

**Modular marvels: the ecology, evolvability, and energetics of bryozoan
polymorphism**

BY

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Abstract

Modularity is a fundamental concept in biology. Most taxa within the colonial invertebrate phylum Bryozoa have achieved division of labor through the development of specialized modules (polymorphs), and this group is perhaps the most outstanding exemplar of the phenomenon. This thesis addresses several gaps in the literature concerning the morphology, ecology, energetics, and evolvability of bryozoan polymorphism.

It has been over 40 years since the last review of bryozoan polymorphism, and here I provide a comprehensive update that describes the diversity, morphology, and function of bryozoan polymorphs and the significance of modularity to their evolutionary success. While the degree of module compartmentalization is important for the evolution of polymorphism in bryozoans, this does not appear to be the case for other colonial invertebrates.

To facilitate data collection, I developed a classification system for polymorphism in cheilostome bryozoans. While classification systems exist for bryozoan colony form, the system presented here is the first developed for polymorphism. This system is fully illustrated and non-hierarchical, enabling swift classification and statistical comparisons at many levels of detail.

Understanding community assembly is a key goal in community ecology, but previous work on bryozoan communities has focused on colony form rather than polymorphism. Environmental filtering influences community assembly by excluding ill-adapted species, resulting in communities with similar functional traits. An RLQ (a four-way ordination) analysis incorporating spatial data was run on a dataset of 642 species of cheilostomes from 779 New Zealand sites, to investigate environmental filtering of colony form and zooid polymorphism. This revealed environmental filtering of colony form: encrusting-cemented taxa were predominant in shallow environments with hard substrata (<200m), while erect-rooted taxa characterized deeper environments with soft substrata (>200 m). Furthermore, erect taxa found in shallow environments with high current speeds were typically jointed. Surprisingly, polymorphism also followed environmental gradients. External ovicells (brood chambers) were more common in deeper, low oxygen water than immersed and internal ovicells. This may reflect the oxygen needs of the embryo or increased predation intensity in shallow environments. Bryozoans with costae (rib-like spines) tended to be found in deeper water as well, while bryozoans with calcified frontal shields were found in shallow environments with a higher concentration of CaCO_3 . Avicularia (defensive grasping structures) were not

related to environmental conditions, and changes in pivot bar structure with depth likely represent a phylogenetic signal. Factors influencing community assembly were somewhat partitioned by levels of organization, since colony form responds to environmental conditions, while the effects of evolutionary history, predation, and environmental conditions were not well-separated for zooid-level morphology. Finally, rootlets may have been a key innovation that allowed cementing taxa to escape hard substrata, potentially contributing to the cheilostome radiation.

Despite the diversity of life on earth, many morphologies have not been achieved. Morphology can be limited by a variety of constraints (developmental, historical, biomechanical) and comparing the distribution of realized forms in a theoretical form-space (i.e. “morphospace”) can highlight which constraints are at play and potential functions. If traits cluster around biomechanical optima, then morphology may be shaped by strong selective pressures. In contrast, a well-explored (filled) morphospace suggests weak constraints and high morphological evolvability. Here, constraints on morphospace exploration were examined for 125 cheilostome bryozoan species from New Zealand. The mandible morphospaces for avicularia (beak-like polymorphs) were visualized using Coordinate-Point Extended Eigenshape analysis. Mechanical advantage, moment of inertia, drag, peak force, and rotational work required to close the mandible were calculated for theoretical ($n=47$) and real mandibles ($n=224$) to identify biomechanical optima. The volume and surface area of the parcel of water passed through by the closing mandible (referred to as the “domain”) was also calculated. The theoretical morphospace of avicularia is well-explored, suggesting they are highly evolvable and have relaxed developmental constraints. However, there may be constraints within lineages. A well-developed fulcrum (complete pivot bar) may be an evolutionary pre/corequisite to evolving mandibles with extreme moments of inertia such as setose and highly spatulate forms. The most common mandible shape, triangular, represents a trade-off between maximizing domain size, minimizing energetic cost (force and construction material), and minimizing the potential for breakage. This suggests that they are well suited for catching epibionts, representing the first empirical evidence for avicularian function. Tendon length and mechanical advantage are limited by tendon width, which itself is constrained by the base width of the mandible. This explains the low mechanical advantage of setose mandibles and suggests that they are unable to grasp epibionts. The calories required to close the mandible of an avicularium (estimated from rotational work) are quite small (1.24×10^{-16} to 8.82×10^{-11} cal).

Overall, this thesis highlights the complexity of bryozoan polymorphism and suggests cheilostome avicularia could provide a unique evolutionary system to study due to their apparent lack of strong

developmental constraints. Future studies into the ecology of polymorphism should focus on the degree of investment (polymorph abundance within a colony) rather than presence or absence.

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Irene and Ronald Schack (Grammy & Grampy)

Ethel and Charlton Webb (Gabby & Papa)

I always strive to make you proud.

Statement of Authorship

This thesis has been written as a series of publications, with unique figure numbering and references provided at the end of each chapter. The two published papers (Chapter 2-3) are presented as they appeared in their respective journals, including their unique citation styles, page numbers, and paragraph numbering. Ken Ryan (KGR) and Dennis Gordon (DPG) provided supervision and advice throughout. Carolann Schack (CRS), KGR, and DPG were involved in the editing and revision of all chapters.

The introduction and discussion (Chapters 1 and 6) were conceptualized and written by CRS.

Chapter 2 was published in *Biological Reviews* (2019), and was thoroughly revised following comments from three anonymous reviewers. This paper was conceptualized by CRS, DPG, and KGR. CRS wrote and illustrated the original draft. DPG provided SEM images.

Chapter 3 was revised based on comments from A. Ostrovsky and published in the *Annals of Bryozoology* (2018). This paper was conceptualized by CRS and DPG. CRS wrote and illustrated the original and draft.

Chapter 4 was submitted to *Science Advances* in February 2019, and underwent major revision following the comments of three anonymous reviewers and the editor J.B.C. Jackson. This paper was conceptualized by DPG and CRS. CRS curated data, conducted formal analysis, visualized the data, and wrote the original draft. This paper will soon be submitted to Proceedings of the National Academy of Sciences of the United States of America (PNAS) and reflects their formatting guidelines and length requirements.

Chapter 5 was conceptualized by CRS. CRS collected data, conducted formal analysis, visualized the data, and wrote the original draft. It is the authors' intent to submit an abridged version of this paper to *Science*.

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Chapter 1: Introduction

Modularity is the degree of compartmentalization between the units of a system (e.g. genes, organs, individuals within a colony, etc.). Modular organization is an important concept in biology, since it allows selection to act separately on each module (rather than on the organism as a whole). The ability to simultaneously respond to different selection pressures, combined with function-preserving redundant molecules (which reduce the impact of deleterious mutations), enhances the adaptive potential of modular organisms [1–3]. In this way, modularity is thought to counter the inherent cost of complexity: the genome size increases with organism complexity, which means a single mutation has less impact [1,4–6].

Bryozoans are colonial, sessile suspension feeders that exhibit a high degree of modularity. Their colonies are composed of physically connected individuals called zooids. The degree of compartmentalization between zooids may be responsible for the presence of polymorphism in some bryozoan taxa [7]. Polymorphic zooids exhibit discontinuous variation in form and contribute the division of labor within a colony by performing different functions [8,9]. Colonies that exhibit greater polymorphism can be considered more complex than monomorphic colonies. Polymorphism is absent in Phylactolaemata (which has relatively uncompartimentalized zooids), but is present to varying degrees in the Ctenostomata, Cyclostomata, and Cheilostomata (which have increasingly more compartmentalized zooids) [7]. The relationship between modularity and polymorphism is worthy of further study, both in bryozoans and other colonial invertebrates. Bryozoan polymorphism in particular is in dire need of an updated review, since it has been 40 years since the last comprehensive review [8].

The degree of polymorphism in bryozoans and other colonial organisms was previously thought to relate to disturbance [10,11]. Fluctuating environmental conditions would render highly specialized polymorphs useless, so polymorphism should only evolve in consistent environments. However, this does not appear to be the case for eusocial insects (incidence of polymorphism is instead related to colony size [12]), or bryozoans [13,14]. While the degree of polymorphism (i.e. degree of complexity) may be unrelated to environmental conditions, it is highly likely that polymorphism has an effect on bryozoan ecology. If bryozoans live an energetically marginal existence [15], then the cost of polymorphic zooids (which are often non-feeding) should significantly influence their energy budget. This could restrict taxa with energetically expensive polymorphs to food-rich habitats. In addition, there

may be environments that are only accessible to taxa with certain polymorphs (i.e. environmental filtering). For example, habitats with high sedimentation rates or an abundance of fouling organisms may only be accessible to colonies with cleaning polymorphs (e.g. vibracula). Understanding the relationship between polymorphs and the environment can be used to predict community responses to environmental change, reconstruct the paleoenvironment, predict the success of invasive species, suggest trait functions, and identify “key innovations” that allow species to bypass environmental filters. These key innovations could have triggered species radiations by providing access to new habitats and niches. Of course, communities with similar traits can also be due to colonization history or phylogenetic relatedness, so it is important to include spatial and phylogenetic data when available [16].

Modularity in bryozoan colonies suggests that polymorphism and colony form (which varies with substratum type and depth [17]) should respond independently to environmental conditions and other selection pressures [18]. However, the relationship between polymorph morphology, colony form, and the environment has yet to be thoroughly investigated.

Bryozoan polymorphs include heterozooids (avicularia, vibracula, kenozooids), autozooidal polymorphs (reproductive zooids, some spines and rootlets), and multi-zooidal complexes (some embryo incubation chambers) [8]. All of these types possess diverse morphologies [19–21], suggesting specializations for different functions within each type [8,22]. For example, avicularia are beak-like structures with an articulated mandible. Pointed mandibles may grasp epibionts, while spathulate ones may direct wastewater over the surface of the colony [11,22]. Lumping polymorphs together by general type, without regard for morphology, can result in noisy data, obscuring underlying trends. A classification system would ensure that morphological variation is captured, and that the criteria used to categorize different morphologies remains constant through the classification process. While classification systems exist for bryozoan colony form [23], none exist for polymorphism.

The morphological variation present in bryozoan polymorphism lends itself to morphospace analysis. A morphospace is a hypothetical space that encompasses the variation in morphology of a particular trait. The axes of a morphospace are typically quantitative measurements (e.g. body length, degree of shell curvature, surface area to volume ratio, etc.). Theoretical morphospaces (usually generated mathematically) can be compared to empirical ones to determine the effect of evolutionary history, developmental constraints, and biomechanical trade-offs on current morphology [24,25]. Modular organization within zooids themselves would reduce development constraints (through the dissociation of components) and may allow for more homogeneous morphospace exploration. In other words,

modular organisms may more easily realize a wider range of theoretical morphologies compared to unitary organisms. Polymorphs are typically morphologically complex, three-dimensional structures. However, much of the variation in avicularian mandible shape can be captured in a two-dimensional plane. This allows the use of geometric morphometric techniques that can provide an objective measure of shape and generate theoretical morphospaces. The simple movement of avicularia also allows for a detailed analysis of their biomechanics, though this has only been attempted once before [26]. An empirical morphospace that is well-aligned with biomechanical optima suggests that morphospace exploration is at least partly driven by selection. In contrast, a patchy morphospace (particularly one that does not approach biomechanical optima) may be driven more by developmental and historical constraints. Identifying biomechanical optima can also suggest functions for different mandible morphologies (many of which are still debated [22]). Finally, these biomechanical analyses can calculate the energetic cost associated with different mandible shapes and sizes. Knowing the energetic investment required to perform certain tasks can provide insight into the life-history strategies and energy budgets of bryozoans. However, the energetic costs associated with avicularia – or any other polymorphism – are currently unknown. It is clear that the energetics, functions, and evolution of avicularia require further study.

Bryozoans, though understudied, are economically and ecologically important. They provide “micro-reef” habitats for diverse communities of epibionts and juvenile fish [27,28], and are important fouling species on docks, in harbors, and (for freshwater species) wastewater treatment plants [29]. Like many overlooked marine organisms, bryozoans are a valuable resource for marine natural products, including anti-cancer and anti-bacterial compounds [30,31]. The results presented in this thesis provide insights into their ecology and evolution, but also highlight why these “modular marvels” are worthy of further investigation.

Chapter 2: Modularity is the mother of invention: a review of polymorphism in bryozoans

This chapter is an extensive literature review, recently published in *Biological Reviews* [32], that compares polymorphism across all bryozoans through the lens of modularity. Plasticity, cormidium construction, and the role of modularity in the evolution of polymorphism are described theoretically.

Chapter 3: Classification of cheilostome polymorphs

The second data chapter details a thorough classification system of cheilostome polymorphism, published in the *Annals of Bryozoology* [33]. This classification system is non-hierarchical, allowing statistical comparisons to be made at any level of detail.

Chapter 4: Community assembly in a modular organism: the impact of environmental filtering on bryozoan colony form and polymorphism

Utilizing the framework created in Chapter 3, this chapter examines whether bryozoan communities are environmentally filtered by their polymorphs. Here an ordination technique (RLQ) is used to link environmental conditions to species traits.

Chapter 5: Cheap labor and high evolvability: A lack of strong developmental constraints on mandible shape of avicularia in New Zealand bryozoans

In this chapter, the morphology, evolvability, and energetics of mandible shape in cheilostome avicularia are examined using geometric morphometrics (Coordinate-Point Extended Eigenshape analysis) and a biomechanical analysis.

Chapter 6: Discussion

Here the results of the prior chapters are synthesized, and future directions are outlined.

Appendix 1: Errata for Chapter 2 and 3

Appendix 2: Errata for Chapter 3

Appendix 3: Supplementary methods and figures from Chapter 4

Appendix 4: Supplementary figures from Chapter 5

Supporting information: Data from Chapters 4-5 and R code from Chapter 5.

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Modularity is the mother of invention: a review of polymorphism in bryozoans

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ABSTRACT

Modularity is a fundamental concept in biology. Most taxa within the colonial invertebrate phylum Bryozoa have achieved division of labour through the development of specialized modules (polymorphs), and this group is perhaps the most outstanding exemplar of the phenomenon. We provide a comprehensive description of the diversity, morphology and function of these polymorphs and the significance of modularity to the evolutionary success of the phylum, which has >21000 described fossil and living species. Modular diversity likely arose from heterogeneous microenvironmental conditions, and cormidia (repeated clusters of associated modules) are an emergent property of the cue thresholds governing zooid plasticity. Polymorphs in a colony have, during phylogeny, transitioned into associated non-zooidal structures (appendages), increasing colonial integration. While the level of module compartmentalization is important for the evolution of bryozoan polymorphism, it may be less influential for other colonial invertebrates.

Key words: modularity, polymorphism, Ctenostomata, Cheilostomata, Cyclostomata, avicularia, kenozooids, ovicells, cormidia.

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I. INTRODUCTION

The high degree of modularity in the organization of many colonial invertebrates is key to their evolutionary success. Modules experience separate selection pressures within the same organism (Kirschner & Gerhart, 1998; Carroll, 2001). The dissociative nature of modular organization enhances adaptive potential by reducing the impact of deleterious mutations (Kirschner & Gerhart, 1998; Carroll, 2001). Module redundancy also enhances adaptive potential, allowing novel forms to arise in non-essential modules (Kirschner & Gerhart, 1998). This is like the International Space Station: each room (module) is separated by a sealed door, which prevents breaches (deleterious mutations) from damaging the rest of the station. The station may be reorganized through the addition, removal, or modification of modules (evolution). The adaptive potential provided through modular organization may be important for overcoming the ‘cost’ of complexity. In most organisms, as complexity increases, the ability to evolve toward optimal characters is reduced owing to the decreasing impact of a single mutation (Orr, 2000; Carroll, 2001). However, the presence of non-modular or slightly modular organisms (e.g. arthropods: integrated individuals with serially homologous limbs (modules) capable of differentiation) and the secondary integration of modules (Krohs, 2009) indicates that modularity does not universally increase adaptive potential. Any change to a module with multiple functions must be compatible with those functions, which limits variation and thus adaptive potential (Krohs, 2009).

The selection pressures, developmental changes, and canalization contributing to module differentiation – as well as the origin of modularity itself – require further study. Bryozoans are highly modular organisms and exhibit division of labour through a wide variety of polymorphs. The functions, development, and evolution of many bryozoan polymorphs are still poorly understood, but they may provide insight into independently developed modularity and module differentiation in different classes.

Silén (1977) published his review of bryozoan polymorphism 41 years ago. Much has been done since then and our aim is to provide an update to bryozoan polymorphism in the context of modularity. Our companion paper (Schack, Gordon & Ryan, 2018), provides a classification system for cheilostome polymorphism to facilitate morphological analyses. An appendix of terms is provided in Table 1.

(1) Bryozoan taxonomy

Phylum Bryozoa comprises three classes: Phylactolaemata, Stenolaemata, and Gymnolaemata. The phylactolaemates are exclusively found in fresh water and are unmineralized, while the opposite is true for the stenolaemates. Class Stenolaemata dominated until the Late Cretaceous, when it was overtaken by the radiation of gymnolaemates (McKinney & Jackson, 1989). Today, the gymnolaemates are extremely diverse and occur mostly in marine environments. They are divided into two orders – the non-calcified Ctenostomata, which are paraphyletic and the calcified, highly abundant Cheilostomata (McKinney & Jackson, 1989; Mukai, Kiyoshi & Reed, 1997). Since phylactolaemates lack polymorphism, this review will focus on the stenolaemates, ctenostomes, and the highly polymorphic cheilostomes.

(2) Autozooids

The basic (primary) module of a bryozoan colony is the self-supporting autozooid (Silén, 1977; McKinney & Jackson, 1989), which consists of a cystid and polypide (Fig. 1). The cystid (body wall) is partially deformable and is composed of a secreted outer layer (ectocyst, generally calcified in cheilostomes and cyclostomes) and an inner cellular lining (endocyst). Enclosed by the cystid is the polypide – which includes the ciliated tentacle crown (‘lophophore’), the U-shaped digestive tract, the intrinsic muscular system, and parts of the nervous system (McKinney & Jackson, 1989; Mukai *et al.*, 1997).

Table 1. Appendix of terms.

<i>Adventitious avicularia</i> :	avicularia that are borne on the frontal side of the autozoooid. Can be sessile or pedunculated.
<i>Alveoli</i> :	hollow structures in cyclostomes that develop between autozoooids, autozoooidal rows, or in association with gonozoooids.
	Walls have a similar structure to autozoooids and share communication pores with neighbouring autozoooids. Typically described as extrazoooidal but may be kenozoooidal. See also: <i>Cancelli</i> , <i>Dactylethrae</i> , <i>Kenozoooid</i> , <i>Mesozoooid</i> , <i>Metapore</i> , <i>Nematopore</i> , <i>Tergopore</i> .
<i>Ancestrula</i> :	the first zoooid in a colony, resulting from the metamorphosis and settlement of a bryozoan larva. Usually a single zoooid but may be a complex of multiple zoooids.
<i>Anascan</i> :	a cheilostome bryozoan lacking an ascus.
<i>Appendage</i> :	a projection from the cystid of an autozoooid that is not separated from the coelom by a pore plate. See also: <i>Autozoooidal polymorph</i> .
<i>Ascus</i> :	a compensation sac/space that allows ascophoran bryozoans to evert their tentacle crowns <i>via</i> hydrostatic displacement.
<i>Ascophoran</i> :	a cheilostome bryozoan possessing an ascus or compensation space.
<i>Autozoooid</i> :	the main unit of a bryozoan colony, capable of feeding and other functions necessary for life.
<i>Autozoooidal polymorph</i> :	zoooid that retains a protrusible tentacle crown (which may or may not feed) but differs from an autozoooid in the form of its cystid, polypide, or both. This includes reproductive zoooids, appendaged autozoooids, and nanozoooids.
<i>Avicularia</i> :	a heterozoooid with a reduced, non-feeding polypide. Its operculum is enlarged into a mandible. These structures have mechanosensory capabilities and are thought to have a mainly defensive function. See also: <i>B-zoooid</i> , <i>Vibraculum</i> .
<i>B-zoooid</i> :	a heterozoooid with a feeding polypide and an enlarged operculum. Thought to be an evolutionary precursor to avicularia. Found in some Steginoporellidae and Calloporidae. See also: <i>Avicularia</i> .
<i>Cancelli</i> :	an ambiguous term for structures in Cancellata (Cyclostomata) that may be vacuoles, extrazoooidal skeleton lacking skeletal walls, or a kenozoooidal chamber. See also: <i>Alveoli</i> , <i>Dactylethrae</i> , <i>Kenozoooid</i> , <i>Mesozoooid</i> , <i>Metapore</i> , <i>Nematopore</i> , <i>Tergopore</i> .
<i>Communication pore</i> :	a hole in interzoooidal walls that allows zoooid-to-zoooid communication. Communication may be direct or <i>via</i> a pore–cell complex (rosette).
<i>Cormidium</i> :	a ‘colony within a colony’ that consists of a feeding zoooid and associated polymorphs. The cormidium is capable of performing most functions vital for life. Multiple types of cormidia may be present within a colony.
<i>Cheilostome</i> :	bryozoans belonging to the gymnolaemate order Cheilostomata.
<i>Cribrimorph</i> :	see <i>Frontal shield</i> .
<i>Cystid</i> :	zoooidal body wall, whether calcified or uncalcified, enclosing the coelomic cavity and housing a polypide (when present).
	The cystid wall is composed of a secreted outer layer (ectocyst) and an inner cellular lining (endocyst).
<i>Dactylethrae</i> :	an ambiguous term for vacuoles in stenolaemate colonies that are closed by a terminal diaphragm which may be extrazoooidal or kenozoooidal. See also <i>Alveoli</i> , <i>Cancelli</i> , <i>Kenozoooid</i> , <i>Mesozoooid</i> , <i>Metapore</i> , <i>Nematopore</i> , <i>Tergopore</i> .
<i>Distal</i> :	in the direction of colony growth (away from the ancestrula and origin of growth).
<i>Encrusting</i> :	a mode of growth where the colony grows along the surface of the substratum.
<i>Erect</i> :	a mode of growth where the colony is held away from the substratum.
<i>Exilapore</i> :	see <i>Exilazoooid</i> .
<i>Exilazoooid</i> :	a vacuole with internal flask-shaped structures. May be an extrazoooidal structure or zoooid with a reduced polypide.
<i>Extrazoooidal structures</i> :	structures that are external to zoooidal boundaries. See also: <i>Mesopore</i> .
<i>Firmatopore</i> :	see <i>Nematopore</i> .
<i>Frontal</i> :	the side of a zoooid bearing the orifice (the hole through which the tentacle crown is protruded).
<i>Frontal shield</i> :	a calcified frontal surface in cheilostomes. This can constitute calcification of the frontal wall itself (gymnocystal), or a protective mesh of fused spines (spinocystal), a calcified wall arched over the primary membranous frontal wall (umbonuloid), or a calcified wall beneath the membranous frontal wall but above the ascus (lepralioid).
<i>Funicular system</i> :	branching semitubular to tubular system of mesenchymatous cords, connecting the gut of the polypide with communication pores, facilitating transfer of nutrients throughout the colony. Present in gymnolaemates.
<i>Funiculus</i> :	tubular muscular cord connecting the stomach and body wall (rarely, communication pore) in Bryozoa. See also: <i>Funicular system</i> .
<i>Gonozoooid</i> :	a voluminous brood chamber present in cyclostomes, formed through the modification of a female autozoooid (which may involve extrazoooidal structures, as in Lichenoporidae). Consists of an enlarged brood cavity (site of embryo incubation) and an oocistostome (aperture). See also: <i>Ovicell</i> .
<i>Heterozoooid</i> :	a specialized zoooid with a non-feeding, vestigial or absent polypide.
<i>Interzoooidal avicularia</i> :	avicularia occurring between zoooids, typically smaller than an autozoooid and resting on the substratum.
<i>Kenozoooid</i> :	a heterozoooid that lacks a polypide. Kenozoooids fulfill many functions within the colony, including space-filling, attachment, and colony support. See also: <i>Rhizoid</i> , <i>Vicariozoooid</i> .
<i>Lepralioid</i> :	see <i>Frontal shield</i> .
<i>Membranous frontal wall</i> :	the frontal surface of a bryozoan cystid. In anascan bryozoans it is flexible enough to be displaced by the parietal muscles, allowing the tentacle crown to be everted.
<i>Mesopore</i> :	see <i>Mesozoooid</i> .
<i>Mesozoooid</i> :	in <i>Paleozoic stenolaemates</i> , a tapering chamber closed by a terminal diaphragm, potentially containing diaphragms or cysts. May be extrazoooidal or kenozoooidal. See also: <i>Alveoli</i> , <i>Cancelli</i> , <i>Dactylethrae</i> , <i>Kenozoooid</i> , <i>Metapore</i> , <i>Nematopore</i> , <i>Tergopore</i> .
<i>Metapores</i> :	slender, deep cavities in the extrazoooidal skeleton of stenolaemates that lack skeletal walls of their own. See also: <i>Alveoli</i> , <i>Cancelli</i> , <i>Dactylethrae</i> , <i>Kenozoooid</i> , <i>Mesozoooid</i> , <i>Nematopore</i> , <i>Tergopore</i> .
<i>Module</i> :	a repeated unit within an organism. Primary modules can be combined to form higher-level structures.

