. 161–8.
30. Lander MA, Guard CP. Creation of a 50-year rainfall database, annual rainfall climatology, and annual rainfall distribution map for Guam. [Internet]. Guam; 2003 Jun [cited 2024 Feb 1]. <https://weri-cdn.uog.edu/wp-content/PDFs/TRs/WERI%20TR%20102%20-%20Lander%20%26%20Guard%202003.pdf>.
31. Braley RD. Reproductive periodicity in the indigenous oyster *Saccostrea cucullata* in Sasa Bay, Apra Harbor, Guam. *Mar Biol* [Internet]. 1982;69(2):165–73. <https://doi.org/10.1007/BF00396896>.
32. Gordon DP. The ageing process in bryozoans. In: Woollacott RM, Zimmer RL, editors. *Biology of bryozoans*. New York: Academic; 1977. pp. 335–76.
33. Winston JE. Behavior. In: Schwaha T, editor. *Handbook of Zoology, Bryozoa*. Berlin: DeGruyter; 2020. pp. 143–88.
34. Ostrovsky AN. Evolution of sexual reproduction in marine invertebrates: Example of gymnolaemate bryozoans. Dordrecht, Heidelberg, New York, London: Springer; 2013. p. 356.
35. Pires A, Woollacott RM. A Direct and Active Influence of Gravity on the Behavior of a Marine Invertebrate Larva. *Science* (1979) [Internet]. 1983;220(4598):731–3. <https://www.science.org/doi/abs/https://doi.org/10.1126/science.220.4598.731>.
36. Cancino JM, Hughes RN, Ramirez C. Environmental cues and the phasing of larval release in the bryozoan *Celleporella hyalina* (L). *Proceedings of the Royal Society London Series B*. 1991;246(1315):39–45.
37. Pires A, Woollacott RM. Serotonin and dopamine have opposite effects on phototaxis in Larvae of the bryozoan *Bugula neritina*. *Biol Bull*. 1997;192:399–409.
38. Freckelton ML, Nedved BT, Hadfield MG. Induction of Invertebrate Larval Settlement; Different Bacteria, Different Mechanisms? *Sci Rep* [Internet]. 2017;7(1):42557. <https://doi.org/10.1038/srep42557>.
39. Hayward PJ, Ryland JS. Growth, reproduction and larval dispersal in *Alcyonidium hirsutum* (Fleming) and some other Bryozoa. *Publicazioni Della Stazione Zool Di Napoli Suppl*. 1975;39:226–41.
40. Keough MJ. Kin-recognition and the spatial distribution of larvae of the bryozoan *Bugula neritina* L. *Evolution* (NY). 1984;38:142–7.
41. Schwaha T, Ruthensteiner B, Melzer RR, Asami T, Páll-Gergely B. Three phyla - two type specimens - one shell: history of a snail shell revealed by modern imaging technology. *J Zoological Syst Evolutionary Res*. 2019;57:527–33.
42. Hayward PJ. Preliminary observations on settlement and growth in populations of *Alcyonidium hirsutum* (Fleming). In: Larwood GP, editor. *Living and fossil Bryozoa*. London: Academic; 1973. pp. 107–13.
43. Marin F, Le Roy N, Marie B. The formation and mineralization of mollusk shell. *Front Biosci*. 2012;10:99–125.
44. Smyth MJ. Incidence of boring organisms in gastropod shells on reefs around Guam (West Pacific Ocean). *Bull Mar Sci*. 1990;46:432–49.
45. Reed CG, Cloney RA. The larval morphology of the marine bryozoan *Bowerbankia gracilis* (Ctenostomata: Vesicularioidea). *Zoomorphology*. 1982;100:23–54.
46. Reed CG. Phylogenetic implications of the morphogenesis of the cystid epithelium during the metamorphosis of gymnolaemate bryozoans. In: Ross JRP, editor. *Bryozoa: Present and Past*. Bellingham: Western Washington University; 1987. pp. 221–8.
47. Decker S, Wanninger A, Schwaha T. Morphology and life cycle of an epiphytic pherussellid ctenostome bryozoan from the Mediterranean Sea. *Org Divers Evol* [Internet]. 2020;20:417–37. <https://doi.org/10.1007/s13127-020-0044372>.
48. Reed CG, Cloney RA. The settlement and metamorphosis of the marine bryozoan *Bowerbankia gracilis* (Ctenostomata: Vesicularioidea). *Zoomorphology*. 1982;101:103–32.
49. Smyth MJ. *Penetrantia clionoides*, sp. nov. (Bryozoa), a Boring Bryozoan in gastropod shells from Guam. *Biological Bulletin* [Internet]. 1988;174(3):276–86. <http://www.biobull.org/cgi/content/abstract/174/3/276>.
50. Decker SH, Saadi AJ, Baranyi C, Hirose M, Lemer S, Sombke A et al. Boring systematics: a genome skinned phylogeny of ctenostome bryozoans and their endolithic family Penetrantiidae with the description of one new species. *Ecol Evol*. 2024;14(4).
51. Ryland JS. Physiology and ecology of marine bryozoans. In: Russell FS, Yonge M, editors. *Advances in marine biology Vol14*. London etc.: Academic; 1976. pp. 285–443.
52. Smith AM. Growth and Calcification of Marine Bryozoans in a Changing Ocean. *Biol Bull* [Internet]. 2014;226(3):203–10. <https://www.journals.uchicago.edu/doi/abs/https://doi.org/10.1086/BBLv226n3p203>.