Table 1. Continued

<i>Nematopore</i> : hollow structures with skeletal walls that have been incorporated into the extrazoooidal skeleton of stenolaemate colonies. Also called firmatopores. May have a thread-like or squat morphology and are typically interpreted as kenozooidal. <i>See also: Alveoli, Cancelli, Dactylethrae, Kenozooid, Mesozooid, Metapore, Tergopore.</i>
<i>Ooecium</i> : two-layered calcified part of an ovicell, comprising an outer ectooecium (partly or mostly membranous in some taxa) and inner endooecium, with a coelomic lumen (sometimes obliterated) between the two layers that is connected to coelom of the originating zooid.
<i>Ovicell</i> : a specialized brood chamber found in cheilostomes, consisting of a calcified ooecium and brood cavity, the entrance to which may be closed by the ooecial vesicle and/or the operculum of the maternal zooid. A multi-zoooidal complex. <i>See also Ooecium, Gonozooid.</i>
<i>Polymorphism</i> : variation in zooid morphology, including heterozooids and autozooidal polymorphs. <i>See also Autozooidal polymorph, Heterozooid.</i>
<i>Polypide</i> : soft body of the bryozoan, principally the tentacle crown and its sheath, gut, and intrinsic musculature and nervous systems.
<i>Pore plate</i> : an interzooidal wall element with one to several communication pores.
<i>Proximal</i> : in the direction of the ancestrula (away from the growing edge, towards the origin of growth).
<i>Rhizoid</i> : an elongated kenozooid typically used for attachment to the substratum or branch support in erect colonies.
<i>Stolons</i> : structures used to support the colony off the substratum or used in overgrowth competition.
<i>Spine</i> : a polymorphic autozooidal module, either an appendage of an autozooid or a kenozooid. Typically slender, hollow, and with or without a cuticular joint at its point of attachment. <i>See also: Appendage.</i>
<i>Tergopore</i> : hollow structures with similar walls to autozooids and interzooidal communication pores. Occur in stenolaemates. May be kenozooidal. <i>See also: Alveoli, Cancelli, Dactylethrae, Kenozooid, Mesozooid, Metapore, Nematopore.</i>
<i>Umbonuloid</i> : <i>see Frontal shield.</i>
<i>Vibraculum</i> : an extreme form of an adventitious avicularium, in which the operculum is elongated as a thin setiform mandible.
<i>Vicarious avicularia</i> : avicularia that take the place of an autozooid in the budding sequence. Usually of a similar size to autozooids.
<i>Vicariozooid</i> : a kenozooid replaces an autozooid and is capable of budding. Used to increase colony rigidity and margin robustness.

The interzooidal walls of both gymnolaemate and stenolaemate zooids are perforated by communication pores (often grouped into pore plates). These pores allow free communication between the exosaccal coeloms of neighbouring stenolaemate zooids (although some may become plugged by epithelial cells or covered by a calcareous plate), while the communication pores of gymnolaemates are each sealed by a cellular plug (or rosette; Mukai *et al.*, 1997).

In all taxa, intrazoooidal nutrient transfer may be facilitated by the funiculus, a fluid-filled semitubular cord that connects the stomach caecum to the body wall (Ryland, 1979; Mukai *et al.*, 1997). Since developing zooids at the edge of a bryozoan colony lack fully formed polypides, nutrients must be shared among individuals. In stenolaemates, interzooidal nutrient transfer is achieved in one of two ways: (i) diffusion or pressure changes through communication pores; or (ii) exchange through confluent, extra-skeletal spaces surrounded by a membranous wall (Ryland, 1979; Boardman, 1998). Interzooidal nutrient transport in gymnolaemates is facilitated by the funicular system, a branched network associated with communication pores that is secondarily evolved from the funiculus. In both taxa, nutrients dissolved in coelomic fluid are transported from actively feeding areas to the closest growing edge (Miles *et al.*, 1995 and references therein; Mukai *et al.*, 1997). Non-feeding modules are resourced in the same way.

II. POLYMORPHISM

In bryozoans, a distinction must be made between sources of continuous variation (astogenetic, ontogenetic,

developmental noise) and polymorphism. The first zooid in a colony (ancestrula) often has a different form from later zooids, and intermediate forms exist between them. Astogenetic variation occurs during maturation of the colony, and ontogenetic variation occurs during maturation of zooids in the same astogenetic zone. Autozooids may also vary within the same astogenetic and ontogenetic zone because of developmental noise or plasticity (Jebram, 1978). Developmental noise is intrinsic variance in developmental processes (Debat & David, 2001) and can result in slight variation in autozooid morphology or 'abnormal' zooids (e.g. double polypides; Jebram, 1978). Polymorphism is typically defined as discontinuous variation in form (i.e. no intermediate forms are present between polymorphic and normal zooids). Therefore, slight gradations in autozooid morphology are not considered polymorphic. Indeed, these sources of variation are also present in polymorphic zooids.

Plasticity, defined as the ability to express different phenotypes in response to environmental conditions, may also produce continuous or discrete variation in zooid morphology. Continuous plastic variation can occur in both regular autozooids and polymorphic zooids. For example, plasticity in zooids allows cheilostomes to form abnormally shaped zooids where zooid growth is constrained by some barrier (Jebram, 1978). Similarly, spinule length in *Membranipora membranacea* increases with increasing concentration of chemical cues from a nudibranch predator (Harvell, 1998). However, it is only when plastic responses produce discontinuous variation that they can be considered polymorphic. Polymorphs may contribute to the division of labour within a colony (Harvell, 1994; Lidgard *et al.*, 2012), which provides a further distinction between true

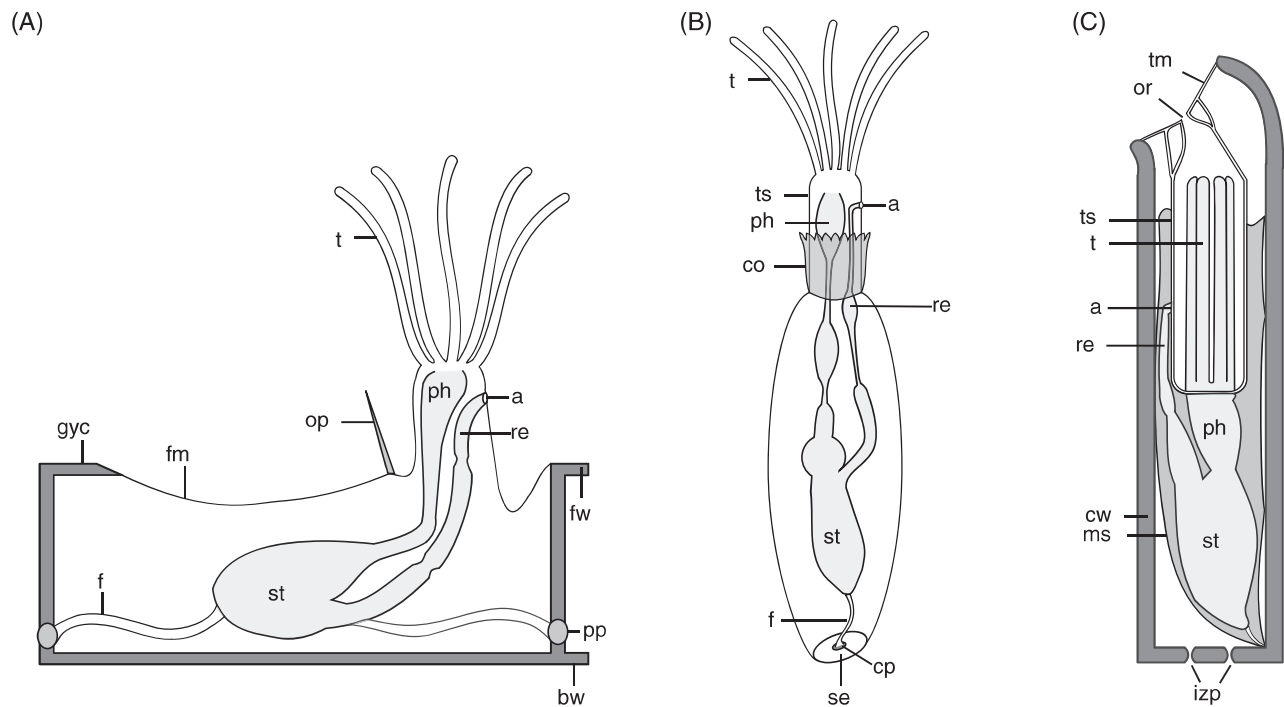


Fig. 1. Simplified zooidal anatomy of (A) an ascan cheilostome, (B) a stoloniferan ctenostome, and (C), a fixed-walled cyclostome. Reproductive organs and musculature not depicted. a, anus; bw, basal wall; co, collar; cp, communication pore; cw, cystid wall; f, funicular cord; fm, membranous part of frontal wall; fw, frontal wall; gyc, gymnocyst (calcified part of frontal wall); izp, interzooidal pores; ms, membranous sac; op, operculum; or, orifice; ph, pharynx; pp, pore plate (hosting a communication pore); re, rectum; se, septum; st, stomach; t, tentacle; tm, terminal membrane; ts, tentacle sheath. A and C redrawn from Ryland (1970), B from Nielsen & Pedersen (1979).

polymorphism and continuous plasticity (where differences in function type are non-existent).

It is also important to consider *when* zooids become polymorphic. Consistent polymorphs are zooids that begin as polymorphs and remain polymorphic throughout their lifespan (e.g. avicularia). Reversible polymorphs are temporary modifications to autozooids, while irreversible polymorphs begin as autozooids but are permanently transformed into polymorphic zooids. Both may be tied to ontogeny and the polypide degeneration–regeneration cycle. While irreversible and reversible polymorphs can be discontinuous plastic responses, it is unclear if consistent polymorphs are generated in response to environmental conditions. Abiotic macroenvironmental conditions do not influence the number and type of consistent polymorphs produced (Hughes & Jackson, 1990; Simpson, Jackson & Herrera-Cubilla, 2017). However, microenvironmental variations within a colony and predatory epibionts (Lidgard, 2008; Lidgard *et al.*, 2012) may induce the production of consistent polymorphs.

(1) Polymorphism classification

Resource sharing within a colony (McKinney & Jackson, 1989) permits the existence of non-feeding polymorphs (and developing zooid buds), although some polymorphic zooids are capable of feeding. Polymorphic zooids are

distinguished from regular autozooids by modifications to their cystid, polypide, or both (Fig. 2). The cystid may be modified without change to the polypide and *vice versa*. However, they are integrated structures that are not fully independent: dwarf or irregularly shaped cystids possess reduced or aborted polypides. In phylactolaemates and cyclostome stenolaemates the polypide is formed before the cystid, while in gymnolaemates the cystid is formed first (Mukai *et al.*, 1997). This suggests that the cystid exerts control over polypide formation in the Gymnolaemata (the zooecium size hypothesis; Harvell, 1994), while the polypide (and its possible absence) exerts control on cystid formation in other bryozoan taxa. The zooecium size hypothesis also operates in the other direction: abnormally large polypides (conjoined or double polypides) have been found only in enlarged cystids (Jebram, 1978; Harvell, 1994), and ‘macrozooids’ in Trepostomata may have contained larger polypides (Boardman & Buttler, 2005).

Despite high structural and physiological integration, the cystid and polypide also exhibit a degree of modularity in polymorphic zooids and can be considered ‘zooidal semimodules’. Polymorphic zooids can therefore be classified according to modifications of their semimodules (Table 2).

Extrazoooidal structures, like polymorphs, are derived phylogenically from autozooids, so the distinction between extrazoooidal and zooidal may be unnecessary (P.D. Taylor,

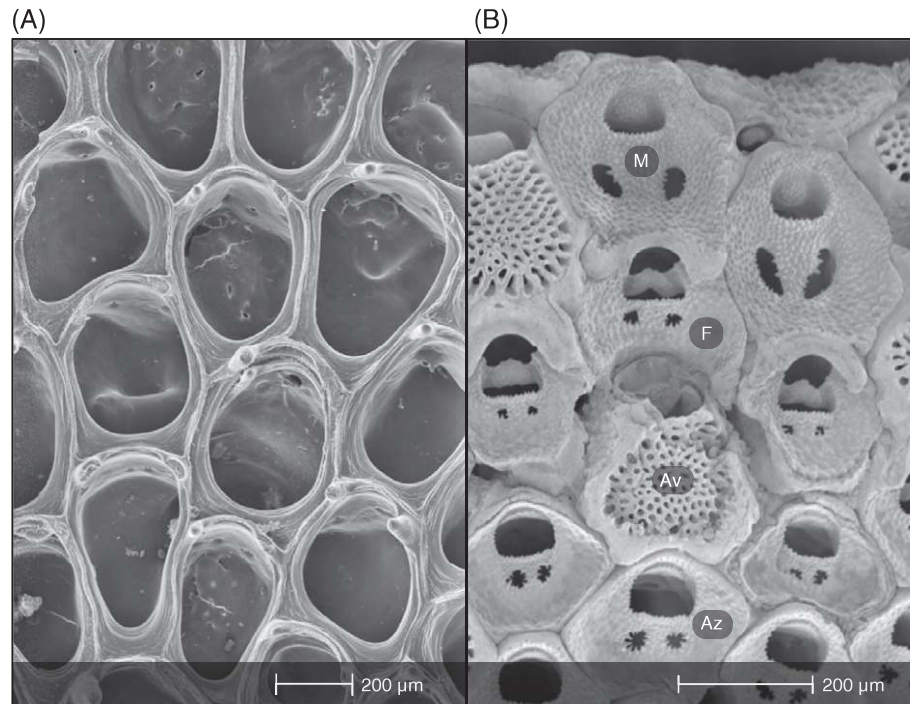


Fig. 2. A monomorphic colony (A, *Membranipora pura*) and a polymorphic colony (B, *Selenaria punctata*). Av, avicularium; Az, autozooid; F, female zooid with oocidium; M, male zooid. A by D.P.G., B by P.E. Bock.

personal communication). However, the distinction is useful for determining structural homology and the evolutionary pathways used to generate similar structures. Distinguishing between different forms is difficult, so polymorphs will be discussed in terms of their unifying similarities to facilitate comparison among polymorph types and bryozoan taxa. These unifying similarities group polymorph types based on structure (although not implying homology) and probable function.

The resources required for the construction and support of polymorphs must be offset by their function. As suspension feeders, bryozoans maintain an energetically marginal existence. Therefore, any adaptation that provides even a small increase in colony efficiency can significantly influence survival and competitive ability (Thorpe, 1979; Lidgard, 1985).

Polymorphic zooids may be arranged with regular autozooids in a cormidium (a colony within a colony; Beklemishev, 1969; Ryland, 1979; Haeckel, 1888; Section VIII.2). The vital functions a cormidium can perform are determined by its submodules, and the number of each submodule type should influence the magnitude of those functions (Schack *et al.*, 2018). Submodules include autozooids, autozooidal polymorphs (i.e. autozooids with cystid modifications), heterozooids, multizooidal complexes (Schack *et al.*, 2018), or extrazooidal structures. Although they can be part of a cormidium, non-feeding groups of heterozooids and extrazooidal structures cannot constitute a cormidium on their own (i.e. they cannot fulfil the basic functions vital for life). These ‘paramodules’ can

occur at any level of modular organization and need not be iterated within the colony (e.g. a kenozooidal attachment stalk).

III. MANDIBULATE POLYMORPHS

(1) Avicularia

Cheilostome avicularia are highly modified zooids: their reduced polypide retains a sensory and potentially secretory function, but can no longer feed (Mukai *et al.*, 1997; Carter, Gordon & Gardner, 2008). Instead, energy is likely obtained from the funicular system (Lutaud, 1983; Carter *et al.*, 2008; Carter, Gordon & Gardner, 2010a). The operculum is enlarged into a mandible while the cystid is distally stretched and tapered (Fig. 3; Mukai *et al.*, 1997). The main types are: (i) vicarious avicularia, generally equal (but can be larger) in size to autozooids and replacing them in the budding sequence; (ii) interzooidal avicularia, smaller than autozooids and fitted between them (i.e. their basal walls rest on the substratum); (iii) adventitious avicularia, formed on the frontal, basal, or lateral walls of the autozooid; and (iv) vibracula, an extreme form of adventitious avicularium with an elongated setiform mandible and unique hinge structure (McKinney & Jackson, 1989; Carter *et al.*, 2010a). Sessile forms can be flush with the surface of the autozooid, while pedunculate forms are elevated on a peduncle or stalk (Fig. 4; McKinney & Jackson, 1989).

Table 2. Classification of polymorphs according to modification of their semimodules.

Polymorphic structure	Definition	Examples	Note
Modified polypide	Cystid identical to that of a regular autozooid. Tentacle (number, length, ciliation), polypide size, and the presence/absence of other structures may be modified.	Male zooids in <i>Odontoporella bisulphi</i> (Cheilostomata; Gordon, 1968; Carter & Gordon, 2007).	Rare, perhaps owing to difficulty of observation (living material required). May be due to polypide degeneration–regeneration cycles (Rogick, 1963; Powell, 1967).
Modified cystid	Polypide identical to that of a regular autozooid. Retains feeding ability. Modifications may be to zooid size, shape, orificial structures, or to ‘appendages’ on the zooid surface	B-zooids in <i>Stegonoporella</i> (Cheilostomata); <i>Membranipora membranacea</i> autozooids with predator-induced spinules on the frontal wall (Cheilostomata; Harvell, 1984).	Referred to as ‘autozooidal polymorphs’ or ‘appendaged autozooids’ (Silén, 1977; Schack <i>et al.</i> , 2018).
Modified cystid + polypide	Cystid and polypide are modified, and zooids may have protrusible but non-feeding polypides	<p>Dwarf reproductive zooids in <i>Calloporella hyalina</i> (Cheilostomata); nanozooids and gonozooids in Cyclostomata</p> <p>Avicularia with reduced polypide</p> <p>Kenozooids</p>	<p>Silén (1977) distinguished ‘autozooidal polymorphs’ from ‘heterozooids’ based on their ability to feed (and thus fulfil the primary function of an autozooid). However, there are several variations in the non-feeding zooids (heterozooids) present. Non-feeding polypides may be capable of other functions (sensory, secretory, sperm gathering, etc.).</p>
Multizoooidal complex	Formed from modifications to two or more zooids	Some ovicells (Cheilostomata)	
Extrazoooidal structure	Structures external to zoooidal boundaries at all stages of development (Boardman & Buttler, 2005). Can be solid skeleton or space-enclosing skeletal structures (McKinney & Jackson, 1989).	Alveoli, styles (Stenolaemata)	

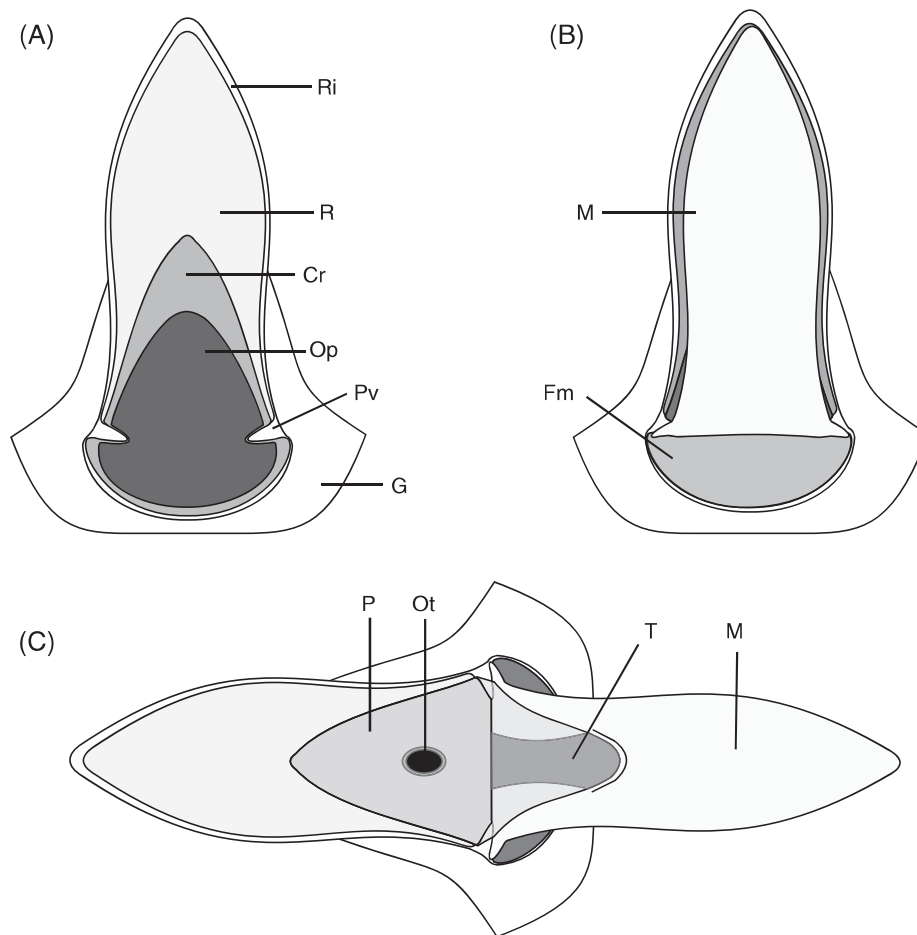


Fig. 3. Structure of a vicarious avicularium, frontal view. (A) skeletal morphology, (B) living state, mandible closed, (C) living state, mandible open (rotated 90°). Cr, cryptocyst; Fm, frontal membrane; G, gymnocyst; M, mandible; Op, Opesia; Ot, opening to tentacle sheath of reduced polypide (palatal foramen); P, palate; Pv, pivot structure (hinge); R, rostral area; Ri, rim; T, adductor muscle tendon.

Mandibles vary from spatulate, triangular, or fan-shaped, to highly irregular forms that bear spine-like projections (Fig. 5; Schack *et al.*, 2018) and the rostrum (part of the cystid that the closed mandible fits into) is often modified to accommodate particular mandible morphology. Avicularia varying markedly in size and shape are often found on the same colony. For example, Carter *et al.* (2008) described avicularia with oval and acute mandibles in the same colonies of *Stephanollona scintillans*, and in many other species of Phidoloporidae.

There is also a high degree of variation present in avicularian orifices and rudimentary polypide structure. While most taxa only have a pore in the membranous wall (palate) below the mandible, in some the tip of the vestigial polypide can protrude when the mandible is open (Carter *et al.*, 2010a). In *Rhynchozoon zealandicum*, this polypide has papillae covered in microvilli and cilia, while those of *Bugulina flabellata* and *Arachnopusia unicornis* possess prominent tufts of cilia. *Nordgaardia cornucopioides*, on the other hand, has a tentacle sheath that is hypertrophied into a secretory plug (a cluster of glandular cells containing secretion vesicles)

and has internal cilia (Carter *et al.*, 2010a). Still other taxa are equipped with tubular orifices or bulbous extensions, providing evidence for the high degree of variation in form (and potential function) present in avicularia (Carter *et al.*, 2008, 2010a). When the sensory cilia of avicularia are triggered, the mandibles are snapped shut using well-developed adductor muscles (Kaufmann, 1971; Mukai *et al.*, 1997). Like mechanosensory ciliary tufts, the secretory plugs are associated with a cerebral ganglion, but the trigger for glandular secretions and their function are unknown (Carter *et al.*, 2010a). Avicularia may also have chemosensory capabilities: *Microporella vibraculifera*, *Parasmittina collifera*, and *Bugulina californica* responded strongly to a mixture of amino acids (known feeding stimulators, also indicative of prey injury; Winston, 1991).

The function of avicularia is still debated, particularly owing to the variation in avicularian morphology. Adaptations in bird's-head avicularia suggest a defensive function: the shape and mineralization of these structures reduces mechanical stress associated with grasping a struggling organism, and nodding behaviour allows them

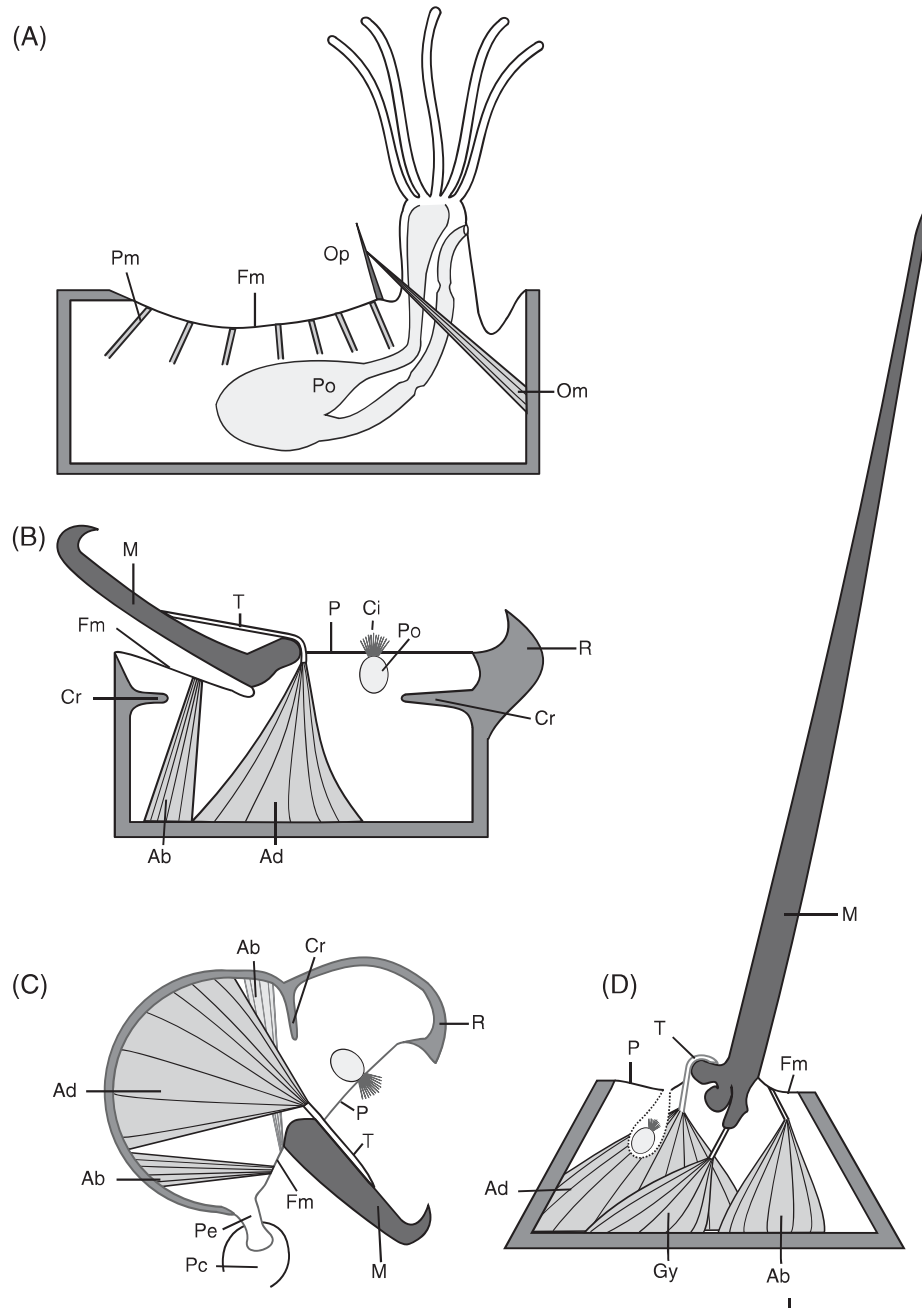


Fig. 4. Comparison of the musculature and polypides of avicularia and an autozooid. (A) Cheilostome autozooid, (B) sessile avicularium, (C) adventitious bird's-head avicularium, (D) vibraculum. The cryptocyst is not shown in D. Ab, abductor muscle (depresses frontal membrane and opens mandible); Ad, adductor muscle (closes mandible); Ci, ciliary tuft, part of the polypide; Cr, cryptocyst; Fm, frontal membrane; Gy, gyator muscle; M, mandible; Om, occlusor muscle of the operculum; Op, operculum; P, palate; Pc, peduncle cushion, provided by autozooid; Pe, peduncle; Pm, transverse parietal muscle (depresses frontal membrane and protrudes polypide); Po, polypide; R, rostrum; T, adductor muscle tendon. A and B modified from Ryland (1970), C from Kaufmann (1971), D from Marcus (1962).

to increase capture rates by 'patrolling' (Kaufmann, 1971). These avicularia can capture large organisms by seizing terminal segments or hairs (e.g. a 4 mm amphipod caught by a 0.2 mm avicularium; Kaufmann, 1971), although captured epibionts typically escape (Kaufmann, 1971; Winston, 1986; Lidgard *et al.*, 2012). This suggests that mitigation of potential

damage, rather than removal or destruction of epibionts, is the main function of such avicularia (Kaufmann, 1971). If defence is the main function of avicularia, variation in mandible morphology may simply reflect specializations for catching different epibionts (Silén, 1977). In addition to mechanical defence, avicularia may provide a chemical

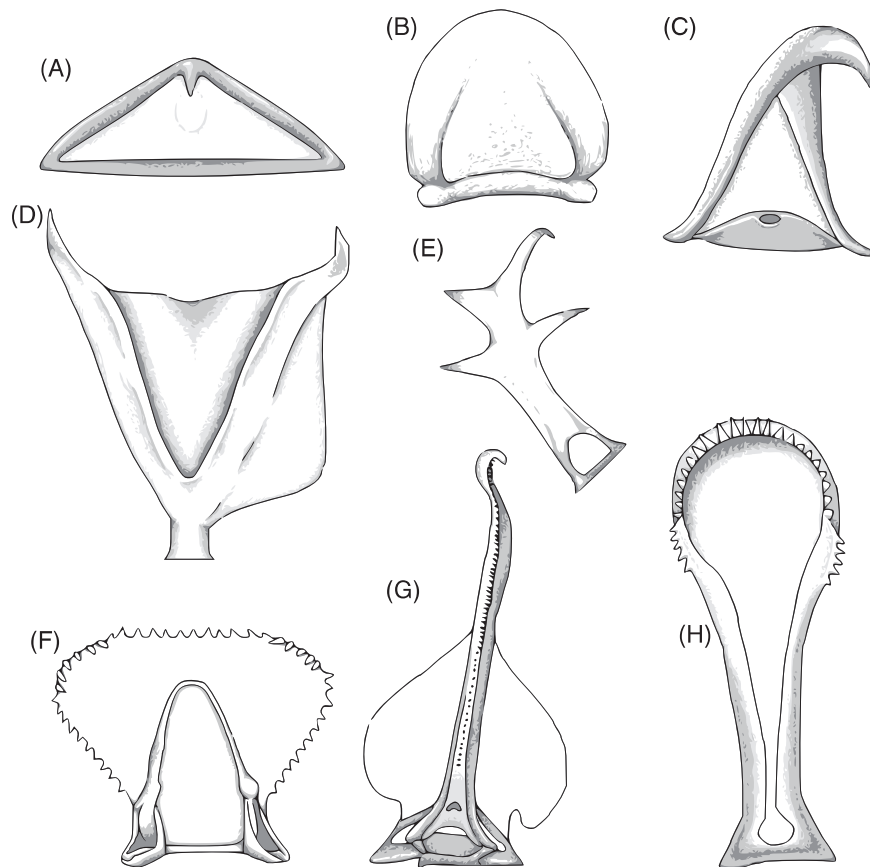


Fig. 5. Diversity of avicularian mandibles. (A) *Cellaria punctata*, (B) *Crassimarginatella harmeri*, (C) *Amastigia rudis*, (D) *Bryopesanser thricyng* (as *Escharina pesanseris*), (E) *Licornia longispinosa* (as *Scrupocellaria*), (F) *Steginoporella dilatata* (B-zooid), (G) *Smittipora cordiformis*, (H) *Labioporella thornelyae*. A, C, and E–H drawn from Harmer (1957), B drawn from Fransen (1986), D drawn from Gordon (1984).

deterrent which would help explain the presence of a secretory plug in some forms of avicularia (Carter *et al.*, 2008, 2010a).

Avicularia may also direct waste-water currents to colony margins and chimneys, aiding in the removal of excretory waste. A waste-removal function may explain spatulate mandible types and sporadic spacing of avicularia (wide spacing may enhance waste-removal currents but would decrease defensive capabilities; Kaufmann, 1971; Winston, 1984). It is also possible that avicularia catch and retain epibionts to create bait for organisms small enough to be consumed by the bryozoan [e.g. bacteria (Goldstein, 1880, cited in Scholz & Krumbein, 1996)]. Several other functions of avicularia have been proposed (nutrient storage, direct food capture, aid in respiration, baffles), but there is little evidence for these (Marcus, 1926, cited in Winston, 1984).

Unlike avicularian mandibles, which are hinged to swing through one plane, the elongate mandibles of vibracula rotate and sweep over the colony (McKinney & Jackson, 1989; Carter *et al.*, 2008). Their extreme morphology (Fig. 4), may indicate a different purpose from other avicularia. Vibracula respond to tactile stimulation and may sweep debris and organisms from the colony surface (McKinney & Jackson, 1989; Winston, 1991). However, some free-living forms use

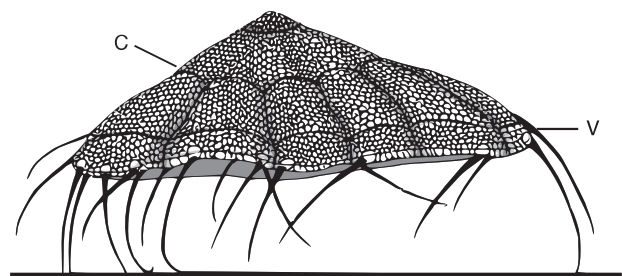


Fig. 6. A 'walking' colony of *Selenaria maculata*. C, colony; V, vibracular seta. Drawn from Cook & Chimonides (1978).

vibraculum-like avicularia to raise their colony above the substratum, unbury or overturn itself, or as a means of locomotion (Fig. 6; Cook & Chimonides, 1978).

(a) B-zooids

Some cheilostomes, mainly in *Steginoporella* (Steginoporellidae) and *Macropora* (Macroporidae), possess polymorphic feeding zooids with enlarged mandibles. These 'B-zooids' have a reinforced orifice, a thick skeletonized operculum, and larger opercular muscles (Banta, 1973; Cheetham *et al.*,

2006; Winston, Vieira & Woollacott, 2014). *Steginoporella mandibulata* is the only species of *Steginoporella* where the B-zooids have non-protrusible polypides and can be considered 'true' avicularia (Harmer, 1957; Banta, 1973). B-zooids may represent an early stage in the evolution of avicularia (Harmer, 1957; Banta, 1973).

The reduction of the polypide in avicularia allows the avicularian cystid also to shrink in size, which in turn permits more integrated forms (e.g. interzooidal and adventitious avicularia), allowing defensive and feeding functions to be fulfilled in the same space. The functional polypide of a B-zooid allows it to feed, while its enlarged operculum allows it to trap organisms (Winston, 2004). The loss of a B-zooid's feeding ability in the transition to a vicarious avicularium would need to be offset by the increased efficiency of another function. The energetic cost of this may be the reason why only *S. mandibulata* has avicularia, while other *Steginoporella* only have B-zooids (or lack mandibulate polymorphs). However, B-zooids are far less prevalent than avicularia in other cheilostome taxa. For example, the genus *Crassimarginatella* has several species with B-zooids (*C. similis*, potentially *C. latensis*; Cook, 1968), while others typically have vicarious avicularia. Although the transition from B-zooid to vicarious avicularium may have initially been more expensive, vicarious avicularia provide several benefits: damage to a vicarious avicularium will not affect colony feeding ability; muscle size is not limited by the presence of a feeding polypide; and rostrum shape is not restricted to accommodate the protruded tentacle crown (e.g. B-zooids with narrow setose mandibles do not exist).

Separating feeding and defensive structures into discrete modular units may be more efficient than a single 'multitasking' unit. Lineages that retain B-zooids either cannot afford the reduction in feeding ability or do not require the increased efficiency.

(b) Evolution of avicularia

The earliest avicularia appear in the Cretaceous (Albian–Cenomanian) genus *Wilbertopora*. These avicularia range from barely modified B-zooids retaining a functional tentacle crown and gut (*W. mutabilis*) to highly modified (pointed and interzooidal avicularia inferred to have lacked the ability to feed (*W. acuminata* and *W. hoadleyae*; Cheetham *et al.*, 2006). However, living genera also possess species with a similar range of avicularian modification (*Crassimarginatella*; Cook, 1968). There are several trends in the modification of autozooids to avicularia: (i) distal elongation and decreased opesia area; (ii) reduction of the polypide and its feeding ability; (iii) possession of an elevated rim; (iv) production of a resting surface for the mandible (e.g. an incomplete shelf or hard, complete palate); (v) inclusion of a pivot structure (condyles or a pivot bar) that separates the orificial and opesia areas; and (vi) transition from a small, rounded operculum to an enlarged, pointed mandible (Fig. 7; Cook, 1968; Cheetham *et al.*, 2006). However, all trends (particularly 4–6) may not necessarily be present in all avicularia. The transition from rounded to pointed mandibles may follow a

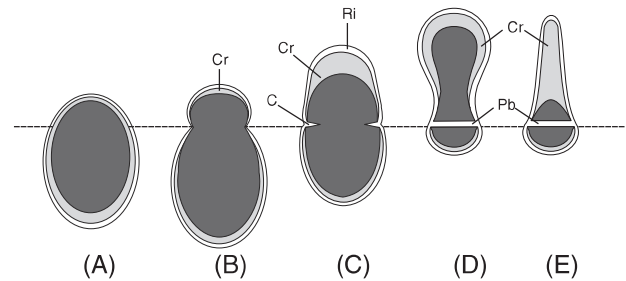


Fig. 7. Increasing modification of avicularian skeletal parts, showing development of the cryptocystal shelf, reduction in the post-mandibular area, elongation of the mandibular area, and the development of hinge structures for the mandible. Below the line is the post-mandibular area, above it is the orificial/mandibular area. (A) autozooid, (B) B-zooid with slight modification, as in *Wilbertopora mutabilis*, (C) B-zooid with moderate modification, as in *W. listokiniae* or *Steginoporella murachbanensis*, (D) spatulate avicularium (strongly modified), as in *W. manubriiformis* or *Crassimarginatella spatulata*, (E) triangular avicularium (strongly modified), as in *W. sanneriae* or *C. falcata*. C, condyles (hinge); Cr, cryptocystal shelf; Pb, pivot bar; Ri, rostral rim.

latitudinal gradient but this has not been tested statistically (Schopf, 1973).

Since Cretaceous *Wilbertopora* and Paleogene–Recent *Crassimarginatella* both show a similar range of avicularian modification, it is likely that avicularia arose independently multiple times over the course of cheilostome evolution (Cheetham *et al.*, 2006). Although vicarious avicularia were the first to evolve, adventitious avicularia occur more frequently and at higher densities. This may reflect a trend towards increased colonial integration and efficiency, allowing colonies to possess highly modified avicularia without losing the energy provided by fully functional autozooids (Carter *et al.*, 2010a; Lidgard *et al.*, 2012). Even if some avicularia no longer provide a solely defensive function, repeated evolution of avicularia may be in response to predation by epibionts (Lidgard *et al.*, 2012). Similarly, a trend for increasing colonial integration and avicularian/vibracular capability in free-living lunulitiform bryozoans is likely. In *Otionellina* (Otionellidae) vibracula clean the colony surfaces, while vibracula in *Selenaria* (Selenariidae; Fig. 6) use elongate mandibles to 'walk' across the substratum, right themselves if overturned, and unbury themselves if covered by sediment (Cook & Chimonides, 1978; Chimonides & Cook, 1981).

(2) Eleozooids and aviculomorphs

The clearest stenolaemate analogue to cheilostome avicularia is found in the extinct Eleidae (Cyclostomata). Eleids are unique among stenolaemates because they possessed a calcareous operculum and avicularium-like heterozooids called eleozooids (Taylor, 1986; Viskova, 2016). Like avicularia, eleozooids may have lacked a feeding polypide. Their preserved opercula are typically hypertrophied, but may also be reduced in comparison with autozooids (Taylor,

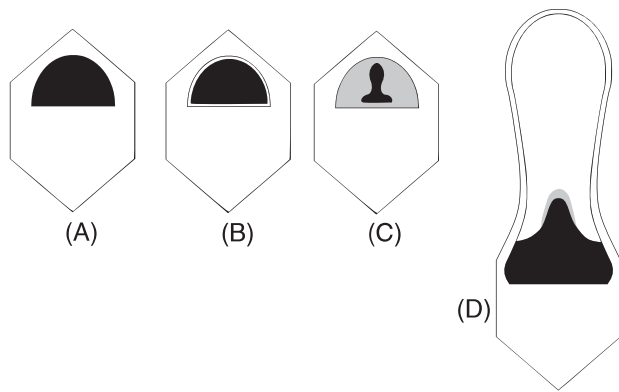


Fig. 8. Eleid autozoid and eleozoids. (A) autozoid, (B) demizoid, (C) trifolizoid, (D) rostrozoid. Redrawn from Fig. 1 of Taylor (1986).

1994). Rostrozooids had an aperture that is enlarged and distally tapered into a rostrum, with similar variations to avicularian mandibles. Trifolizoids had small apertures resembling an inverted 'T'. Demizoids had D-shaped apertures and opercula. Both trifolizoids and demizoids are smaller than autozooids but can replace them in a series (Taylor, 1986, 1994) (Fig. 8).

There were no adventitious eleozoids (all are analogous to vicarious or interzooidal avicularia), but there is still a trend towards increasing colonial integration of polymorphs, similar to that in cheilostomes (Lidgard *et al.*, 2012). After their initial appearance [100.5–113.0 million years ago (Mya)], when they were randomly arranged within colonies, a regular arrangement of eleozoids arose in free-walled (93.9–100.5 Mya) and fixed-walled (86.3–89.8 Mya) species (Taylor, 1986). Although typically reserved for cheilostomes, the term *cormidium* could be applied to these patterns of autozooids and eleozoids (especially in the genus *Atagma*, which has autozooids surrounded by smaller demizoids; Taylor, 1986).

It is likely that eleozoids performed similar functions to avicularia (Viskova, 2016). However, eleozoids have no analogue in Recent cyclostomes (Taylor, 1986, 1994; Viskova, 2016), so their exact function cannot be observed.

The mandibles of both avicularia and eleozoids are derived from opercula, which are present only in cheilostomes and eleids. In this way, eleids may be convergent with cheilostomes (Taylor, 1986). It is clear that the operculum is a key structure in the evolution of avicularian-like polymorphs (Schopf, 1973; Taylor, 1986), although the existence of an operculum does not automatically ensure that these structures will arise, since eleids lacking eleozoids persisted until the Maastrichtian (66.0–72.1 Mya; Taylor, 1986) and Recent non-polymorphic cheilostomes [and one operculum-bearing genus of Ctenostomata (*Penetrantia*); Pohowsky, 1978] still exist.

An operculum may not be necessary to evolve a mandibulate polymorph. In *Fenestropora*, a Devonian genus of stenolaemates, avicularian-like structures are present on the underside of colony branches. These 'aviculomorphs'

consist of a concave pit surrounded by a triangular outline with an acute tip and may have had a cuticular mandible (McKinney, 1998). Aviculomorphs may have arisen from modifications to the laminar extrazoooidal skeleton, like cyclozoecia (McKinney, 1998). Cyclozoecia are a similar size to aviculomorphs and occur primarily on the underside of branches (McKinney, 1998). The difference between the circular outline of cyclozoecia and the triangular outline of aviculomorphs is reminiscent of the change from a circular operculum to a pointed mandible in some avicularia.

IV. KENOZOIDS, AUTOZOOIDAL APPENDAGES AND EXTRAZOOIDAL STRUCTURES

Kenozooids lack a polypide. These zooids can either be vicarious (budded between zooids) or adventitious (budded on zooids). In gymnolaemates, kenozooids are not 'empty', but are enervated by the funicular system and may contain musculature (Cheetham & Cook, 1983; Lutaud, 1983; Gordon & Parker, 1991b). The funiculus is formed from an epithelial layer of the cystid basal wall (at least in *Membranipora membranacea*; Lutaud, 1983), not from the developing polypide. Similarly, muscles external to the polypide, such as apertural and parietodepressor muscles, are formed from groups of myocytes on the walls of the cystid buds (Lutaud, 1983). Since the cystid walls are formed before the polypide bud itself, the construction of a gymnolaemate kenozooid should not require a polypide bud during development.

In stenolaemates the polypide forms before the cystid (Mukai *et al.*, 1997), suggesting that initial development of the polypide and subsequent degeneration are required in kenozooid formation. Further evolution of kenozooidal structures in stenolaemates may have resulted in genetically 'preprogrammed' kenozooids that skip polypide formation entirely. However, doing so likely required more changes in developmental timing (*via* further mutations) than in gymnolaemates.

The apparent ease by which gymnolaemates can develop kenozooids may contribute to their high level of polymorphism. Since the kenozooid is unconstrained by the need to accommodate a polypide (both during and after development), it can fulfil a wide range of morphologies. In cheilostomes, these kenozooidal morphs were likely responsible for two important taxonomic novelties: the frontal shield and the oecium (see Sections IV.5 & V.2; Dick *et al.*, 2009; Ostrovsky & Taylor, 2005a; Lidgard *et al.*, 2012). High polymorphism arising from kenozooids is apparent in a cyclostome family with kenozooids: species of Crisiidae can have branches of vicarious kenozooids (e.g. *Bicrisia abyssicola*), hollow spines (*Crisidia cornuta*), and even kenozooidal rhizoids (*Crisia ramosa*; Hayward & Ryland, 1985). While the presence of kenozooids does not automatically ensure the evolution of such structures, it provides an important module necessary

for their construction (similar to operculate zooids in the development of avicularia).

Kenozooids are easily confused with extrazoooidal structures in stenolaemates. The main distinction between kenozooids and extrazoooidal chambers is that the space inside a kenozooid cystid is homologous to that of an autozooid, while the space in an extrazoooidal chamber is created *de novo* by delimiting extrazoooidal walls and is not homologous to that of an autozooid. Given the potential difficulty in developing kenozooids in stenolaemates, developing extrazoooidal structures may have been an evolutionarily easier solution, with the extrazoooidal skeleton serving as an ‘unconstrained module’. The evolution of extrazoooidal structures requires further investigation.

Kenozooids may also be confused with autozooidal appendages. Autozooidal appendages are not polymorphic zooids themselves but are modifications to the cystid of an autozooid. These appendages vary in form (e.g. spines, peristomes, tubercles) but make an autozooid polymorphic only if they exhibit discontinuous variation with other zooids in the same astogenetic and ontogenetic zone.

(1) Space-filling and strengthening structures

In cheilostomes, space-filling kenozooids are budded at branch edges, colony stalks, and between zooids when space is limited. In erect species, kenozooids often appear in a consistent pattern (e.g. *Spiralaria florea* and *Chelidozoum ternarium*) (Silén, 1977; McKinney & Jackson, 1989; Gordon & d’Hondt, 1991), although this can also occur less frequently in encrusting species (*Hesychoxenia praelonga*; Gordon & Parker, 1991b). In *Chelidozoum ternarium* the main colony structure is provided by slender kenozooidal chains (Gordon & d’Hondt, 1991), while in *Hesychoxenia praelonga* kenozooids are budded along the colony margin and alternate with pairs of autozooids (Gordon & Parker, 1991b). These vicarious kenozooids (‘vicariozooids’ of Silén, 1977) can be irregular or similar in shape to autozooids, but are typically smaller than autozooids. Cheilostome kenozooids may bud spines (as in *Chorizopora ferocissima*) or adventitious avicularia (e.g. *Chaperiopsis cristata*), and may be the daughter zooid in an ovicell complex (Gordon, 1984; Ostrovsky *et al.*, 2009). In general, the function of space-filling kenozooids may be to increase colony rigidity and the robustness of colony margins (Silén, 1977; McKinney & Jackson, 1989). This function explains the presence of elongated spicule bundles in the kenozooids of *Hesychoxenia praelonga*, which encrusts flexible seagrass stems (Gordon & Parker, 1991b).