53. Taylor PD. *Bryozoan Paleobiology*. London, UK: Wiley; 2020.
54. Smith AM, Key MM. Growth geometry and measurement of growth rates in marine bryozoans: a review. In: Jackson P, Zagorsek K, editors. *Bryozoan studies 2019*. Prague, Czech Republic: Czech Geological Society; 2020. pp. 139–56.
55. Achilleos K, Smith AM. Proof of concept for measuring growth of shelf marine calcifiers: 'a Bryozoan odyssey'. *Mar Freshw Res* [Internet]. 2023;74(14):1262–73. <https://www.publish.csiro.au/paper/MF23114>.
56. Wood V, Seed R. Reproduction and growth of *Alcyonidium hirsutum* (Fleming) and *Flustrellidra hispida* (Fabricius) (Bryozoa: Ctenostomata) within a *Fucus serratus* L. community. *Cah Biol Mar*. 1992;33:347–63.
57. Jebram D. Stolonen-Entwicklung und systematik bei den Bryozoa Ctenostomata. *J Zoological Syst Evolutionary Res*. 1973;11:1–48.
58. Jebram D. Thigmic growth reactions in *Victorella*. *Zoologischer Jahrbücher, Abtheilung für Anatomie und Ontogenie der Tiere*. 1987;115:255–62.
59. Amui-Vedel AM, Hayward PJ, Porter JS. Zoid size and growth rate of the bryozoan *Cryptosula pallasiana* Moll in relation to temperature, in culture and in its natural environment. *J Exp Mar Biol Ecol* [Internet]. 2007;353(1):1–12. <http://www.sciencedirect.com/science/article/B6T8F-4R0CR5J-1/1/39be38d7b02032380ec4f93a63576034>.
60. Saunders MI, Metaxas A. Effects of temperature, size, and food on the growth of *Membranipora membranacea* in laboratory and field studies. *Mar Biol* [Internet]. 2009;156(11):2267–76. <https://doi.org/10.1007/s00227-009-125476>.
61. Kuklinski P, Sokolowski A, Ziolkowska M, Balazy P, Novosel M, Barnes DKA. Growth Rate of Selected Sheet-Encrusting Bryozoan Colonies Along a Latitudinal Transect: Preliminary Results. In: Ernst A, Schäfer P, Scholz J, editors. *Bryozoan Studies 2010* [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013. pp. 155–67. https://doi.org/10.1007/978-3-6427164178_11.
62. Cancino JM, Hughes RN. The effect of water flow on growth and reproduction of *Celleporella hyalina* (L) (bryozoa: cheilostomata). *J Exp Mar Biol Ecol* [Internet]. 1987;112(2):109–30. <http://www.sciencedirect.com/science/article/B6T8F7488CFNY7143/1/e75c253edccf509e946794f156097189>.
63. Harvell CD, Grosberg RK. The timing of sexual maturity in clonal animals. *Ecol*. 1988;69:1855–64.
64. Taylor JD, Reid DG. Shell microstructure and mineralogy of the Litorinidae: ecological and evolutionary significance. *Hydrobiologia*. 1990;193(1):199–215.
65. Fabry VJ, Seibel BA, Feely RA, Orr JC. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J Mar Sci*. 2008;65(3):414–32.
66. Iglukowska A, Przytarska J, Humphreys-Williams E, Najorka J, Chelchowski M, Sowa A, et al. Shell mineralogy and chemistry – Arctic bivalves in a global context. *Mar Pollut Bull*. 2023;189:14759.
67. Wisshak M, Schönberg CHL, Form A, Freiwald A. Sponge bioerosion accelerated by ocean acidification across species and latitudes? *Helgol Mar Res* [Internet]. 2014;68(2):253–62. <https://doi.org/10.1007/s10152-014-038574>.
68. Golubic S, Perkins RD, Lukas KJ. Boring Microorganisms and Microborings in Carbonate Substrates. In: Frey RW, editor. *The Study of Trace Fossils: A Synthesis of Principles, Problems, and Procedures in Ichology* [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 1975. pp. 229–59. https://doi.org/10.1007/978-3-64276592372_12.
69. Golubic S, Friedmann EI, Schneider J. The lithobiontic ecological niche, with special reference to microorganisms. *Journal of Sedimentary Research* [Internet]. 1981;51(2):475–8. <https://doi.org/10.1306/212F7CB6-2B24-11D7-8648000102C1865D>.
70. Tribollet A, Golubic S. Cross-shelf differences in the pattern and pace of bioerosion of experimental carbonate substrates exposed for 3 years on the northern Great Barrier Reef, Australia. *Coral Reefs* [Internet]. 2005;24(3):422–34. <https://doi.org/10.1007/s00338-005-000377>.
71. Enochs IC, Manzello DP, Tribollet A, Valentino L, Kolodziej G, Donham EM et al. Elevated Colonization of Microborers at a Volcanically Acidified Coral Reef. *PLoS One* [Internet]. 2016;11(7):e0159818. <https://doi.org/10.1371/journal.pone.0159818>.
72. Tribollet A, Golubic S. Reef Bioerosion: Agents and Processes. In: Dubinsky Z, Stambler N, editors. *Coral Reefs: An Ecosystem in Transition* [Internet]. Dordrecht: Springer Netherlands; 2011. pp. 435–49. https://doi.org/10.1007/978-94-007-011474_25.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.