In stenolaemates, space-filling structures may be arranged between zooids to ensure sufficient distance between apertures and tentacle crowns (as in the cyclostome suborders Cancellata, Rectangulata, and Cerioporina; Taylor & Weedon, 2000). Such structures may provide an alternative colony-strengthening method to thickened autozooid walls (Key, 1991) or an energetically expensive extrazoooidal skeleton. Space-filling polymorphs may also be formed in response to occlusion by gonozooids (see Section V.4; Schäfer, 1991). Many types occur in stenolaemates:

mesozooids/mesopores, metapores, nematopores, cancelli, alveoli, dactylethrae, and exilazooids/exilapores in addition to kenozooids. Most of these structures are not clearly zooidal, kenozooidal, or extrazoooidal (Table 3), and the terms are often ambiguous, and further revision of space-filling polymorphs is necessary (e.g. Batson & Smith, 2018). Not only will this clarify terminology, but it will also determine the taxonomic extent of kenozooidal and extrazoooidal space-filling structures.

(2) Stolons

Stolons are elongated, cylindrical structures that are typically kenozooidal (Silén, 1977) but may be adventitious appendages (e.g. *Immergentia*: Ctenostomata and *Aetea*: Cheilostomata; Pohowsky, 1978; Hayward & Ryland, 1998). In many ctenostomes stolons budded distally generate the main architecture of the colony, creating branching patterns, while autozooids and adventitious stolons are budded laterally on a principal stolon (Fig. 9; Pohowsky, 1978; Cheetham & Cook, 1983; Souto, Fernández-Pulpeiro & Reverter-Gil, 2010; Souto *et al.*, 2011). In cheilostomes, terminal kenozooidal stolons at colony margins and frontal stolons form from an evagination of the frontal wall (Gordon, 1972). Both can be produced within a single colony (e.g. *Schizoporella unicornis*). Their morphology is variable: frontal stolons in *Celleporaria* sp. are short and bulbous (Osborne, 1984), but those in *C. apiculata* can be 30 times the length of the autozooid (Tzioumis, 1994). Both types can be produced in response to contact between encrusting bryozoan colonies (Fig. 9). Stolon production can result in cessation or redirection of growth in addition to competitive overgrowth (Osborne, 1984; Tzioumis, 1994). Large colonies of *M. membranacea* use stolons to slow the growth of small intraspecific competitors and allow the larger colony to surround it (Padilla *et al.*, 1996).

In addition to kenozooidal stolons, *M. membranacea* produces vertical growths that may be analogous to frontal stolons (Osborne, 1984). These ‘tower zooids’ are kenozooids ontogenetically derived from autozooids (Cook & Chimonides, 1980; Xing & Qian, 1999). Tower zooids lack a polypide and orifice and, most notably, possess an evaginated cuticular frontal wall that can grow up to 8.4 mm high (Fig. 9; Cook & Chimonides, 1980; Hayward & Ryland, 1998). These structures may control water currents above the colony surface or provide protection from abrasion (Cook & Chimonides, 1980; Xing & Qian, 1999). This latter function is particularly important for *M. membranacea* since it encrusts red algae and may be frequently scraped across other fronds or substrata (Cook & Chimonides, 1980; Xing & Qian, 1999). Frontal stolons are reversibly induced polymorphs, only lasting for around 6 weeks (Osborne, 1984; Tzioumis, 1994), while tower zooids are irreversible polymorphs. When comparing these structures, it is tempting to suggest that the reversible frontal stolon may have given rise to the irreversible tower zooid, or *vice versa*.

Table 3. Descriptions of space-filling polymorphs in stenolaemates.

Space-filling polymorph	Description	Taxa	Author	Condition
Metapore	Deep cavities in the extrazoooidal skeleton that lack skeletal walls of their own.	Rhabdomesina, Cryptostomata; <i>Petalopora costata</i> , <i>Grammascoecia dichotoma</i> , <i>Reteporidae lichenoides</i> (Petaloporidae: Cancellata)	Boardman (1983, 1998); Schäfer (1991)	Extrazoooidal
Nematopores (syn. firmatopores)	Slender, thread-like kenozooids.	Tubuliporina	Brood (1972); Boardman (1983)	Kenozooidal
Tergopore	Squat, unclosed kenozooids with strong skeletal walls. Pores with walls that are structurally similar to autozooids and are perforated with interzooidal communication pores.	<i>Discoyctis ecentrica</i> ; <i>Discoyctis infundibuliformis</i> <i>Crisina</i> , <i>Plauronea</i>	Schäfer (1991) Brood (1972)	Kenozooidal Kenozooidal
Alveoli	Shallow polymorphs with walls perforated by communication pores. Morphological differentiation of the extrazoooidal skeleton.	<i>Disporella</i> Rectangulata	Boardman (1998) Schäfer (1991)	Extrazoooidal Extrazoooidal
Cancelli	Vacuoles with walls that have a similar microstructure to autozooidal walls. Syn. cancelli	<i>Disporella</i> ; <i>Lichenopora</i> : Rectangulata	Brood (1972)	Kenozooidal
	Kenozooids often sealed by a calcified exterior wall. Syn. cancelli	Cyclostomata	Taylor & Weedon (2000)	Kenozooidal
	Vacuoles in the extrazoooidal skeleton without skeletal walls.	Homerids	Boardman (1998)	Extrazoooidal
	Terminally occluded, short kenozooidal chambers.	<i>Crisina waltersi</i> (Petaloporidae: Cancellata); <i>Discoyctis canadensis</i> (Cytididae: Cancellata)	Schäfer (1991)	Kenozooidal
	Alveoli occluded by calcaerous diaphragms. Vacuoles with walls that have a similar microstructure to autozooidal walls. Syn. alveoli	<i>Lichenopora</i> : Rectangulata <i>Lichenopora</i> : Rectangulata	Harner (1896) Brood (1972)	Not given Kenozooidal
Dactylethrae	Kenozooids often sealed by a calcified exterior wall. Syn. alveoli	Cyclostomata	Taylor & Weedon (2000)	Kenozooidal
	Short kenozooids.	<i>Tenbellaria</i> , Cyclostomata	Brood (1972); Boardman (1983)	Kenozooidal
	Small, club-like chambers, always closed by a terminal diaphragm.	Ceritoporina	Schäfer (1991)	Extrazoooidal
Mesozooid/ mesopore	Tapering vacuoles, which are too small to house a functional polypide and may be closed by a diaphragm. Can possess internal diaphragms or cysts.	Palaeozoic Stenolaemata	Boardman (1983, 1998)	Kenozooidal
		Cyclostomata Trepotomata	Schäfer (1991) Boardman & Butler (2005)	Extrazoooidal Extrazoooidal
Exilazoooid/exilapore	Vacuoles with few or no diaphragms, but possessing internal flask-shaped structures.	Trepotomes	Boardman & Butler (2005)	Extrazoooidal
	Small zooids with flask-shaped structures and potentially reduced polypides.	Palaeozoic Stenolaemata	Utgard (1973); Boardman (1983)	Zoooidal (non-kenozooidal)

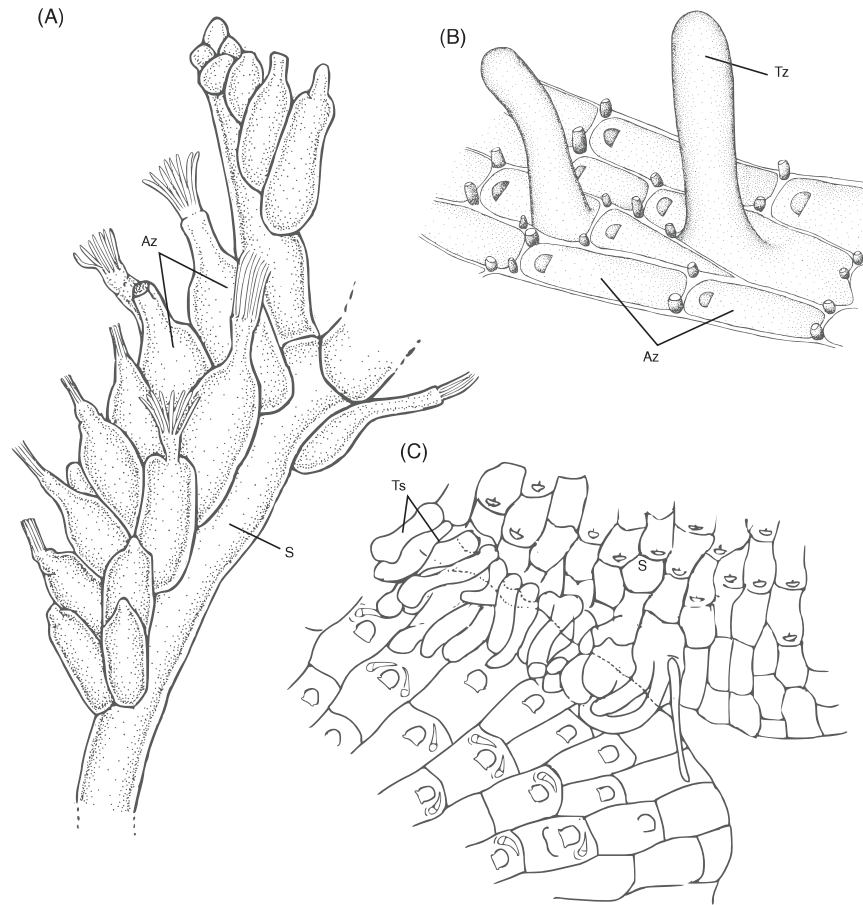


Fig. 9. Gymnolaemate stolons. (A) Kenozooidal stolons supporting autozooids in the ctenostome *Amathia pustulosa*, (B) tower zooids in *Membranipora membranacea* (side view), (C) intercolonial competition between the cheilostomes *Stylopoma duboisii* (top right, generating stolons) and *Hippopodina feegeensis* (bottom left). Az, autozooid; S, kenozooidal stolon; Ts, terminal stolon (kenozooidal); Tz, tower zooid (autozooidal appendage). A drawn from Souto, Fernández-Pulpeiro & Reverter-Gil (2011), B drawn from Hayward & Ryland (1998), C drawn from Osborne (1984).

(3) Rhizoids

Rhizoids (rhizozooids, rootlets, and radical fibres) are thin tubes that can anchor erect colonies or raise planar colonies above soft substrata (e.g. Beaniidae, Petraliidae, *Retiflustra*; Harmer, 1926; Cook *et al.*, 2018). These structures possess a variety of terminal ends to anchor colonies (single tip, fimbriate, holdfasts; Fig. 10; Vieira *et al.*, 2014; Schack *et al.*, 2018), which can be adhesive (Cook & Chimonides, 1981, 1985; Cook *et al.*, 2018). Ctenostome rhizoids may bore through organic substrata (e.g. echinoid spines and sipunculan cuticles by *Triticella minini* and *T. maiorovae* respectively; Grischenko & Chernyshev, 2015, 2017), perhaps *via* biochemical erosion (e.g. some shell-boring taxa; Pohowsky, 1978). Some small free-living colonies may use turgor pressure in rhizoids to elevate their colonies above the substratum (Fig. 11; Hirose, 2011).

In cheilostomes and cyclostomes, rhizoids are typically modified kenozooids that bud adventitiously from autozooids or vibracular chambers (Taylor & Weedon, 2000). In ctenostomes, rhizoids develop as a series of septa-separated

tubes, the first of which is an evagination of a stolon or autozooid wall (Souto *et al.*, 2010, 2011). This is similar to the jointed rhizoids in the cyclostome family Crisiidae (Hayward & Ryland, 1985). While most rhizoids are kenozooidal, they can also be appendages formed from evaginations of the body wall ['props' as in *Chaperiopsis uttleyi* (Gordon, 1992) and *Favosipora* (Gordon & Taylor, 2010); Fig. 10] or the frontal cuticle [e.g. *Steginoporella neozelanica* (Gordon, Voje & Taylor, 2017)]. Extrazoooidal rhizoids also occur: sand-dwelling colonies of *Lanceopora* and *Sphaeropora fossa* use a single rhizoid to elevate their colonies (Fig. 11). The rhizoid is formed from the cuticular body wall and is able to repair damage and maintain turgor pressure (provided by coelomic fluid) (Cook & Chimonides, 1981, 1985; Cook *et al.*, 2018).

(a) Articulated colonies

Erect bryozoan colonies may possess articulated joints. The joints may be indeterminate (provided by dorsal rhizoid bundles; Hageman *et al.*, 1998) or determinate (autozooidal appendages; Schäfer, Bader & Blaschek, 2006). Determinate

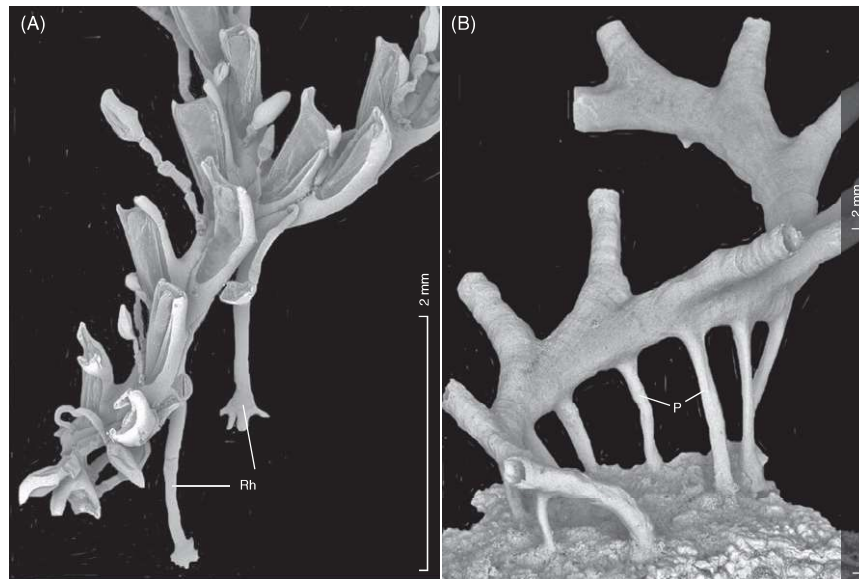


Fig. 10. Rhizoids (zooids) *versus* props (autozooidal appendages). (A) *Camptoplites* sp. (Cheilostomata) with rhizoids (Rh), (B) *Pandanipora helix* (Cyclotomata) with props (P). Photographs by A.V. Grischenko.

articulation is most common in cheilostomes (particularly *Cellaria*) but is also found in *Crisia* (Cyclotomata) and in Paleozoic stenolaemates (Wyse Jackson, Ernst & Andrés, 2017). In *Cellaria sinuosa* the joints are chitinous, while in *Crisia eburnea* they appear to be collagenous (Schäfer *et al.*, 2006).

Articulation allows erect colonies to resist mechanical stress through flexibility (McKinney & Jackson, 1989; Poluzzi, Masini & Capozzi, 1991) rather than investing in skeletal strength, extrazoooidal calcification, or branch thickening (Cheetham, 1986). This may be an adaptive strategy for high-energy environments (McKinney & Jackson, 1989; Poluzzi *et al.*, 1991; Schäfer *et al.*, 2006). If mechanical stress results in joint breakage, the internodes (zooid clusters) can generate new colonies and function as asexual propagules (as in *C. sinuosa*; Bader, 2000).

(4) Spines

‘Spine’ refers to a variety of skeletal and cuticular structures. These are typically hollow with an inner lining of epithelial cells, and possess varying degrees of calcification (unmineralized to fully calcified) (Silén, 1977; Ostrovsky, 1998; Lidgard *et al.*, 2012). Spines can have a cuticular basal articulation or can be unjointed costae. Spines exhibit a wide range of variation (tubercles, branching spines, shield-like scuta) (Silén, 1977; Gordon, 1984, 1986; Vieira *et al.*, 2014) that generally bud adventitiously on zooids and are incapable of budding zooids.

(a) *Gymnolaemate* spines

Most cheilostome spines are autozooidal appendages (Ryland, 1979; Cheetham & Cook, 1983). True kenozooidal spines (‘spinozooids’ of Silén, 1977) around the opesia have been found only in *Bellulopora* (Ostrovsky & Taylor, 2005a),

where they form a costate frontal shield. While kenozooidal spines are separated from the autozooid by communication pores, spines formed as appendages of the autozooid are not (Silén, 1977; Cheetham & Cook, 1983; Ostrovsky & Taylor, 2005a; A.N. Ostrovsky, personal communication). Other spine-like kenozooids exist in a variety of taxa [e.g. terminal kenozooids in *Chelidozoum ternarium* (Gordon & d’Hondt, 1991); and the ctenostome *Amathia wilsoni* (Chimonides, 1987)], although these structures are not budded adventitiously as true kenozooidal spines. In some cheilostomes spines project internally from cystid walls; they may be needle-like, branching, or form a robust scoop-like ‘plectriform apparatus’ to guide the polypide as it everts (Gordon & Parker, 1991a, 1991c).

Although they may not possess structural homology, most spines are functionally homologous. It is likely that spines first evolved in response to predation (Dick *et al.*, 2009). The earliest known cheilostome with spines is *Charixa burdonaria*, which had a pair of latero-oral spine bases (Taylor, Lazo & Aguirre-Urreta, 2009). This suggests that oral spines evolved first 135.0–135.8 Mya, before spreading to the periopodial margin to protect the membranous frontal wall as in *Spinicharixia* 124.5–112.0 Mya (Taylor *et al.*, 2009).

The clearest evidence for a defensive role is in *M. membranacea*, which produces cuticular spinules (short spine-like appendages, non-homologous to most spines) on the membranous frontal wall in response to grazing by nudibranchs (Harvell, 1984, 1986). Interestingly, three genotypes are present in *M. membranacea*: colonies with inducible spinules (irreversible polymorphs), and colonies that produce and do not produce spinules regardless of nudibranch-related cues (consistent polymorphs) (Harvell, 1998). When nudibranchs are removed, colonies with the inducible genotype cease spinule production within 1 day.

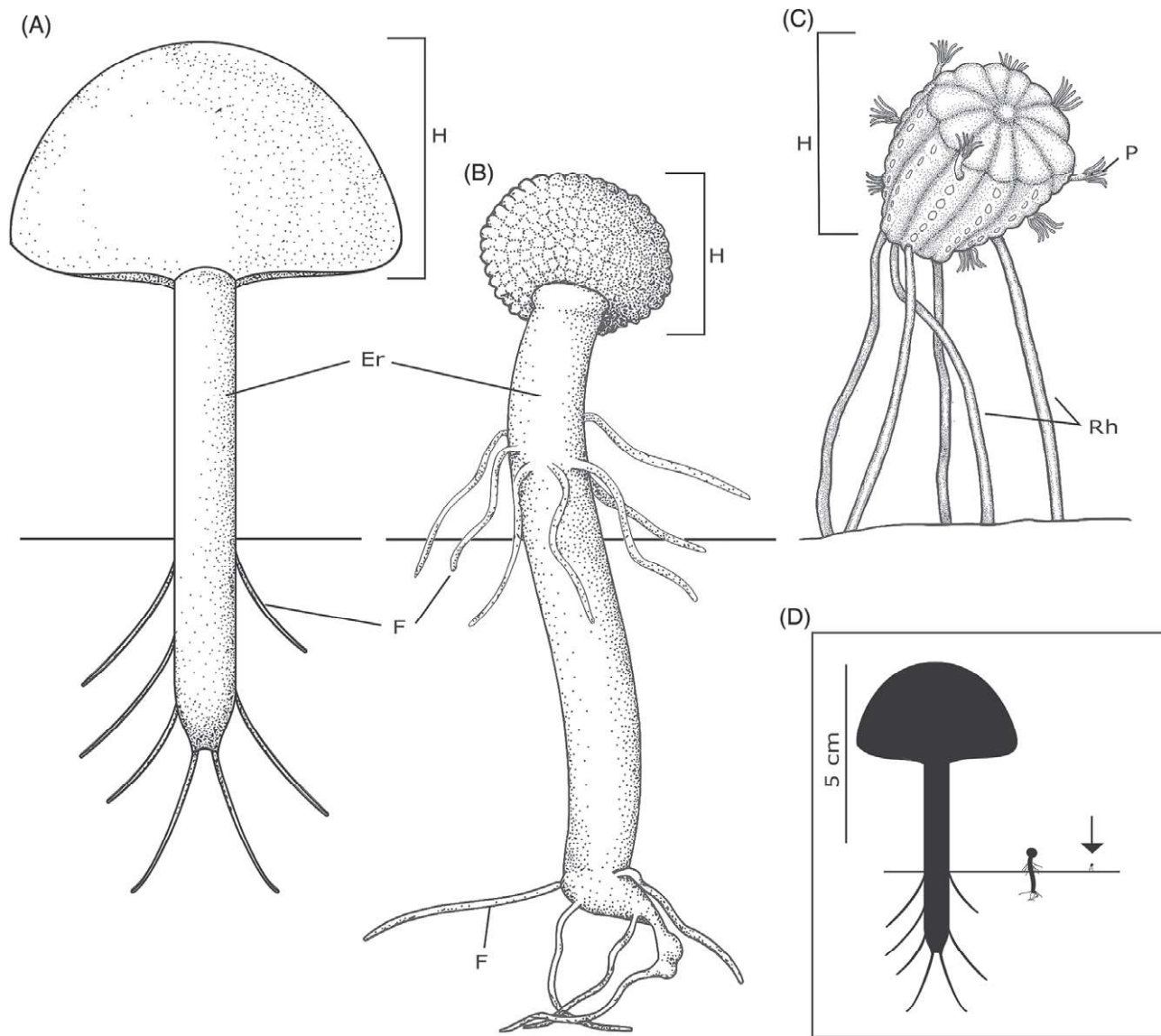


Fig. 11. Colonies supported and anchored by turgid rhizoids (not to scale). (A) *Lanceopora* sp., (B) *Sphaeropora fossa*, (C) *Conescharellina catella* (rhizoids below the sediment not shown), (D) relative sizes of colonies (arrow points to *C. catella*). Er, extrazoooidal rhizoid; F, fibril of extrazoooidal rhizoid (an appendage, not a new rhizoid); H, head of the colony, containing autozooids; P, everted tentacle crown of polypide; Rh, rhizoid (zoooidal). The top of the sediment is represented by a horizontal black line. A drawn from Cook & Chimonides (1985), B drawn from Cook & Chimonides (1981), C drawn from Hirose (2011).

Although spinule production decreases colony growth rate by ~14% (Harvell, 1986), the cost is likely balanced by the reduction in damage: spinulated colonies were largely undamaged and experienced lower predation rates than unspinulated colonies (Harvell, 1984). Spines in *Electra* spp. deter nudibranchs by 'spearing' them (Cook, 1985). Defence against nudibranchs is important since many nudibranchs feed only on bryozoans (Lidgard, 2008).

Although marginal spines on the edges of colonies appear important in preventing overgrowth by other organisms (Stebbing, 1973; Scholz, 1995), spines alone provide no significant advantage in interspecific competition between bryozoans (Barnes & Rothery, 1996).

While defence is likely their main function, spines may also provide protection against abrasion. This is particularly useful for species that often experience tumbling and those that live on kelp fronds [like *Electra pilosa* and the ctenostome *Flustrellidra hispida* (Stebbing, 1973; Whitehead, Seed & Hughes, 1996)]. Protection from abrasion and predation may have different structural requirements (e.g. pointed tip *versus* rounded tubercle). Oral spines may also increase local turbulence, trapping food particles near the tentacle crown or by increasing boundary layer thickness and allowing tentacle crown protrusion into higher-velocity waters (Riedl & Forstner, 1968, cited in Whitehead *et al.*, 1996).

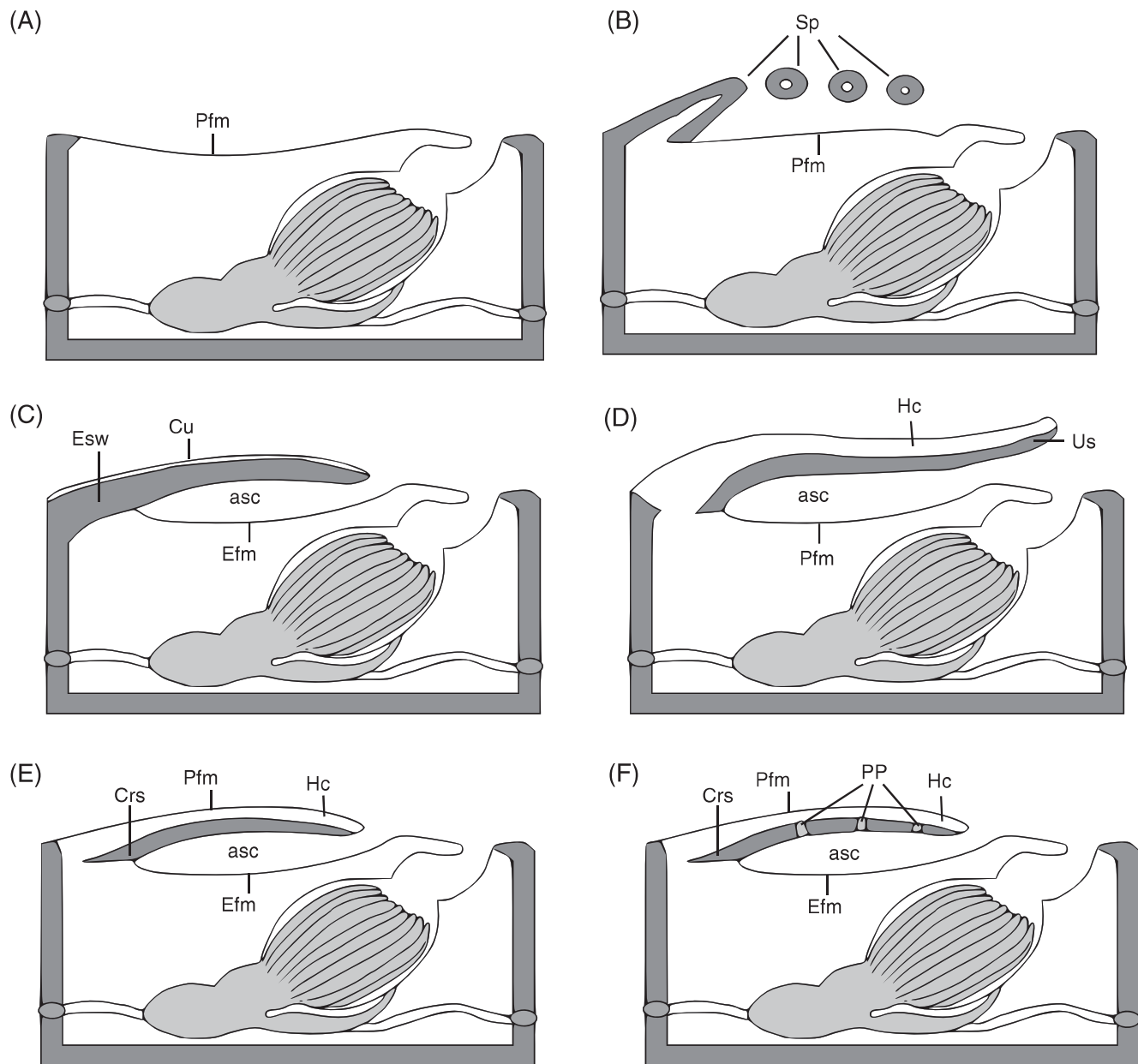


Fig. 12. Frontal shields of cheilostomes. (A) Basic anascan condition (no shield), frontal wall wholly or principally membranous (cuticular), (B) membranous frontal wall protected by a shield of overarching spines (spinocyst), (C) ascophoran with gymnocystal shield, i.e. calcified frontal body wall (cuticle overlies the skeletal layer), (D) ascophoran with an umbonuloid shield, (E) ascophoran with a lepralioid shield, (F) ascophoran with a lepralioid shield with pseudopores. Note that the frontal shield in D–F has an overlying hypostegal coelom and cuticular epitheca. Asc, ascus; Crs, cryptocystal shelf; Cu, cuticle; Efm, homologous extension of the membranous frontal wall, the epithelial cells of which peel back from the cryptocystal shelf in lepralioid shields (Cheetham & Cook, 1983); Esw, external (gymnocystal) skeletal wall; Hc, hypostegal coelom; Pfm, primary cuticular frontal wall (reinforced in C by calcifying beneath it to form a gymnocyst); PP, pseudopores plugged with tissue; Sp, spines; Us, umbonuloid shield. Drawn from Mukai *et al.* (1997).

(b) *Stenolaemate spines*

In stenolaemates, kenozooids may be modified to form ‘spinose kenozooids’ (Silén, 1977; Taylor & Weedon, 2000). These heterozooids are analogous to the spinozooids of Silén (1977). These spines may be single kenozooids or slender chains separated by cuticular joints (e.g. *Crisidia cornuta*;

Hayward & Ryland, 1985). Apertural spines can surround the aperture of a zooid, although typically only one spine is present. These spines are projections of the inner body wall and only occur in free-walled autozooids that lack a peristome (Taylor & Weedon, 2000). Like the plectiform apparatus, mural spines (spinules) and pustules extend into

the zooidal chamber from the skeletal wall. These spines may be attachment structures for the polypide [e.g. some *Disporella* species (Boardman, 1998)].

Extrazoooidal spine-like 'stylets' may also be present in stenolaemates, which unlike spines, possess a solid core enclosed by laminating sheaths (Blake, 1983; Boardman & Buttlar, 2005). A few rare forms arise from zooidal boundaries [zooecial styles, exozonal styles (Boardman & Buttlar, 2005)], and should be considered autozooidal appendages instead of extrazoooidal structures. Stylets may have supported tissues of confluent coeloms, facilitating interzooidal communication (Blake, 1983; Boardman, 1983). The loss of stylets in fixed-walled stenolaemates, which possessed communication pores, supports this theory (Boardman, 1983). However, prominent stylets may have served a defensive purpose (Blake, 1983).

(5) Frontal walls

Kenozooids and spines both contributed to the evolution of ascophoran frontal shields with a hypostegal coelom. Periopiesal spines (around the frontal membrane) became rigid costae through the loss or calcification of their cuticular joint base. Fusions between costae led to the creation of the cribrimorph costal shield during the Late Cretaceous [although costal shields have evolved independently many times (Dick *et al.*, 2009; Lidgard *et al.*, 2012)]. The cribrimorph costal shield then acted as a surface for overlying kenozooids, allowing thicker shields to be created (Gordon & Voigt, 1996; Lidgard *et al.*, 2012). This can be observed in the Cretaceous *Ascancestora bretoni*, which possesses broad interzooidal kenozooids that overgrow the lateral and frontal walls of autozooids (Gordon & Voigt, 1996). Subsequent reductions in the underlying spines (costal field) and the frontal membrane produced umbonuloid and lepralioid shields (Fig. 12). This restricted the hydrostatic mechanism used to protrude the tentacle crown and required the development of an ascus (a compensation sac that evolved independently many times; Gordon, 2000). Development of the costal shield and ascus likely increased survival and contributed to the ascophoran radiation (Gordon & Voigt, 1996; Dick *et al.*, 2009; Lidgard *et al.*, 2012).

V. REPRODUCTIVE POLYMORPHISM

Reproductive polymorphism includes morphological variation in both zooids and brood chambers. Excellent reviews exist for Gymnolaemata (Ostrovsky, 2013) and Stenolaemata (Schäfer, 1991), summarized here (see also Reed, 1991).

(1) Gonochoristic zooids

Autozooids can be sterile, hermaphroditic (testes and ovaries developing together), male, or female. Hermaphroditic zooids typically undergo reversible changes to the polypide (e.g. development of intertentacular organ) (Reed, 1991). In

colonies with gonochoristic zooids (most gymnolaemates and stenolaemates), male and female zooids can be differentiated based on modifications to the polypide, cystid, or both (Reed, 1991). Cystid modification can include changes in size (dwarf females of *Haplopoma sciaphilum*; Silén & Harmelin, 1976), shape (*Reptadeonella violacea*; Hayward & Ryland, 1999), or in characteristics of the orifice (some *Calyptotheca*; Ryland, 1976). For example, *Celleporella hyalina* and *Antarctothoa bougainvillei* (both Hippothoidae) possess autozooids, ovicelled female zooids, and male zooids that are all distinct in terms of their skeleton and polypide structure. Male and female zooids are dwarfed in comparison to sterile autozooids, with reduced polypides, the female's being more rudimentary (Marcus, 1938, and Hughes, 1987, cited in Ostrovsky, 1998). The most extreme gonochoristic changes result from the formation of brooding structures.

In *Odontoporella bishopi* (Fig. 13), autozooids and male zooids have identical cystids but different polypides (Carter & Gordon, 2007). The egg-producing autozooids have 15–16 tentacles, while males have only eight unciliated tentacles (four short, four long) and a reduced polypide filled with spermatozoa [Gordon, 1968 as *Hippopodina adpressa*]. The identical skeletal morphology of autozooids and males may be a consequence of cyclic polypide degeneration and regeneration, since in *A. bougainvillei* and *A. tongima* autozooids may regenerate as males (Rogick, 1963; Powell, 1967 as *Hippothoa bougainvillei*).

Although the vast majority of bryozoans are colonial hermaphrodites, there may be dioecious cyclostome colonies composed of only male autozooids or sterile autozooids, female autozooids (future gonozooids) and gonozooids, which are incapable of self-fertilization [e.g. *Filicrisia geniculata* (Jenkins *et al.*, 2017)]. The sex ratio within colonies can also be influenced by environmental conditions: when exposed to stress the cheilostome *Celleporella hyalina* increased production of males and suppressed investment in brooding females (Hughes *et al.*, 2003).

(2) Cheilostome brood chambers

Cheilostome bryozoans may either spawn embryos (as planktotrophic cyphonautes larvae) or incubate embryos before release (lecithotrophic coronate larvae) (Ostrovsky, 2013). Cheilostome brooding methods include: external membranous sacs (ovisacs), calcified chambers (ovicells), uncalcified internal brood sacs, or the intracoelomic space (Ostrovsky, 2013). The origin of ovisacs is unclear (likely a sticky fertilization envelope; Ström, 1977; Ostrovsky, 2013), but they are present only during the reproductive period (e.g. *Aetea* species; Ström, 1977). Since the ovisac does not involve modification of the maternal zooid it should not be considered a polymorphic appendage. External ovisacs also appear in ctenostomes and may represent the most primitive brooding method (Ostrovsky, 2013).

Most cheilostomes brood their embryos in external calcified chambers called ovicells. These consist of the oecium (protective hood), the brood cavity (incubation chamber), and a closing mechanism (Ostrovsky, 2013). Eggs pass into

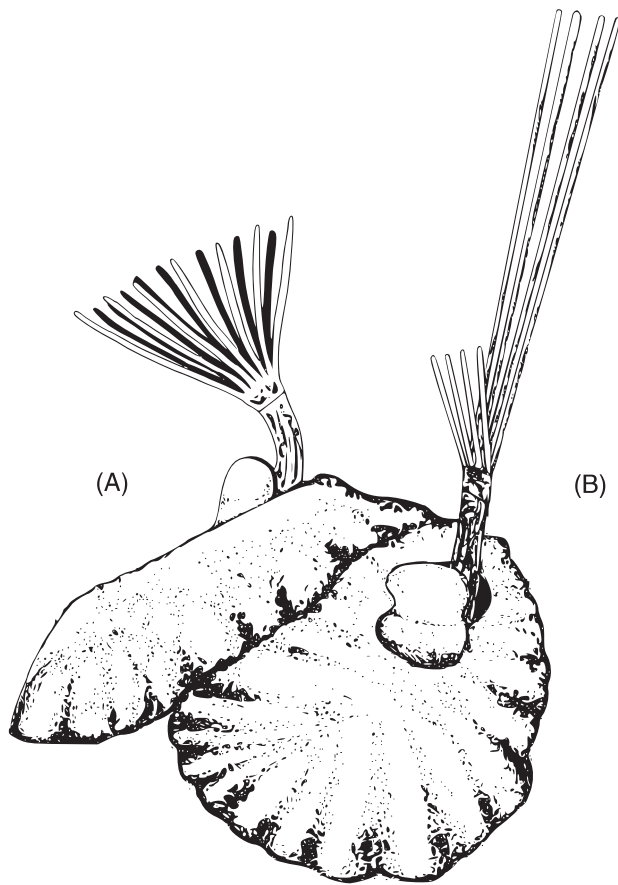


Fig. 13. *Odontoporella bishopi*, showing differences between the polypides of (A) an autozooid and (B) male zooid. Redrawn from Gordon (1968). Note that the four short tentacles of the male zooid (B) are drawn offset for clarity.

the ovicell through a gonopore of the maternal zooid, and the ovicell may be closed by an ooecial vesicle, the operculum, or both. The vesicle is a membranous evagination of the maternal zooid's distal wall, the interior of which is confluent with the coelom of the maternal zooid, and is operated by special musculature (Ostrovsky, 2013). In some taxa the ooecial vesicle aids in the delivery of extraembryonic nutrition (Moosbrugger *et al.*, 2012). The maternal zooids can be considered polymorphic on the basis of the ooecial vesicle alone.

The double-walled ooecium is not formed by the maternal (egg-producing) zooid, but by a proximal-facing outgrowth of a distal zooid (Fig. 14). This distal zooid can be an autozooid, an avicularium, or a kenozooid (Ostrovsky, Dick & Mawatari, 2008; Ostrovsky *et al.*, 2009; Ostrovsky, 2013). In many cases, the distal zooid forms the floor of the brood chamber in addition to the ooecium ['type 1' ooecia of Ostrovsky, 2013]. 'Type 2' ooecia are formed by a kenozooid that is budded interzooidally or adventitiously by the maternal zooid. In these cases, the whole (or nearly the whole) of the kenozooid constitutes the ooecium, and the floor of the brood chamber is provided by the maternal zooid (Fig. 14; Ostrovsky, 2013). Here, a 'type 1' ooecium is an adventitious appendage of the distal daughter. This makes 'type 1' ooecia

similar to adventitious spines, which have been documented on autozooids as well as kenozooids (e.g. *Petalostegus bicornis*, *Chorizopora ferocissima*; see Section IV.4; Gordon, 1984) and avicularia (e.g. *Valdemunitella fraudatrix*, *Corbulella translucens*; Gordon, 1984, 1986). By contrast, a 'type 2' ooecium is a complete kenozooid and is a full polymorphic zooid similar to adventitious avicularia or kenozooidal spines in *Bellulopora* (Ostrovsky & Taylor, 2005a). Regardless of ooecium type, the ovicell represents a multizoooidal complex in which the ooecium originates from a distal zooid while the eggs and closing structure (when present) originate from the maternal zooid (Lidgard *et al.*, 2012; Ostrovsky, 2013).

Other brood chambers are formed entirely by overarching spines (acanthostegal brood chambers), or by ooecia constructed from multiple lobes or plates. Acanthostegal brood chambers are multizoooidal complexes, but represent an alternate path in brood-chamber evolution rather than an early stage of modern ovicells (Ostrovsky, 2013). Multilobed ooecia can be formed from kenozooids budded by the maternal zooid ('type 2' ooecia, as in *Catenicula*) or by projections of the maternal frontal wall (as in *Thalamoporella*) (Ostrovsky, 2013). This makes the ovicells of *Thalamoporella* unique: they are not multizoooidal complexes but integrated, single-zooid structures.

Internal brood chambers (endozooidal) are contained entirely within the maternal zooid. The walls and floor of the brood cavity are formed from an uncalcified invagination of the distal wall (Ostrovsky, 2013). Brood cavities open to the environment are closed by an ooecial vesicle (likely homologous to that in ovicells), while those opening to the vestibulum may have a flap to isolate the brood cavity (Ostrovsky, 2013). The ability to brood internally relies partly on the ability to 'make room' for the developing embryo within the cystid of the maternal zooid. This can result in maternal zooids with enlarged cystids (e.g. *Chlidonia pyriformis*), but this does not necessarily need to occur (e.g. *Watersipora subtorquata*) (Ostrovsky, 2013). In both cases the maternal zooid may be considered polymorphic owing to the presence of a brood cavity (internal cystid modification).

(a) Evolution of brood chambers

The ooecium likely evolved from spines on the proximal opesial rim of the daughter zooid (Fig. 15; Ostrovsky & Taylor, 2005a, 2005b), instead of spines from the maternal zooid as postulated by Silén (1977). In addition to protecting the frontal membrane, spines of a distal zooid may have also protected membranous ovisacs produced by a proximal zooid (Ostrovsky, 2013). Increased embryo protection could arise from curving spines towards the proximal ovisac/maternal zooid. This was likely the first step to a complete ooecium and ovicell (Ostrovsky & Taylor, 2005a). Following that, fusion (or reduction in number) and flattening of spines, loss of articulation and relocation of spine bases, filling openings in the ooecium, and forming a concave ovicell floor are all trends toward the evolution of non-spinose ovicells (which have evolved at least twice; Ostrovsky & Taylor, 2005a).

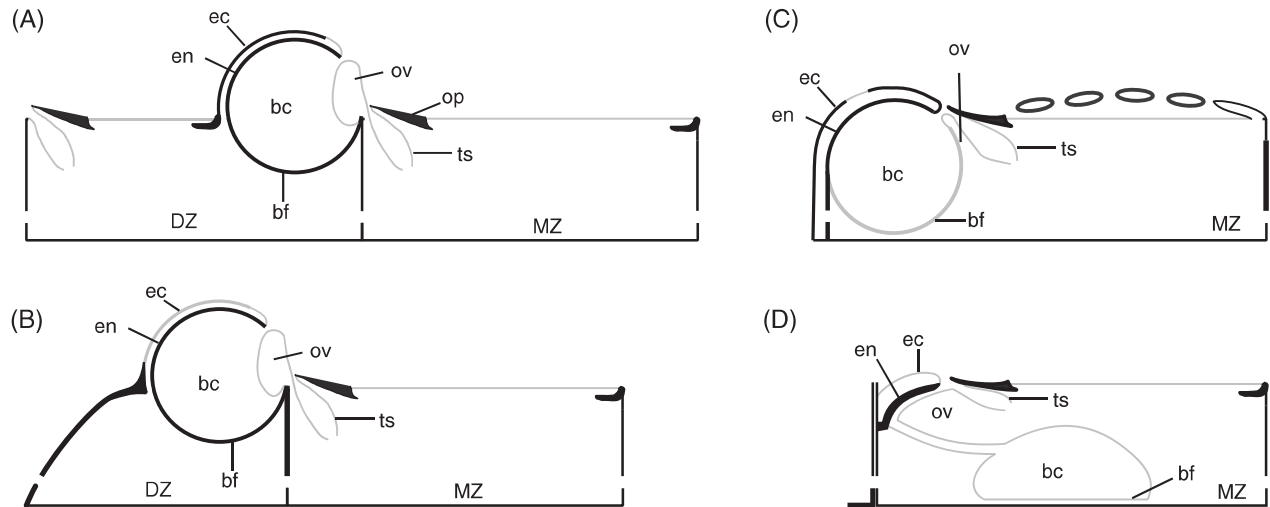


Fig. 14. Type 1 (appendage) and type 2 (zooidal) oöcia. (A) Type 1 oöcium of *Callopora lineata*, provided by a distal autozoid, (B) type 1 oöcium of *Tegella armifera*, provided by a distal kenozooid, (C) type 2 oöcium of *Cribilina annulata*, (D) type 2 oöcium of *Cauloramphus spinifer*. In A and B the oöcium is an appendage of the daughter zooid, the brood chamber floor is provided by the daughter zooid, and the ovicell is closed by an oöcial vesicle (acleithral). In C and D the oöcium is a kenozooid budded by the maternal zooid, the brood chamber floor is provided by the maternal zooid, and the immersed ovicells are closed by the operculum and oöcial vesicle (cleithral). bc, brood chamber; bf, brood chamber floor; DZ, distal zooid; ec, ectooecium; en, endooecium; MZ, maternal zooid; op, operculum; ov, oöcial vesicle; ts, tentacle sheath. Calcified walls in black, membranous walls in grey. Drawn from Ostrovsky (2013).

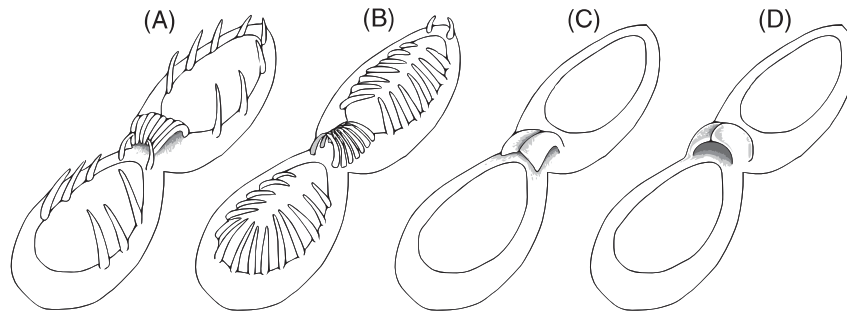


Fig. 15. Steps in the evolution of the oöcium from periopodial spines. (A) *Distelopora bipilata*, (B) *D. spinifera*, (C) *Gilbertopora larwoodi*, (D) *Wilbertopora mutabilis*. Redrawn from Ostrovsky & Taylor (2005b).

In addition to the formation of non-spinose ovicells, there is trend towards the immersion of the brood chamber in the maternal zooid (Ostrovsky *et al.*, 2008; Ostrovsky, 2013). Ovicells can be hyperstomial (prominent: more than half of the brood cavity is above the frontal surface), subimmersed, or immersed (entirely below the surface; Ostrovsky *et al.*, 2008). As the brood cavity becomes immersed, the oöcium is reduced to a caplike, vestigial structure and the walls and floor of the brood cavity become uncalcified (Ostrovsky, 2013). It is highly probable that uncalcified internal brood sacs were derived (multiple times) from immersed ovicells (Ostrovsky, 2013). Internalization of the brood chamber also results in the increased integration of the cormidium through the reduction of the component zooid: the brood chamber is no longer a multizoooidal complex (ovicell) but a structure formed by a single maternal autozoid.

Lecithotrophic larvae typically have higher survival rates than planktotrophic larvae (Mercier, Doncaster & Hamel,

2013), but brooding lengthens their exposure to motile benthic predators. In the absence of protective structures, predation on embryos and larvae is stronger in the benthos than in the plankton (Allen & McAlister, 2007). Benefits of yolk larvae are realized only if they survive the brooding stage. Therefore, the construction of a calcified ovicell and the immersion of the brood cavity were likely driven by predation from small epibionts (Lidgard *et al.*, 2012; Ostrovsky, 2013). The development of ovicells and lecithotrophic larvae likely coincided, and poorly dispersing larvae may have resulted in population fragmentation and increased speciation, thus triggering the cheilostome radiation (Ostrovsky & Taylor, 2005a; Lidgard *et al.*, 2012).

(3) Ctenostome brood chambers

Ctenostomes may brood embryos externally or internally. Most external brood chambers in ctenostomes are simply the

fertilization envelope of the embryo and therefore are not polymorphic structures of the maternal zooid. This envelope may attach directly to the surface of the maternal zooid [e.g. *Triticella koreni* and *Paludicella articulata* (Ström, 1977)] or may be attached by flexible mucus [*Pottsiella erecta* (Smith, Werle & Klekowski, 2003)]. Most ctenostomes house the developing embryo in a vestibule (e.g. *Bulbella abscondita*), tentacle sheath (e.g. *Amathia verticillata*), or the diaphragm between the two (e.g. *Alcyonidium duplex*) (Ström, 1977; Micael *et al.*, 2018), and as such are not considered polymorphic.

Specialized, temporary structures may be developed in maternal zooids after polypide degeneration. Maternal zooids in *Amathia gracilis* develop ‘ciliated gutters’ to move the oocyte to the tentacle sheath (Reed, 1988), while other species develop ‘brood pouches’ between the degenerated tentacle sheath and vestibule [e.g. *Alcyonidium polyoum* (Ström, 1977)]. After adhering to the maternal zooid, embryos may also be brooded in epithelium-closed invaginations of the vestibule body wall (as in *Victorella muelleri* and *Nolella dilatata*; Ström, 1977). Since these modifications are temporary, maternal zooids producing specialized structures are reversible polymorphs.

While most ctenostomes exhibit brooding methods that do not require polymorphic zooids, some shell-boring species possess ovicells or gonozooids (Soule, 1950a, 1950b; Pohowsky, 1978). These structures exist in the genus *Penetrantia*, the only operculate ctenostome genus, and the fossil species *Spathipora cheethami* (Pohowsky, 1978). The brood chambers are globular, cuticular structures (Silén, 1946; plate 12 in Pohowsky, 1978). In *P. concharum* and *P. sileni* the brood chamber opens into the tentacle sheath, while in *P. densa* it opens directly to the environment via a widened aperture (Silén, 1946; Soule, 1950b; plate 11 in Pohowsky, 1978). Like other internally brooding ctenostomes, the polypide degenerates (Silén, 1946; Soule, 1950b), potentially to supply extraembryonic nutrition. However, maternal zooids hosting these brood chambers are consistent polymorphs owing to their modified polypides: while there are 12 tentacles in normal autozooids, maternal zooids only possess eight (Silén, 1946). The brood chambers themselves, unlike cheilostome ovicells, are probably non-kenozooidal appendages of the maternal zooid. The evolutionary drivers for brood chambers in *Penetrantia* are unclear, especially since internal brooding is possible in other boring bryozoans (Soule, 1950a).

(4) Cyclostome gonozooids

Gonozooids are the external brood chambers of cyclostomes. Unlike the multizoooidal brood chambers of cheilostomes, gonozooids are formed through the modification of a single zooid and are less numerous within the colony. The gonozooid consists of a proximal undifferentiated section, an enlarged area forming the brood cavity where the embryos are incubated, and the brood chamber aperture (oeciostome) homologous to the autozooidal peristome (Ström, 1977; Schäfer, 1991). Typical cyclostome gonozooids are lacking in only two families: Cinctiporidae, which has very large autozooids (Boardman, McKinney & Taylor,

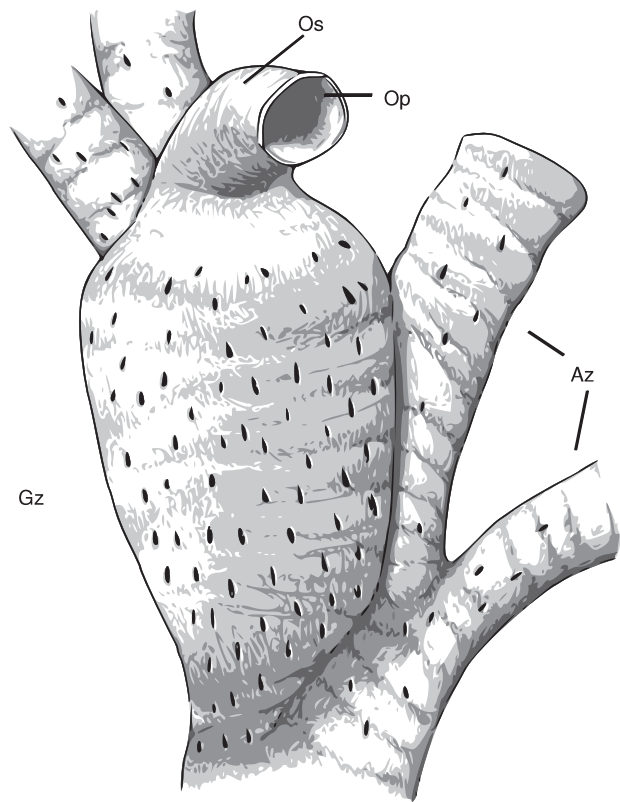


Fig. 16. Club-shaped gonozooid of *Filicrisia geniculata*. Az, autozooidal peristomes; Gz, gonozooid; Op, oeciopore, or gonozooidal aperture; Os, oeciostome, which is homologous to the zooidal peristome. Drawn from Jenkins *et al.* (2017).

1992) that might function reproductively (unconfirmed) and Anyutidae, which has autozooids possessing modified peristomes with inferred reproductive function (Grischenko, Gordon & Melnik, 2018).

Spatial competition between gonozooids and autozooids results in a variety of gonozooid forms. The simplest of these have pyriform (pear-shaped) brood cavities (Fig. 16). In Tubuliporina, the expansion of the pyriform brood cavity results in the dwarfism or elongation of neighbouring autozooids. Enlargement of elongate/pyriform brood cavities in Cerioporina and Eleidae results in the occlusion of both autozooids and mesopores (Schäfer, 1991).

Since feeding is vital to colony survival, it is advantageous to limit obstruction of autozooids by brood-cavity growth (Fig. 17). Loosely arranged zooid apertures (e.g. Articulata), dorsal budding (*Tervia*: Tubuliporina), and peristomial budding (only in *Stomatopora gingrina*: Tubuliporina) all limit the interference of pyriform brood cavities and allow them to expand freely. Grouping autozooidal apertures into fascicles (tight bundles) can also promote this (e.g. *Fasciculipora ramosa*), but zooids may still be occluded (Schäfer, 1991). To avoid autozooid occlusion, gonozooids may expand laterally by forming lobes between aperture rows or may become perforated by autozooids (which form their peristomes on the brood cavity roof). Since lobate and perforated forms

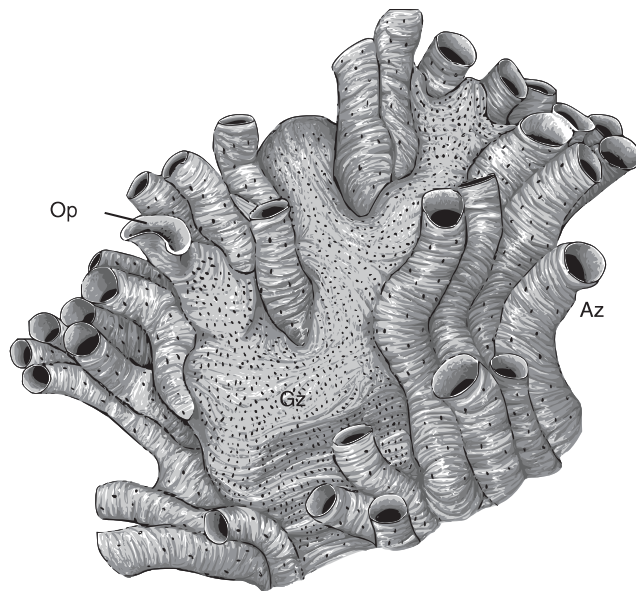


Fig. 17. Irregular gonozooid of *Tubulipora plumosa*. Az, autozooid; Gz, gonozooid; Op, oocypore. Drawn from Jenkins *et al.* (2017).

are common in colonies with alternating zooid rows and quincunxially arranged zooids respectively, the shape of a brood cavity may be controlled by the organization of neighbouring zooids (Schäfer, 1991).

Space-filling polymorphic structures are typically occluded by the expanding brood cavity. However, some gonozooids incorporate these structures into their brood cavities. In Petaloporidae (Cancellata) the roof and walls of brood cavities are covered in mesopores, while in *Discoecyis canadensis* (Cytididae: Cancellata) the brood cavity walls contain cancelli. Most striking are the brood cavities of *Patinella* (Lichenoporidae: Rectangulata): the fertile zooid grows alongside extrazoooidal skeletal structures (alveoli), and eventually the walls separating the fertile zooid and the alveoli are reabsorbed to create a confluent brood cavity (Fig. 18; Schäfer, 1991). The gonozooids of *Patinella* are therefore 'multimodular' structures, similar to the ovicells of Cheilostomata (although the potential extrazoooidal character of the alveoli means that they may not be multizoooidal complexes).

Gonozooids are irreversible polymorphs. Some egg-producing autozooids are transformed to gonozooids initially possessing a fully formed or non-feeding polypide (with short tentacles and a reduced digestive tract) that is everted from the orifice (Borg, 1926; Reed, 1991). It is likely that a tentacle crown is necessary to collect sperm (Silén, 1972), potentially through the supraneural pore as in gymnolaemates (Ryland, 1976). Once an egg in a gonozooid is fertilized, the polypide degenerates and the embryos receive nutrition from a mass of cells generated by the surrounding membranous sac (Borg, 1926; Ström, 1977; Reed, 1991). Regardless of shape, gonozooid walls are highly punctate, potentially ensuring adequate respiration for the embryos

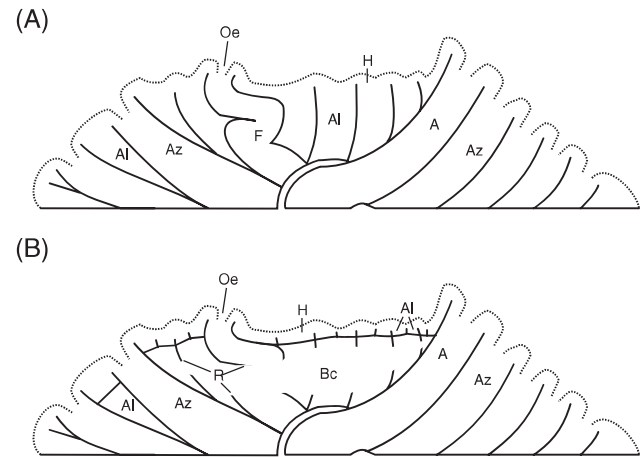


Fig. 18. Brood chamber development of the free-walled cyclostome *Patinella radiata*. Interzooidal communication pores are not shown. (A) The female zooid is separated from adjacent alveoli by skeletal walls, (B) a large brood chamber is constructed through the reabsorption of skeletal walls of the female zooid and adjacent alveoli. The brood chamber possesses a calcified roof with additional alveoli developing on the surface. A, ancestrula; Al, alveolus; Az, autozooid; Bc, brood chamber; F, female zooid; H, hypostegal coelom; Oe, oocystostome; R, sites of reabsorption. Drawn from Schäfer (1991).

within (Ryland, 1976). Cyclostomes have polyembryonic development, so the primary embryo undergoes cloning by multiple fission (Borg, 1926). Lecithotrophic larvae (Ström, 1977) are released from the gonozooid *via* the oocystostome, which is curved to direct larvae away from the feeding currents of autozooids (Schäfer, 1991). Larvae are released over an extended period (≤ 69 days in *Filicrisia geniculata*), suggesting prolonged budding of the primary embryo (Jenkins *et al.*, 2017).

Gonozooids are expensive to maintain. It is unlikely that a single degenerated polypide could provide sufficient nutrients for the development of over 150 larvae (Kluge, 1962, in Jenkins *et al.*, 2017) during an extended period. Therefore, the gonozooid and embryonic multiplication and growth must be supported *via* resource transfer from autozooids (Jenkins, Bishop & Hughes, 2015). The high cost of brooding may limit the number of gonozooids present in a colony (Hughes *et al.*, 2005; Jenkins *et al.*, 2015), and species of cyclostomes reduce female investment in the absence of alien sperm. When reproductively isolated, *Tubulipora plumosa* reduced both gonozooid production and the number of larvae (produced through intraclonal self-fertilization), while female *Filicrisia geniculata* formed incomplete gonozooids (Jenkins *et al.*, 2015).

Polyembryony is a paradoxical strategy because it combines the disadvantages of both cloning and sexual reproduction (Hughes *et al.*, 2005). However, polyembryony may be advantageous for cyclostomes. Bryozoans broadcast their sperm in the water column, resulting in severe sperm dilution and rare fertilization events. Polyembryony may allow colonies to capitalize on infrequent fertilization events

(Ryland, 1976), potentially aided by retention of allosperm in the feeding current (Hughes *et al.*, 2005). The extended release of cloned larvae would allow a genotype to be tested over a variety of temporal environments, some of which may be more favourable for larval survival (Jenkins *et al.*, 2017).

(5) Brood chambers in other stenolaemates

Structures resembling brood chambers exist in some Paleozoic stenolaemates. Zooids with ovicell-like swellings have been found in fenestrate colonies. These brood chambers are associated with a single zooid and may have retained a feeding polypide. Since these structures are small and numerous they probably each contained a single embryo, rather than being sites of polyembryony (Schäfer, 1991). Cystoporate brood chambers are more similar to cyclostome gonozooids with enlarged 'brood cavity' sections likely lacking a feeding polypide, but were unlikely to be polyembryonic (Schäfer, 1991). While it is difficult to determine when polyembryony evolved, the presence of brood chambers in Paleozoic taxa indicates that lecithotrophic larvae were already present (Schäfer, 1991).

VI. ASEXUAL PROPAGULES

Bryozoan colonies may reproduce asexually. This is best known in the Phylactolaemata, which produce statoblasts (Wood, 1983). Statoblasts develop from epithelial cells in the funiculus (Wood, 1983; Mukai *et al.*, 1997). Once mature, statoblasts detach from the funiculus and either remain in the coelom until the colony dies or are released through a temporary pore (Wood, 1983). Their chitinous outer layer allows statoblasts to survive harsh conditions [desiccation, ingestion (Wood & Marsh, 1996; Figuerola *et al.*, 2004)] thus helping to maintain the population. Statoblast variants include dispersing floatoblasts and non-dispersing sessoblasts, and both typically go through a dormant phase before germinating (Wood, 1983).

While statoblasts are produced inside a zooid, asexual propagules in gymnolaemates are budded from other zooids. Asexual propagules in ctenostomes are called hibernacula (Fig. 19). These structures are budded like zooids but lack a polypide. Instead, they are filled with granular reserves that are coloured like embryos and may consist of similar material (Jebram, 1975). These granular reserves may also be similar to the 'nutrient storage cells' associated with the embryos and funicular system of some cheilostomes (Ostrovsky, 2013). Since hibernacula lack a polypide, and secondary formation of hibernacula after polypide degradation has not been reported, nutrient reserves must be supplied by neighbouring autozooids.

Like sessoblasts, hibernacula have strengthened outer walls, remain cemented to the substratum after the death of the colony, and are only dispersed through colony breakage (Jebram, 1975; Wöss, 1996). Germination of hibernacula

may be triggered by changing environmental conditions (e.g. increasing temperature; Carter *et al.*, 2010b). During their germination, the granular reserves become transparent, suggesting that they are metabolized autozooids or stolons (Jebram, 1975). Despite evidence for seasonal changes in hibernacula production (Carter *et al.*, 2010b), temperature and salinity do not have a significant influence (Jebram, 1975). Instead, insufficient or low-quality food can trigger the formation of hibernacula (forming after only a day of starvation). This suggests that seasonal variations in food supply, rather than the direct influence of abiotic factors, drive colonies to produce hibernacula (Jebram, 1975).

'Sac-zooids' are found in shell-boring ctenostome genera (*Ropalonaria*, *Spathipora*, and *Terebripora*). These kenozooidal structures are filled with granules (Pohowsky, 1978), suggesting a nutrient-storage function and possible homology with hibernacula. A nutrient-storage function would allow a colony to survive during periods of food shortage, but whether a sac-zooid could bud a new zooid after colony death is unclear. Such an ability would allow a colony to remain in a favourable substratum and would make the distinction between hibernacula and sac-zooids irrelevant. In the ctenostome *Timwoodiellina natans*, small buds break off from the colony and swim using their tentacle crown. However, nautizooids are probably produced through sexual reproduction and are not polymorphic zooids (Wood & Okamura, 2017).

In Cheilostomata, dispersing asexual propagules ('sacculi' occur only in *Aetea*. Species in this genus are often epiphytes on seagrass (Balduzzi *et al.*, 1991). These are laterally budded from the autozooid. Sacculi have a small stalk that widens into a punctate body that is either narrowly ovate with a single adhesive tip (Type A) or obtriangular and forked into thin appendages, each of which possess an adhesive tip (Type B) (Simma-Kreig, 1969; Balduzzi *et al.*, 1991). Their small size suggests that they have a reduced polypide or lack one entirely. After breaking off from their parent zooid, sacculi attach to the substratum and generate a new autozooid (Simma-Kreig, 1969; Balduzzi *et al.*, 1991).

While hibernacula and sessoblasts typically ensure recolonization of an old substratum, dispersing sacculi and floatoblasts enable the colonization of new substrata. This suits the ephemeral nature of the seagrass fronds on which sacculiferous *Aetea* typically grow (Pergent *et al.*, 2008) and is a more viable strategy than over-wintering.

The swollen attachment kenozooids of *Caulibugula tuberosa* (Cheilostomata) may function similarly to hibernacula by storing nutrients and allowing the colony to regrow after annual dieback (Hastings, 1939).

Although some phylactolaemates can produce both floatoblasts and sessoblasts (e.g. *Plumatella*; Wöss, 2008), the ability to generate both dispersing and non-dispersing asexual propagules has not been found in gymnolaemates. This may be related to the freshwater habitat of phylactolaemates: lakes and rivers undergo strong seasonal variation (maintenance challenge) and may pose difficulties for colonization (dispersal challenge).

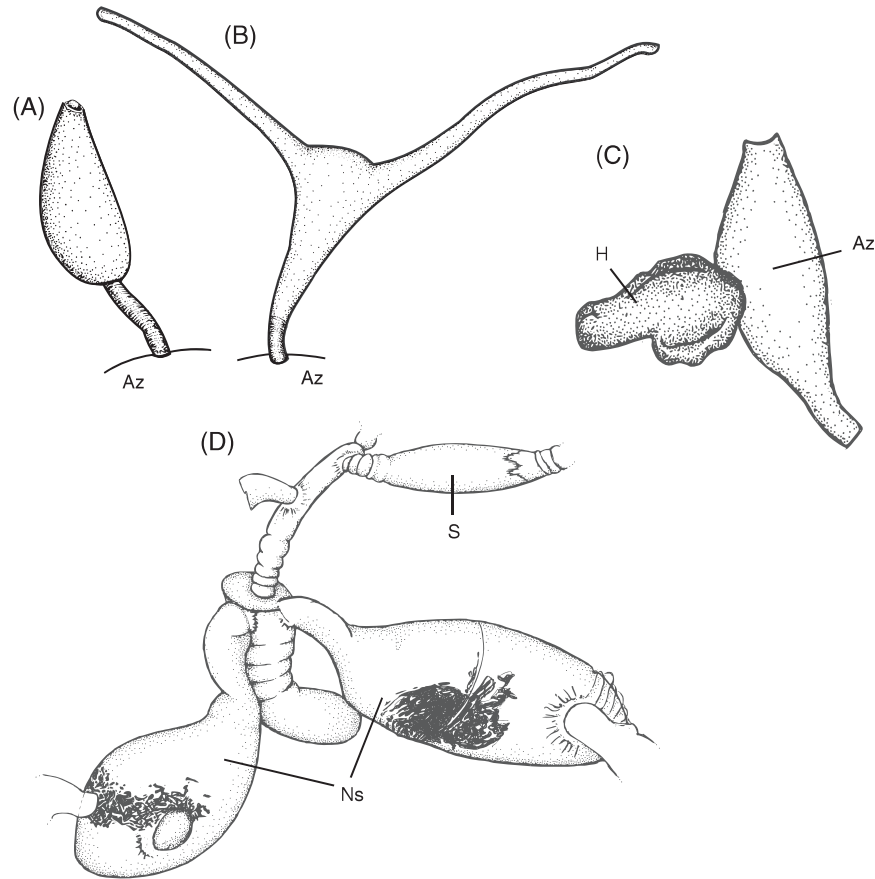


Fig. 19. Asexual propagules. (A) Type A sacculus of *Aetea lepadiformis*, (B) type B sacculus of *A. lepadiformis*, (C) *Paludicella articulata* zooid budding a hibernaculum, (D) base of a *Caulibugula tuberosa* colony with over-wintering stolons. Az, autozooid; H, hibernaculum; Ns, nutrient-storing stolon; S, structural stolon. A and B drawn from Balduzzi, Barbieri & Gristina (1991), C from Rogick & van der Schalie (1950), D from Hastings (1939).

(1) Evolution of asexual propagules

Asexual propagation is important for bryozoans in unstable conditions (Okamura & Hatton-Ellis, 1995; Carter *et al.*, 2010b). The statoblasts of phylactolaemates are not polymorphic zooids, since they are budded within a zooid. By contrast, hibernacula and sacculi both bud from zooids and are considered zooidal. In some species of *Amathia*, stolons can also become filled with yolk reserves, and function in a similar manner to hibernacula [as in *Bowerbankia* (Jebam, 1975)]. This suggests that hibernacula may have evolved from the kenozooidal stolon.

Kenozooids *per se* have not been described in *Aetea* (Hayward & Ryland, 1998), so sacculi may have arisen directly from autozooids. The homology of sacculi with autozooids (Silén, 1977) is supported by the existence of 'free autozooids' in *Aetea*. Free autozooids do not cement to the substratum, but are capable of budding normal encrusting zooids (Simma-Kreig, 1969). It is unclear whether these free autozooids possess a feeding polypide. Like sacculi, the free autozooids are punctate, exhibit a different colour to the rest of the colony, attach to the substratum *via* tubular projections instead of the main body, and may be laterally budded

(Simma-Kreig, 1969). Perhaps free autozooids represent an intermediate step between sacculi and regular autozooids.

VII. POLYMORPHS OF UNKNOWN FUNCTION

(1) Nanozooids

Nanozooids are dwarf zooids unique to cyclostomes and possess a reduced, but protrusible, polypide. Primary nanozooids are restricted to the cyclostome genus *Diplosolen* (Silén & Harmelin, 1974; Taylor & Weedon, 2000). They possess a single, long unciliated tentacle (Silén & Harmelin, 1974; Fig. 20) that is responsive to touch (indicating a potentially sensory function) and is able to remove detritus from the colony surface. A cleaning function would make the primary nanozooid a functional analog of vibracula (Silén & Harmelin, 1974). In *Diplosolen obelium*, the primary nanozooids are regularly budded with autozooids (Silén & Harmelin, 1974) and together may be considered a corium composed of two submodules.

Unlike primary nanozooids and other heterozooids, secondary nanozooids are originally budded as autozooids

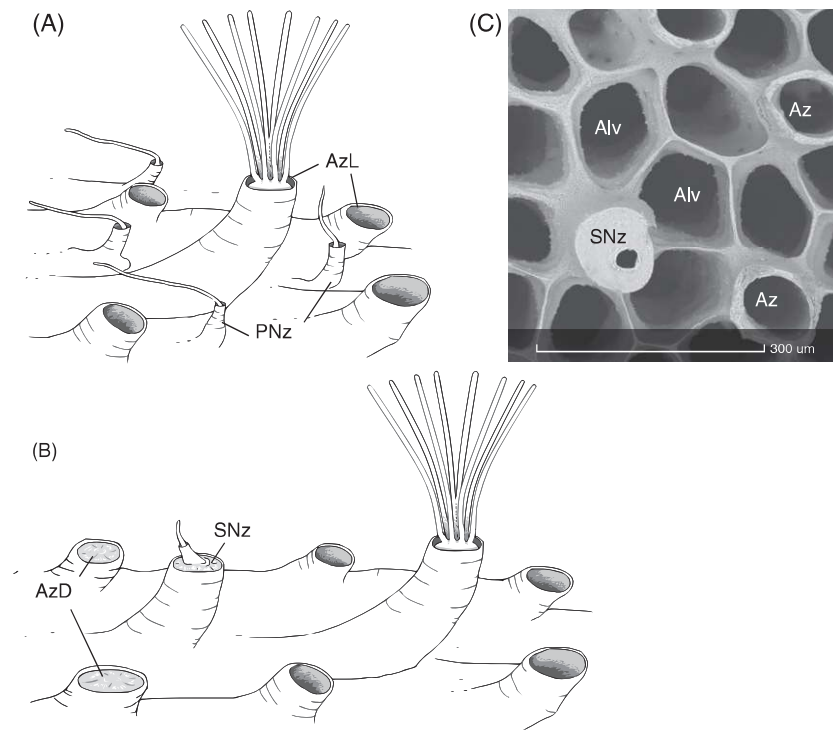


Fig. 20. Cyclostome nanozooids. (A) *Diplosolen* sp. with primary nanozooids, (B) *Plagioecia* sp. with secondary nanozooids, (C) *Favosipora candida* with secondary nanozooids (top-down view). Alv, alveolus; Az, autozoid; AzL, living autozooids; AzD, autozooids with degenerated polypide and orifices sealed by a calcified terminal diaphragm; PNz, primary nanozooids; SNz, secondary nanozooids. A and B drawn from Figs 11 and 16 in Silén & Harmelin (1974), photograph in C taken by D.P.G.

(Silén & Harmelin, 1974) and are an example of ontogenetic polymorphism (Taylor & Weedon, 2000). When the polypide in these autozooids degenerates, the peristome falls off and the original orifice is sealed by a terminal diaphragm that is typically calcified. In secondary nanozooids, this terminal membrane hosts a secondary orifice through which a severely shortened unciliated tentacle is protruded (Fig. 20; Silén & Harmelin, 1974). The function of secondary nanozooids is particularly obscure: they cannot feed (tentacle unciliated) or clean (tentacle too short), they are not reproductive (no male sex cells observed), and no secretory glands or special organs have been observed (Silén & Harmelin, 1974). Instead, secondary nanozooids may have a sensory function. Although the tentacle is short, it remains protruded for long periods, even when autozooids' tentacle crowns have been retracted (Silén & Harmelin, 1974). This behaviour would enable the colony to gather sensory information without danger to the autozooids. Either way, secondary nanozooids may have given rise to primary nanozooids (Silén & Harmelin, 1974), and similar structures are found in other cyclostomes and stenolaemates (e.g. Bancroft, 1986).

Nanozooids may also be generated in response to disturbances in growth, whether external (e.g. substratum irregularities), or because of reduced space from colony structuring (e.g. swelling of gonozooids, see Section V.4) (Silén & Harmelin, 1974). These irregularly formed structures can resemble primary nanozooids or secondary nanozooids (e.g. *Plagioecia sarniensis* and *P. dorsalis*,

respectively), although a protrusible polypide has not been observed (Silén & Harmelin, 1974).

(2) Zooeciules

Zooeciules are dwarf zooids in the cheilostome superfamily Hippothooidea (particularly in *Hippothoa*, *Plesiothoa*, and *Trypostega*). They possess an orifice (sometimes with condyles) and an operculum, suggesting that they at least possess opercular muscles and a polypide to create the official aperture (Lutaud, 1983) (Fig. 21). Zooeciules can replace autozooids (budding distally or laterally), bud laterally on cauda, or adventitiously on the frontal wall (D.P.G., personal observations). They may also bud autozooids, female zooids or other zooeciules (Hastings, 1979). Although zooeciule size and shape varies, the orifice remains minute (Osburn, 1952) so the structure is unlikely to contain a protrusible polypide.

Zooeciules may be male zooids, but definitive evidence for this (e.g. sperm, protrusible tentacles) is lacking (Hastings, 1979), despite the presence of dimorphic females (Hastings, 1979; Hayward & Ryland, 1999). Instead, zooeciules may be analogous to avicularia (Osburn, 1952). Vicarious avicularia have been found in species with zooeciules (*Hippothoa distans*; Hastings, 1979). These avicularia are larger than autozooids and have extensive musculature, but their mandibles are relatively unmodified. If vicarious avicularia were developed first, then zooeciules may be more derived forms of avicularia (Osburn, 1952).

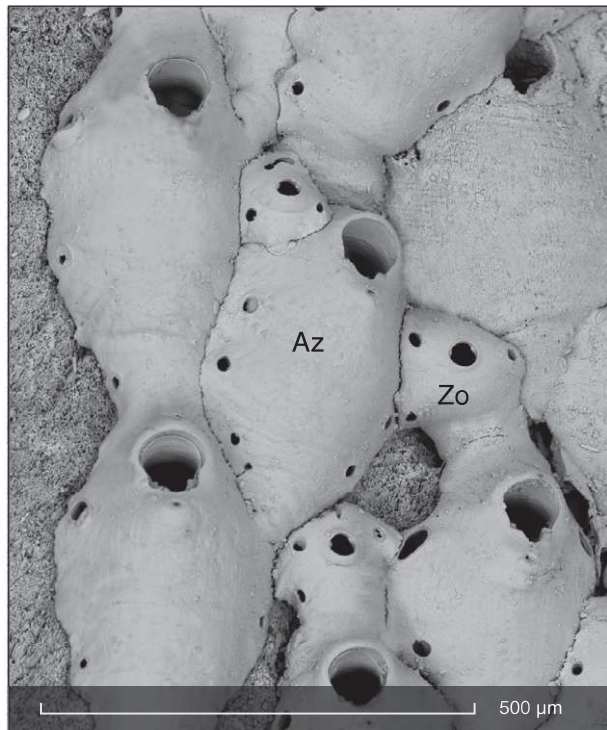


Fig. 21. Zooeciules of *Plesiothoa trigemma*. Az, autozooid; Zo, zooeciule.

VIII. DEVELOPMENT OF POLYMORPHISM

(1) Origin and evolution of polymorphism

Modularity sets the stage for differentiation and polymorphism. Modularity increases adaptive potential by allowing modules to experience selection separately and by limiting the damage from deleterious mutations (Kirschner & Gerhart, 1998; Carroll, 2001). Adaptive potential is further enhanced through function-preserving redundant modules (Kirschner & Gerhart, 1998; Budd, 2006).

Novel phenotypes are generated from new genotypes or the plastic (not necessarily adaptive) response of the original genotype to a new environment (West-Eberhard, 2003). Since zooids in a bryozoan colony are genetically identical, a genotypic change will essentially affect all colony members. While new genotypes create novel ‘colony-wide’ phenotypes, the development of zooidal polymorphism requires phenotypic variance among colony modules. This variation is provided by the plastic response of zooids to microenvironmental changes within colonies (e.g. differences in flow, substratum irregularities, crowding). In contrast to ‘random’ variation resulting from the imprecision of developmental machinery, plasticity provides predictable variation: if the magnitude of each environmental effect and the rules governing developmental responses are known, one could predict zooid form.

Both new genotypes and plastic responses are important to the origin of polymorphism: new genotypes can result in novel structures or pathways, while plasticity

provides phenotypic variation within a genotype (i.e. a single colony). Plastic phenotypes (including polymorphisms) are generated through the modification of existing structures (Silén, 1977). This modification can involve deletion, duplication, reorganization, fusion/segregation, or heterochrony (changes in developmental timing) (West-Eberhard, 2003). For example, the production of kenozooids involves deletion of the polypide, the formation of an avicularium likely involved heterochrony (enlargement of operculum, reduction of polypide) (Harvell, 1994; Cheetham *et al.*, 2006), and the development of some ooecia and frontal shields involved the fusion of spines (Dick *et al.*, 2009; Lidgard *et al.*, 2012; Ostrovsky, 2013).

The changes in gene expression that control bryozoan polymorphism are currently unknown. Hox genes control oral–aboral regionalization of body sections and are ideal for examining differences in body-plan formation (Halanych & Passamanek, 2001). In hydroids, the development and maintenance of specialized zooids may be controlled by differential expression of the Hox gene *Cnox-2* (Cartwright, Bowsher & Buss, 1999). In gastrozooids (and solitary forms) there is a low expression of *Cnox-2* in the hypostome and tentacular region, and a high expression in the body column.

While dactylozooids and gonozooids (expanded body columns) have high *Cnox-2* expression, tentaculozooids (expanded hypostome and tentacles) have low *Cnox-2* expression (Cartwright *et al.*, 1999). By analogy, this type of differential Hox-gene expression may also play a role in the development of bryozoan polymorphs. In addition to Hox genes, differential expression of genes related to polymorph-specific processes or structures [e.g. nematocysts in siphonophore gastrozooids (Siebert *et al.*, 2011); calcium deposition by axial polyps in *Acropora* corals (Hemond, Kaluziak & Vollmer, 2014); mandibular glands in termite soldiers (Miura, 2005)] should also be investigated for bryozoan polymorphs.

Once zooidal plasticity modifies colony fitness, selection (or stochastic processes) can maintain plasticity or result in the canalization of a response. Selection for intracolony plasticity can only occur if the benefit of possessing plastic zooids is greater than the benefit of non-plastic zooids exhibiting one level of the plastic response (Van Kleunen & Fischer, 2005). For example: there are two non-plastic variants in *M. membranacea* (always unspined or always spined) and a plastic genotype where peripheral autozooids can be induced to form spines (Harvell, 1998). In this case, the benefit of only producing spines when needed should be greater than the costs of plasticity: the lag (~2 days; Harvell, 1984) between unspined and spined states leaves the colony vulnerable to predation from nudibranchs, while misinterpreting a cue can result in accidental expenditure of resources [although accidental spine production may be rare because of cue thresholds (Harvell, 1984)] (Van Kleunen & Fischer, 2005). Additional costs of plasticity may include increased developmental instability and the maintenance of sensory structures to detect cues (Van Kleunen & Fischer, 2005). If plasticity is too costly, the responses of zooids may

become invariant to environmental cues (Pigliucci, Murren & Schlichting, 2006). Unfortunately, the mechanisms of canalization (i.e. genetic assimilation) are currently unknown (Harvell, 1994; West-Eberhard, 2003; Pigliucci *et al.*, 2006).

Complete canalization is impossible when the plastic response of zooids produces non-feeding forms, as is the case with kenozooids and avicularia. Instead, selection can change the sensitivity of the response and remove intermediate forms. As a hypothetical example, a colony produces a continuous range of zooid types (X_0 – X_{10}). Differing levels of the cue across the colony produce different zooids (none = X_0 , intermediate = X_1 – X_9 , high = X_{10}), resulting in a patchwork of responses within the colony. However, if X_1 – X_9 zooids perform worse than X_0 and X_{10} zooids in their respective tasks, selection may change the sensitivity threshold so that only X_0 and X_{10} zooids are produced. This would result in discrete zooidal plasticity, i.e. polymorphism. While X_0 and X_{10} forms could become relatively invariant to the external environment, internal rules would be necessary to govern when and where each zooid type is produced (e.g. to prevent an imbalance of feeding: non-feeding forms). These internal rules govern the formation of cormidia and the zooidal composition of colonies.

While complete canalization of non-feeding zooidal polymorphs is not possible, canalization can be achieved through the transformation of a zooidal polymorph to an autozooidal appendage. Such a transformation is seen in the diverse evolution of non-cribrimorph frontal shields *via* overgrowth of the frontal surface by kenozooids, which eventually lose their zooidal nature (Gordon & Voigt, 1996; Taylor, Casadio & Gordon, 2008). Similarly, spines may have been derived from spine-like kenozooids. The mechanisms underlying the change from zooid to appendage are unclear, but likely involve strict cormidial assembly rules, adventitious budding, and fusion of zooids to create a confluent coelom. Regardless of the method, it provides an ‘evolutionary workflow’ for developing novel structures: a kenozooid is derived from an autozooid and modified into a novel structure that is then incorporated into an autozooid as an appendage. This transition increases colonial integration, a trend also observed in the transition from vicarious to adventitious avicularia. However, it is unlikely that adventitious avicularia could ever become true appendages (possessing a confluent coelom with the host autozooid) since they require their own sensory mechanisms and musculature [although autozooids’ musculature can provide nodding behaviour in birds-head avicularia (Kaufmann, 1971)].

Thus, the origin and continued evolution of zooidal polymorphism in bryozoans requires: (i) modular units with modifiable structures; (ii) plastic responses to cues that are heterogenous across the colony; (iii) removal of intermediate forms by changes to cue sensitivity thresholds; and (iv) development of cormidial and colony-level assembly rules to control polymorph budding. Whether these rules hold true for other colonial taxa is worth investigation.

(2) The cormidium: zooid assembly rules

Colonies are built module by module, rather than through ‘top-down’ control exerted by the colony as a whole. This means that bryozoan zooids must react individually to stimuli (including interzooidal communication). However, each zooid follows the same ‘instructions’, which results in the formation of secondary and tertiary structures through module iteration (Hageman, 2003). Few reactions are possible for zooids in monomorphic colonies although diverse colony forms are still possible, but zooids in polymorphic colonies require additional reactions (to various cue thresholds) to assemble cormidia and organize different cormidial types. These cormidial assembly rules reflect both selection at the zooid level (microenvironmental) and at the colony level (macroenvironmental). Ultimately, cormidia and colony form are emergent properties of zooid modularity and plasticity.

Cormidial assembly requires interzooidal communication and may be loose or rigid. For example, two simple rules govern zooid patterns in *Hesychoxenia praelonga*: (i) marginal zooids must be kenozooidal; and (ii) only 2–3 autozooids may bud next to each other (Gordon & Parker, 1991*b*). Similarly, cormidial assembly rules can ensure adequate water flow *via* the production of maculae (Banta, McKinney & Zimmer, 1974; Anstey, Pachut & Prezbindowski, 1976). These structures, found in some living and fossil stenolaemates and cheilostomes, consist of autozooids, polymorphs, and an extrazooidal skeleton (Banta *et al.*, 1974; McKinney & Jackson, 1989; Taylor & Weedon, 2000).

Cormidial assembly rules may also vary with environmental conditions (reflecting the plasticity inherent in polymorphism). For example, in *Celleporella hyalina* (Cheilostomata) environmental stress induces formation of male zooids and suppression of female zooids (Hughes *et al.*, 2003). Allocating energy to male zooids may be a ‘reproductive bailout’ in conditions where the parent colony (and any brooded offspring) have a high mortality risk (Hughes *et al.*, 2003). Aside from ‘reproductive bailouts’, cormidial assembly rules in bryozoans may be invariant along macroenvironmental gradients (Hughes & Jackson, 1990; Simpson *et al.*, 2017).

In addition, colonies with multiple types of cormidia require rules that dictate the organization of different cormidia types. The most striking example is the cheilostome *Corbulipora tubulifera*, which was thought to be three separate species owing to its unique cormidial morphologies (Fig. 22). The base of the colony is formed of cribrimorph autozooids and rhizoids. From this, a kenozooidal stalk is produced that eventually buds ovicelled autozooids with membranous frontal walls and vicarious avicularia. These membranous autozooids alternate with cribrimorph autozooids as the colony grows (Bock & Cook, 1994). This complicated colony structure must rely on a set of assembly rules to dictate the organization of each cormidium type, and the location and duration of cormidial phases (which could be invariant or dynamic).

Cormidia are found in a variety of taxa besides bryozoans. In most siphonophores, cormidial rules are so

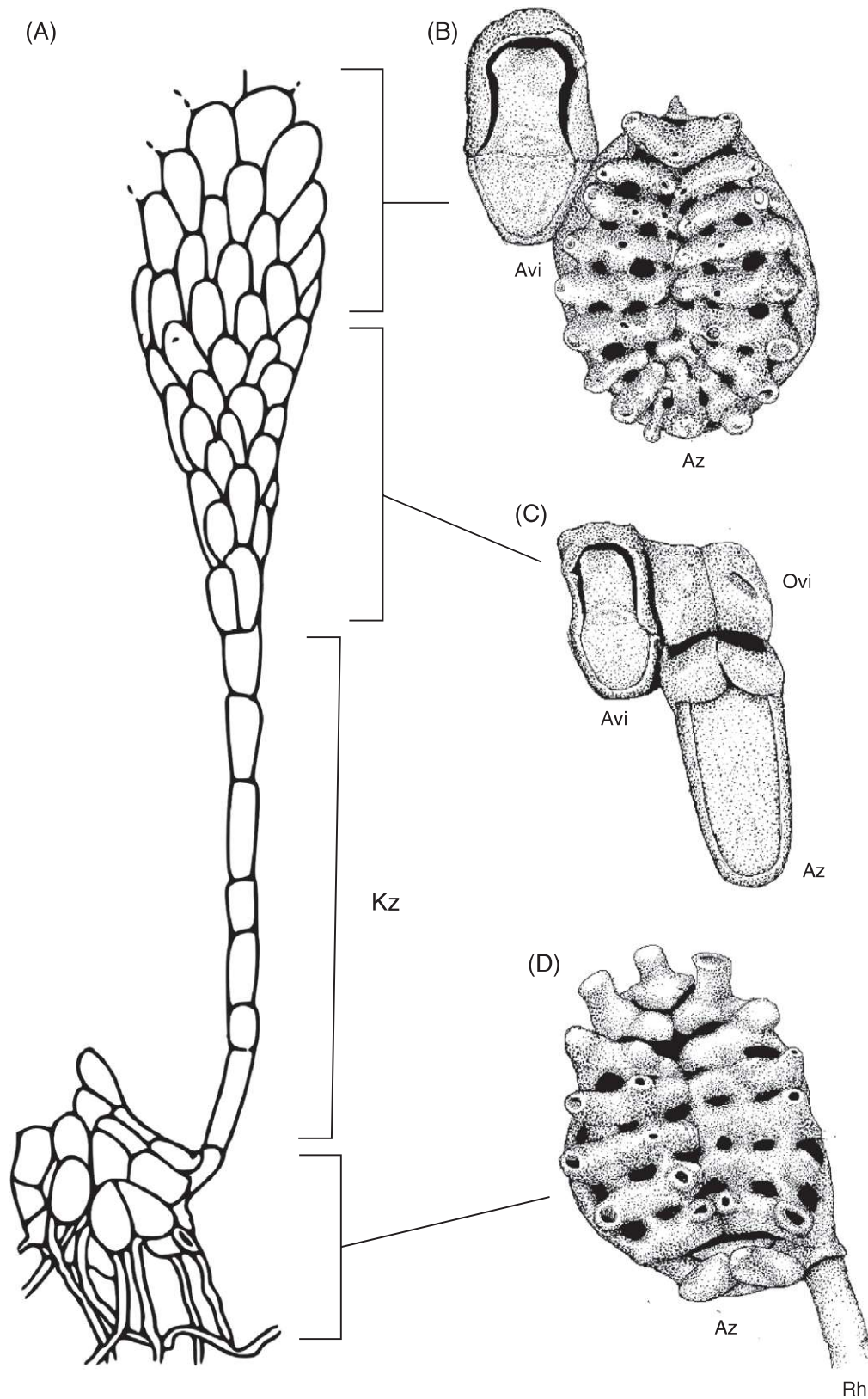


Fig. 22. *Corbulipora tubulifera*. (A) Colony structure, (B) bilaminar phase, (C) flustrine phase, (D) encrusting phase. Avi, avicularium; Az, autozooid; Kz, kenozooidal stalk; Ovi, ovicell; Rh, rhizoid. Drawn from Bock & Cook (1994).

strict that they may be considered semi-canalized. For example, the siphonophore *Bargmannia elongata* produces polymorphic polyps through the division of a probud, which always differentiates into a gastrozoid, a tentaculozoid, a gonozoid, five bracts, and several small buds in that order (Dunn & Wagner, 2006). By contrast, some colonial hydrozoans have dynamic cormidial rules. The hydroid *Thecocodium quadratum* has two polymorphs: a large gastrozoid with a mouth, digestive ability, and no tentacles; and a mouthless dactylozoid with four tentacles to capture prey. Gastrozooids typically stimulate the production of nearby dactylozooids and suppress the budding of nearby gastrozooids. However, when gastrozooids are fed dead material (capture by dactylozooids not required) then dactylozoid production is suppressed and more gastrozooids are budded, illustrating a dynamic response to environmental conditions (Pfeifer & Berking, 2002). While eusocial insect colonies do not have cormidia *per se*, they do regulate the relative proportions of polymorphic castes. In stingless bees (*Melipona beecheii*) workers execute females that develop into extra queens (Wenseleers *et al.*, 2004). However, insect colonies are composed of modules that are not genetically identical: this means that some taxa have caste ratios that are more dependent on genotypes of colony individuals, rather than cue-mediated regulation (Schwander *et al.*, 2010).

(3) Incidence of polymorphism and degrees of modularity

Many different degrees of modular organization exist. The ability to evolve polymorphism in bryozoans may be tied to the level of module dissociation, which may explain why polymorphism is not present in all bryozoan classes (Ryland, 1979). Phylactolaemates lack polymorphism and have low module dissociation, while gymnolaemates have the highest level of polymorphism (Mukai *et al.*, 1997). Stenolaemates have a moderate degree of polymorphism, but varying levels of module dissociation (lower in free-walled than fixed-walled stenolaemates) (Ryland, 1979; Boardman, 1998). In addition, bryozoan taxa have different levels of developmental dissociation: the polypide is formed first in stenolaemates and phylactolaemates, while in gymnolaemates the cystid is formed first (Lutaud, 1983; Mukai *et al.*, 1997). It appears that module dissociation is tied to polymorphism in bryozoans. However, a close examination of polymorphism in different genera and quantitative variation in interzooidal communication is warranted [see Bone & Keough, 2010 and Miles *et al.*, 1995].

Across colonial marine taxa, increased module compartmentalization may be related to the incidence of polymorphism (Venit, 2007). For example, colonial entoprocts have weakly dissociated modules [common vascular system (Borisanova & Malakhov, 2011)] and lack polymorphism (Brusca & Brusca, 2003). Conversely, the strongly compartmentalized zooids in doliolids (Thaliacea) can be polymorphic: in the asexual pelagic phase of their life cycle, doliolid colonies have feeding gastrozooids and reproductive phorozoids that detach and bud gonozooids (Alldredge &

Madin, 1982). This polymorphism may result from nutrient transduction across a 'placenta-like' membrane between adjacent zooids (Venit, 2007).

However, weak compartmentalization of modules does not prevent the evolution of polymorphism. Colonial hydroids, siphonophores, and sea pens all have common gastrovascular cavities, and therefore have a level of module dissociation similar to phylactolaemates. Despite this, these taxa can be highly polymorphic, possessing polyps specialized for reproduction, defence, feeding, colony structure, and asexual propagation (Table 4) (Cartwright *et al.*, 1999; Cartwright, 2003; Dunn, 2005; Dunn & Wagner, 2006; Williams *et al.*, 2012; Sanders *et al.*, 2014). By contrast, scleractinian corals have a wide range of modular dissociation: polyps may be completely separated by the skeleton, share an overlying tissue (cenosarc), or may possess gastrovascular connections (coelenteric canals) through voids in the skeleton (Swain *et al.*, 2018). Despite this range in zooid compartmentalization, polymorphism is rare and poorly developed when present: apical polyps in *Acropora* colonies are sterile, possess fewer tentacles and have lower symbiont levels than gamete-producing radial polyps (Hemond *et al.*, 2014; Swain *et al.*, 2018).

While strong dissociation of modules may increase the ability to generate polymorphs, some module integration is necessary to evolve non-feeding polymorphs: without nutrient transfer at some stage, non-feeding forms are impossible. Pelagic salps (Thaliacea), for example, form chains of zooids that are attached together by special plaques that can propagate nerve impulses (Mackie & Bone, 1977 in Mackie, 1986). However, the attachment plaques do not transmit nutrients and colonies can easily break apart in response to a stimulus (Mackie, 1986), indicating high module dissociation. Pyrosome colonies (Thaliacea) also lack vascular, muscular, and nervous connections between zooids. As such, no polymorphic zooids have been found in salp or pyrosome colonies (Venit, 2007).

Eusocial insect colonies are an exception to the rule that colonies with fully compartmentalized modules lack polymorphism. These colonies have modules that are physically compartmentalized but behaviourally integrated. This allows eusocial insects to produce non-feeding forms: for example, in termites the queen may be unable to move through the subterranean colony and workers must provide food (e.g. *Serritermes serriker*; Barbosa & Constantino, 2017). Despite high compartmentalization, eusocial insects exhibit only a moderate level of polymorphism (polymorphic workers found in only ~15% of ant genera and one bee species; Wheeler, 1991; Grüter *et al.*, 2012).

While strongly dissociated modules may make it easier physically to develop polymorphs, it is neither a guarantee nor a requirement for their evolution. However, complete compartmentalization of modules will prevent polymorphism unless colony members are behaviourally integrated. Trends in dissociation of modules and the incidence of polymorphism in colonial invertebrates may be hampered by differences in morphology across taxa.

Table 4. Polymorphism in colonial invertebrate taxa, showing level of module compartmentalization, level of polymorphism, and the functions the colony can perform. Functions fulfilled by structures that are not equivalent to an individual module (e.g. non-zooidal structures) are indicated by an asterisk.

Phylum	Taxa	Compartmentalization	Polymorphism	Feeding	Reproduction	Structure	Defence	Asexually produced propagules	Other	Source
Bryozoa	Ctenostomata	High	Moderate	Autozooids	Gonochoeristic zooids, ovicells	Kenozooids, stolons, rhizoids, extrazoooidal skeleton	Spines	Hibernacula		Silén (1977); Pohowsky (1978); Boardman (1983); Wood (1983); Schäfer (1991); Taylor & Weedon (2000); Ostrovsky (2013)
	Chelostomata	High	high				avicularia, B-zooids	sacculi	Vibracula	
	Fixed-walled Stenolaemata	Moderate	Moderate		Gonochoeristic zooids, gonozooids		spines, stolons			
	Free-walled Stenolaemata	Low	Low–moderate				Spines, styles, cleistozooids		Nanozooids	
Entoprocta	Phylactolaemata	Low					Spines, styles			
	Coloniales	Low	None	Autozooids						Brusca & Brusca (2003); Borisanova & Malakhov (2011)
Cnidaria	Colonial hydroids (Hydrozoa)	Low	Moderate	Gastrozooids, dactylozooids	Gonozooid + gonophores		Dactylozooids, tentaculo-zooids	Medusa		Cartwright <i>et al.</i> (1999); Cartwright (2003); Sanders, Shcheglovitova & Cartwright (2014)
	Siphonophores (Siphonophora; Hydrozoa)	Low	High	Gastrozooids, dactylozooids	Gonozooid + gonophores	*Primary buds in siphosome and nectosome		*Whole cornidia break off	Nectophores, palpons	Dunn & Wagner (2006); Mapstone (2017)
	Sea pens (Octocorallia; Anthozoa)	Low	Moderate	Autozooids	Autozooids	Oozooid	Autozooids	Acrozooids	Siphonozooid, mesozooid	Kastendiek (1976); Williams, Hoeksema & Olwegen (2012)
	<i>Amphora</i> hard corals (Hexacorallia; Anthozoa)	Low	Low	Radial zooids		Apical zooids				Hemond <i>et al.</i> (2014); Swain <i>et al.</i> (2018)

Table 4. Continued

Phylum	Taxa	Compartmentalization	Polymorphism	Feeding	Reproduction	Structure	Defence	Asexually produced propagules	Other	Source
Tunicata	<i>Echinophyllia aspera</i> , <i>Myccidium</i> <i>elephantinus</i> (Hexacorallia: Anthozoa)	Low	Low	Confluent gastrovascular cavity through aligned costosepta	Zooids with polymorphic corallite morphology (flat <i>versus</i> protruded; polymorphic central corallite)					Arrigoni <i>et al.</i> (2016); Swain <i>et al.</i> (2018)
	Other scleractinian corals	High—complete	None	Zooids separated by skeleton, may share overlying tissue (renosart)	Zooids					Swain <i>et al.</i> (2018)
	(Hexacorallia: Anthozoa)	High	Low—moderate	Zooids separated by a permeable barrier	Gonozooids	Oozooids		Phorozoid		Allredge & Madin (1982)
	Doliolids (Thaliacea)	Complete	None	No vascular, muscular, or nervous connections between zooids	Zooids					
	Salps (Thaliacea)	Complete	None							
Hemichordata	Pyrosomes (Thaliacea)	High—complete	None	Zooids contiguous, may share a common tunic	Blastozooids					Venit (2007)
	Colonial ascidians (Ascidacea)	Low	None	Zooids share a common tunic and blood vasculature						Sabbadin (1979); Brown & Swalla (2012)
	<i>Rhabdopleura</i> (Pterobranchia)	Complete	?None	Zooids thecate, interzooidal stolon restricted by diaphragms	Zooids	Gonochoristic zooids		?Hibernacula (may be juvenile zooids)		Urbanek & Dilly (2000); Maletz (2017)
	Graptolites (Pterobranchia)	?High	?Unclear	Zooids thecate, connected by a stolon running through a common canal	Autotheca	?Bitheca				Maletz (2017)
Arthropoda	Eusocial aphids (Hemiptera)	Complete	Low	Individuals not physically connected	Workers	Sex mothers, non-soldiers	Soldiers	Alate clones, *overwintering eggs		Wheeler (1991); Tyerman & Roitberg (2001); Mura (2005); Grüter <i>et al.</i> (2012)
	Eusocial termites (Blattodea)									
	Eusocial ants and bees (Hymenoptera)					alate (winged) or ergatoid (wingless) queens				

To address this, a quantitative approach should be used to determine module dissociation, e.g. by measuring nutrient transduction (Mackie, 1986; Miles *et al.*, 1995), and to quantify morphological and genetic polymorph differentiation (e.g. Hemond *et al.*, 2014). Such a study may reveal that other aspects of modularity are more important than module dissociation. Although module dissociation may be influential for Bryozoa (Ryland, 1979), it may have little bearing on the level of polymorphism in other taxa.

(4) Types of polymorphs

While all zooidal polymorphism must arise from morphological plasticity in autozooids (or other polymorphs), the types of polymorphs are dependent on the modifiable structures present within a zooid, and the functions required by a colony. For example, definite mandibulate structures (avicularia, eleozooids) exist only in bryozoan taxa that have an operculum. The operculum and its associated musculature are preadaptations for a grasping function (Winston, 2004) and thus make mandibulate polymorphs more likely to evolve in operculate taxa (Cook, 1968; Cheetham *et al.*, 2006). Similarly, the nematocyst-laden tentacles of zooids in colonial cnidarians easily lend themselves to defence (e.g. the sea pansy *Renilla kollikeri*; Kastendiek, 1976) and few ‘steps’ should be required to derive defensive polymorphs from them (e.g. tentaculozooids and dactylozooids in colonial hydroids and siphonophores). Once derived, polymorphs can be further modified to perform different functions (e.g. cleaning vibracula from defensive avicularia). Kenozooids and the extrazoidal skeleton in particular provide modifiable structures since they are no longer constrained by the presence of a polypide. The ability to form a ‘free module’ may explain the high level of polymorphism in bryozoans, even compared to polymorphic cnidarians.

The two functions every colonial organism must fulfil are feeding and reproduction, and these are the most common polymorphs (Table 4) – particularly because the ‘machinery’ required to perform these functions is present in monomorphic zooids. Specialized reproductive modules have developed in most polymorphic colonial taxa except sea pens [which are gonochoristic at the colony level (Edwards & Moore, 2008; Williams *et al.*, 2012)] and *Acropora* stony corals [polyps are simultaneous hermaphrodites and spawn rather than brood (Harrison, 2011)].

While polymorphs probably originate under heterogeneous microenvironmental conditions, their continued evolution can be shaped by both micro- and macroenvironmental stressors (in addition to stochastic processes). Defensive polymorphs may arise in environments with many predators, competitors, or abrasive substrata. Changes in morphology may reflect environmental pressures for different functions or increased efficiency [e.g. the potential latitudinal gradient in avicularian mandible shape (Schopf, 1973)]. Similarly, cleaning polymorphs could arise in areas with high fouling and sedimentation rates, asexual propagules may develop in response to harsh seasonal conditions, and attachment structures should be developed based on

colony form and substratum type. However, changes along macroenvironmental gradients may not affect the level of polymorphism in bryozoans (Hughes & Jackson, 1990; Simpson *et al.*, 2017), even though high levels of stress can trigger a polymorphic response [e.g. increased production of male zooids (Hughes *et al.*, 2003)].

It is assumed that division of labour increases efficiency and is therefore favourable under selection (Nyhart & Lidgard, 2011; Lidgard *et al.*, 2012; West & Cooper, 2016). Increased efficiency may stem from the elimination of task-switching costs (Goldsby *et al.*, 2012) in addition to increased performance from physiological specialization. Since many polymorphs are non-feeding, an increase in efficiency would offset the cost of such structures, particularly in the energetically marginal lifestyle of bryozoans [Thorpe, 1979; Lidgard (1981) in Gordon, Clark & Harper, 1987; Lidgard *et al.*, 2012]. However, the magnitudes of these efficiency gains, if any, have not been determined. It is possible that polymorphisms may provide neutral efficiency, indicating a lack of directional selection. Interestingly, polymorphic bryozoan colonies typically succeed ephemeral non-polymorphic ones, suggesting that polymorphism represents increased investment in colony survival and competitive ability (Simpson *et al.*, 2017).

IX. SUMMARY

This review has described the forms and functions of bryozoan polymorphisms. Limitations on the realized morphospace of bryozoan polymorphisms have yet to be determined (Schack *et al.*, 2018). Polymorphisms do not arise *de novo* but are formed from the modification of fundamental autozooid modules (Silén, 1977). Modifications can be made to the polypide (in reproductive polymorphs), the cystid (appendaged autozooids) or both (heterozooids). The realized morphospace of bryozoan polymorphism and the extent to which modules can be structurally differentiated may be limited by the structure of the base autozooid. For example, the limited bryozoan nervous system may prevent avicularia, which have intrinsic musculature, from becoming ‘true’ limbs. The grasping long-stalked avicularia in *Camptoplites* (Bugulidae) look like protolimb, and can nod using the musculature of the autozooid-provided peduncle (Kaufmann, 1971) but the stalks themselves have no independent power of mobility. While high module dissociation may be related to the level of polymorphism in bryozoans, this is not always the case for other colonial taxa [e.g. colonial cnidarians (*cf.* Venit, 2007)]. The development of polymorphism does rely on ‘true’ coloniality: modules must be behaviourally or physically connected, otherwise non-feeding polymorphs cannot be supported.

Most polymorphisms have unknown or highly debated functions. It is important to note that a polymorph may have a variety of sub-functions in addition to (although less efficiently than) its main function (e.g. a knife can be used to drink soup, albeit very inefficiently). Determining these functions,

along with their scope and effectiveness, will require *in vivo* observations and empirical testing. Understanding form and function will provide information on polymorphism diversity and their division of labour (e.g. potential overlaps in function between polymorphisms). Combined with evolutionary phylogenies, knowledge of function will allow the construction of 'differentiation sequences' showing whether certain polymorphs always evolve first or are more likely to evolve (e.g. reproductive polymorphs are common in most polymorphic colonial organisms).

Three interrelated aspects of polymorphism urgently require further study: developmental mechanisms, energetics, and trait–environment relationships. Understanding these, in addition to form and function, would contribute greatly to our understanding of the evolution of modularity, module differentiation, and the division of labour; concepts which, in turn, are key to understanding the evolution of complexity (Kirschner & Gerhart, 1998; Carroll, 2001). One hopes that interest in bryozoans – and their polymorphisms – will continue to increase, for it is clear that modularity and polymorphism have been key to their evolutionary success.

X. CONCLUSIONS

(1) Modularity is vital to the evolution of polymorphism: the dissociation between modules allows heterogeneous selective pressures to act independently and facilitates the division of labour.

(2) Module dissociation between zooids and dissociation between the cystid and polypide may be related to the level of polymorphism in bryozoans. However, this may not be the case for other colonial organisms.

(3) Cormidia, the organization of cormidia types, and colony form are emergent properties arising from the modularity and plasticity of bryozoan zooids.

(4) Superficially similar polymorphs (e.g. spines, rhizoids, space-filling polymorphs) can be kenozooidal, autozooidal appendages, or extrazoooidal structures. Careful study of their development and internal anatomy is required to separate the different types.

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Appendix 1

Errata for:

Chapter 2, Modularity is the mother of invention. Page numbers are given as they appear in the thesis, while journal page numbers are given in parentheses.

Page	Original text	Corrected text
(787)	...damage and maintain turgor pressure...	...damage and maintain turgor pressure...
(793)	In addition to the formation of non-spinose ovicells, there is trend towards...	In addition to the formation of non-spinose ovicells, there is a trend towards...
(800)	However, each zooid follows the same 'instructions', which results the formation...	However, each zooid follows the same 'instructions', which results in the formation...

Classification of cheilostome polymorphs

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1. Introduction

It is still debated whether apparent increases in biological complexity are driven by natural selection for greater efficiency¹ or are merely due to ‘diffusive’ processes away from minimum complexity.² The continued existence of simple bacteria and the reduction of complexity in many species (such as eye degeneration in cave fish,³ digit loss in squamate reptiles,^{4,5} and the evolution of lungless salamanders⁶) favor a diffusive explanation over long time scales.^{7,8,9} Evolution does not ensure that the “end point” of a lineage will be more complex than its ancestors. Despite this, backtracking through some grades of complexity appears to be impossible: no multicellular organism has produced

a unicellular lineage, and no eukaryote has generated a prokaryotic line.¹⁰

Perhaps complexity should be selected for because it allows organisms greater efficiency through division of labor (via cell types, organ systems, polymorphs, etc.).¹¹ Evidence for active trends in increasing complexity can be observed through changes in minimum complexity, subclade skewness, and ancestor-descendant comparisons.¹² Indeed, directional selection for increased complexity has been found within clades¹³ (suture structures in ammonoids,¹⁴ limb tagmosis in arthropods,^{15,16} and hinge geometry of brachiopods^{17,18}), though these trends can depend on the measure of complexity used.^{19,20}

There is also an apparent cost to complexity: increases in the number of traits in an organism results in a decrease in the amount of “progress” a single mutation can achieve toward optimal trait values.²¹ Although mutation rate does increase with complexity, it does not do so quickly enough to eliminate the cost.^{22,23} This means that more complex organisms are less adaptable than their simpler counterparts.²⁴ However, the cost of complexity can be alleviated by features that increase adaptive potential. This evolvability can be enhanced either by reducing the lethality of mutations or reducing the number of mutations required to produce a new trait.²⁵ One of the most important ways to do this is through modular construction.²⁶ Modularity (compartmentalization) increases adaptive potential by allowing modules to experience and respond to selection separately.²⁷ This is similar in principal to fire doors in a building: if there is a fire (a deleterious mutation) the damage is contained within a certain area (module). Adaptive potential and decreases in structural interdependence are further enhanced by module redundancy, which allows function to be preserved while new traits and functions arise in the redundant modules.²⁸

It is clear that modularity can facilitate the evolution of complexity by reducing its cost, but both concepts require further study. Evolutionary questions regarding the existence of less-than-maximal modularity and the secondary integration of modules,²⁹ and those conditions that may select for (or against) modular and complex organisms still need to be addressed. Bryozoans, with their rich fossil record and modular colonial construction, are excellent taxa for exploring these questions. Competitive interactions³⁰ and indirect measures of fecundity (ovicells) are also preserved in bryozoan fossils, allowing researchers to determine the relative success of bryozoan body plans through evolutionary timescales. The Cheilostomata are particularly useful for studying the evolution of complexity and modularity because of their polymorphic zooids and derivative or associated units.³¹ These polymorphs (divergent modules that exhibit discontinuous variation) have arisen independently multiple times in cheilostome evolution,^{32,33} providing multiple temporal/ environmental settings to examine (potential) selection for increased complexity.

However, cheilostome polymorphs are currently described using ambiguous and broad terminology. To rectify this, we have created an extensive classification system for cheilostome polymorphs. To the best of our knowledge this has not been attempted before, despite being sorely needed. The classification system presented here provides comprehensive and standardized terminology along with illustrations of key terms. In addition to evolutionary studies, this system will also be useful for taxonomists, ecologists, and zoologists investigating the often unknown or debated functions of these polymorphs.

Readers unfamiliar with bryozoan anatomy should examine Figure 1, which provides an overview of the standard anatomical descriptions used in this classification system. Briefly, an autozoid consists of a cystid (body wall) and a polypide (moveable soft body), which includes the tentacle crown, digestive tract, musculature, and parts of the nervous system.^{34,35} There is a variety of frontal wall morphologies present in cheilostomes (membranous, gymnocystal cribrimorph, umbonuloid, lepralioid, and cryptocystal). Although some cheilostome frontal shields were kenozooidal in origin, they are now so derived that they are no longer recognizable as separate modules or polymorphs. In contrast, the cribrimorph shield differs in comprising discrete spines (costae) and does not appear to be as integrated as other frontal shields. Therefore umbonuloid and lepralioid shields are not considered polymorphs and will not be discussed here. The frontal morphology most pertinent to this classification system is the cribrimorph shield, which is composed of costae (modified spines). Anatomical details of polymorphs are discussed in the specific sections on each.

1.1 Standardized terms and system design

Ambiguous terminology is a threat to good science. The presence of synonyms and vague descriptions introduces an unwanted degree of subjectivity and can invalidate comparisons between studies. Moreover, the terms currently used to describe cheilostome polymorphism neglect the full range of morphological variation present in these structures.

The system complements the classification of colony growth forms by providing a zoid-level and cormidium-level view of polymorphism morphology (discussed in section 1.2).³⁶ Following Hageman *et al.* (1998), it is nonhierarchical in form, allowing statistical comparisons to be made at many levels of detail. A non-hierarchical form allows polymorphs to be described even when important characters are missing (useful for fossil bryozoans), and allows the system to be easily updated. A flexible and updatable system is desirable, since many bryozoans — perhaps with highly irregular morphology — remain to be discovered.³⁷ Our classification system is also comprehensive: it encompasses 86 different traits (with 289 levels) and includes numeric, binary, ordinal, and factor data types. Over two-thirds (~70%) of these traits can be observed in fossil specimens, though this relies strongly on good preservation. A comprehensive system provides increased ecological resolution to examine trait-environment relationships,³⁸ and the inclusion of many datatypes facilitates its statistical use.

This classification attempts to preserve the terminology already in use by bryozoan taxonomists, while providing more stringent definitions. When new terminology had to be introduced, particularly for avicularia, it was drawn from the morphological classifications of plants.³⁹ Parts of the classification follow the work of Vieira *et al.*⁴⁰ and Ostrovsky,^{41,42} though much is based on our extensive examination of specimens, Scanning Electron Microscope (SEM) micrographs, and taxonomic literature.^{43,44,45,46} Illustrations for this system were created using Microsoft Paint, Krita, Inkscape, and ImageJ.

Finally, this system creates the groundwork for a database of polymorphism morphology to be created. Such a database, paired with information on colony form, specimen location, and ecological data will, we hope, be of great use to the bryozoology research community.

1.2 Polymorphism

Variation in bryozoan zooids can be divided into three categories; astogenetic, ontogenetic, and polymorphic.⁴⁷ Astogenetic variation encompasses the differences in shape and size between the ancestrula, zooids in the zone of astogenetic change, and zooids within the zone of astogenetic repetition. Ontogenetic variation refers to changes in shape and size that occur as a zooid develops. Both astogenetic and ontogenetic variation are continuous, which means there is transitional gradient between the different shapes and sizes of zooids present within a colony. This is in contrast to polymorphic variation, which is discontinuous and displays abrupt changes in shape, size, and other characteristics (see Table 1).^{48,49} Like autozooids, polymorphs may also exhibit astogenetic and ontogenetic variation. There are two main categories of bryozoan polymorphism—1) autozooidal polymorphs and 2) heterozooids.⁵⁰

An autozooidal polymorph retains a protrusible tentacle crown, though it may or may not be able to feed. Autozooidal polymorphs include reproductive zooids, appendaged autozooids, and cyclostome nanozooids (not discussed). Reproductive zooids (specialized male and female zooids) can be distinguished by changes to their cystid and/or polypide (see Table 1). These zooids are typically non-feeding, using their tentacle crowns to facilitate reproduction (e.g. male zooids releasing sperm).⁵¹ Although tentacle number and length can be necessary to distinguish autozooids from reproductive morphs (as in *Odontoporella bishopi*)⁵², the primary concern of this paper is the classification of skeletal characteristics for use in neontology and paleontology.

Appendaged autozooids are capable of feeding and are distinguished from feeding autozooids only by the presence of non-zooidal adventitious modules. These modules are projections from the cystid that 1) are not separated from the zooidal coelom by a pore plate, 2) display reduced integration in form/function, and 3) exhibit variation separately from the parent zooid. A non-kenozooidal spine is an example of a non-zooidal adventitious module because it is a projection of the body wall that is clearly separable from the autozooid (via cuticular spine-bases) and can exhibit a variety of forms (cervicorn, antenniform, fused, etc.). Note that an appendaged autozooid may also host zooidal adventitious modules (heterozooids) in addition to its non-zooidal adventitious modules.

Unlike autozooidal polymorphs, heterozooids do not have a protrusible tentacle crown and are unable to feed.^{53,54} Heterozooids include avicularia, kenozooids, and cyclostome gonozooids (not discussed). In avicularia, which possess a highly modified cystid and musculature, the tentacle crown is reduced to a vestige. In kenozooids the polypide is completely absent.^{55,56}

Zooid Type	Zooid Subtype	Cystid Change	Polypide Change	NZAM	ZAM	Example
Ancestrula	kenozooidal	none	absent	no	no	some <i>Hippothoa</i>
	resembling daughter autozooids	none	var. in T#, feeding	yes/no	yes/no	most taxa
Autozooid (feeding, post ancestrular)	standard	no change	no change	yes/no	yes/no	most taxa
	B-zooid	various shape & size	var. in T#, feeding	yes/no	yes/no	many <i>Steginoporella</i>
Reproductive zooid	male	none	var. in T#, non-feeding	no	yes?	<i>Odontoporella bishopi</i>
		reduced	reduced, non-feeding	yes/no	no	many Hippothoidae
	female	none	var. in T#, feeding	yes/no	yes/no	many taxa
		enlarged zooid	var. in T#, non/feeding	yes/no	yes/no	many Adeonidae
		dwarf zooid	vestigial, non-feeding	no	yes	<i>Haplopoma sciaphilum</i>
Avicularium	vicarious	enlarged mandible, distal tapering, etc.	vestigial	yes/no	yes/no	many taxa
	interzooidal		vestigial	yes/no	no	most <i>Micropora</i>
	interzooidal vibracular		vestigial	yes/no	no?	<i>Setosella</i>
	adventitious		vestigial	no	no	<i>Chaperiopsis</i>
	adventitious vibracular		vestigial	yes/no	yes/no	many Candidae
Kenozooid	space filler - determinate	various	absent	yes/no	yes/no	<i>Spiralaria florea</i>
	space filler - indeterminate	stochastic changes	absent	yes/no	yes/no	many taxa
	spiniform	various	absent	yes/no	no	<i>Bellulopora</i>
	rhizoids	various	absent	yes/no	no	many taxa

Table 1. Variation in the cystid and polypide of cheilostome zooids. Astogenetic and ontogenetic variation in shape and size occur in all zooid types except for irregularly budded space-filling kenozooids, so this is not included in the table. **Cystid change** and **Polypide Change** refer to differences between the zooid and a feeding zooid in the zone of astogenetic repetition. Variation in tentacle number is shortened to “var in T#”, and zooids that can either be feeding or non-feeding are marked as “non/feeding”. **NZAM** shows whether a zooid is able to host non-zooidal adventitious modules (“appendages”), while **ZAM** shows whether a zooid can host zooidal adventitious modules (heterozooids); “yes/no” signifies that both states are manifested, depending on taxon.

1.3 Modularity and the cormidium

Modularity relies on the concept of the “primary module”, which is the basic repeated unit within a structure. In monomorphic bryozoans, which have colonies that consist only of autozooids, the primary module is simply an autozoid. In polymorphic taxa, the primary module is expanded to include the autozoid and its associated polymorphs, which repeat together within the colony. This unit of repeating autozooids and polymorphs is referred to as a cormidium (a “colony within a colony”) since it can carry out most functions vital for life.^{57,58} To reduce ambiguity we are introducing two new terms associated with the cormidium: submodule and paramodule. Submodules are the components of a cormidium and can be autozooids, autozooidal polymorphs, non-zooidal adventitious modules (e.g. non-kenozooidal spines on an autozoid), heterozooids, or multi-zooidal complexes (ovicells, see section 2.6 and Figure 2D, E for submodule examples). The fundamental basis of the cormidium is the autozoid (which provides the energy necessary to carry out other life functions), and therefore a cormidium cannot be composed entirely of non-feeding heterozooids in Bryozoa. Repeating heterozooids within colonies that are not associated with autozooids are referred to as paramodules, (e.g. kenozooids making up a kenozooidal stalk). Therefore a colony can consist of base autozooids, cormidia, and paramodules.

Polymorphic taxa are further complicated by the presence of different cormidia. An extreme version of this can be seen in *Corbulipora tubulifera* Hincks, 1881 which has three discrete cormidial phases.⁵⁹ The phases (1-3) are easily discernible because they occur in distinct bands, possess unique combinations of submodules, and even have different frontal shields.⁶⁰ However, clear cormidial differences do not always occur. Cormidial types may be interspersed with each other (or autozooids and paramodules) seemingly at random, and the changes in submodule composition may be minute. This raises the question of what minimum difference should be used to distinguish cormidia. Distinguishing traits should either change those vital functions that the cormidia can perform, or the magnitude of those functions. Submodule composition directly affects the vital functions of a cormidium, while the number of each submodule type should change the magnitude of those functions.⁶¹ Changes in submodule morphology between cormidia may influence both type and magnitude of vital functions. Here, the key word is “may” – to the best of our knowledge, there have been no investigations into how morphological changes (particularly of heterozooids) influence function. It is likely however, that changes in shape or discontinuous jumps in size would influence function. Therefore, all three distinguishing traits (submodule composition, number of each submodule, and submodule morphology) should be used to distinguish cormidial types.

An appendaged autozoid is a single polymorphic zooid that functions like a cormidium owing to the presence of its non-zooidal adventitious modules. Since the non-zooidal adventitious modules change either the number or magnitude of functions an autozoid can fulfil, the non-zooidal adventitious modules and the autozoid are considered as distinct submodules when describing cormidia. This puts appendaged autozooids in a

grey area between being a single zooid and a cormidium. However, a grey area here facilitates comparisons between autozooids with non-zooidal adventitious modules and autozooids with zooidal adventitious modules (e.g. non-kenozooidal vs kenozooidal spines), and should be maintained.

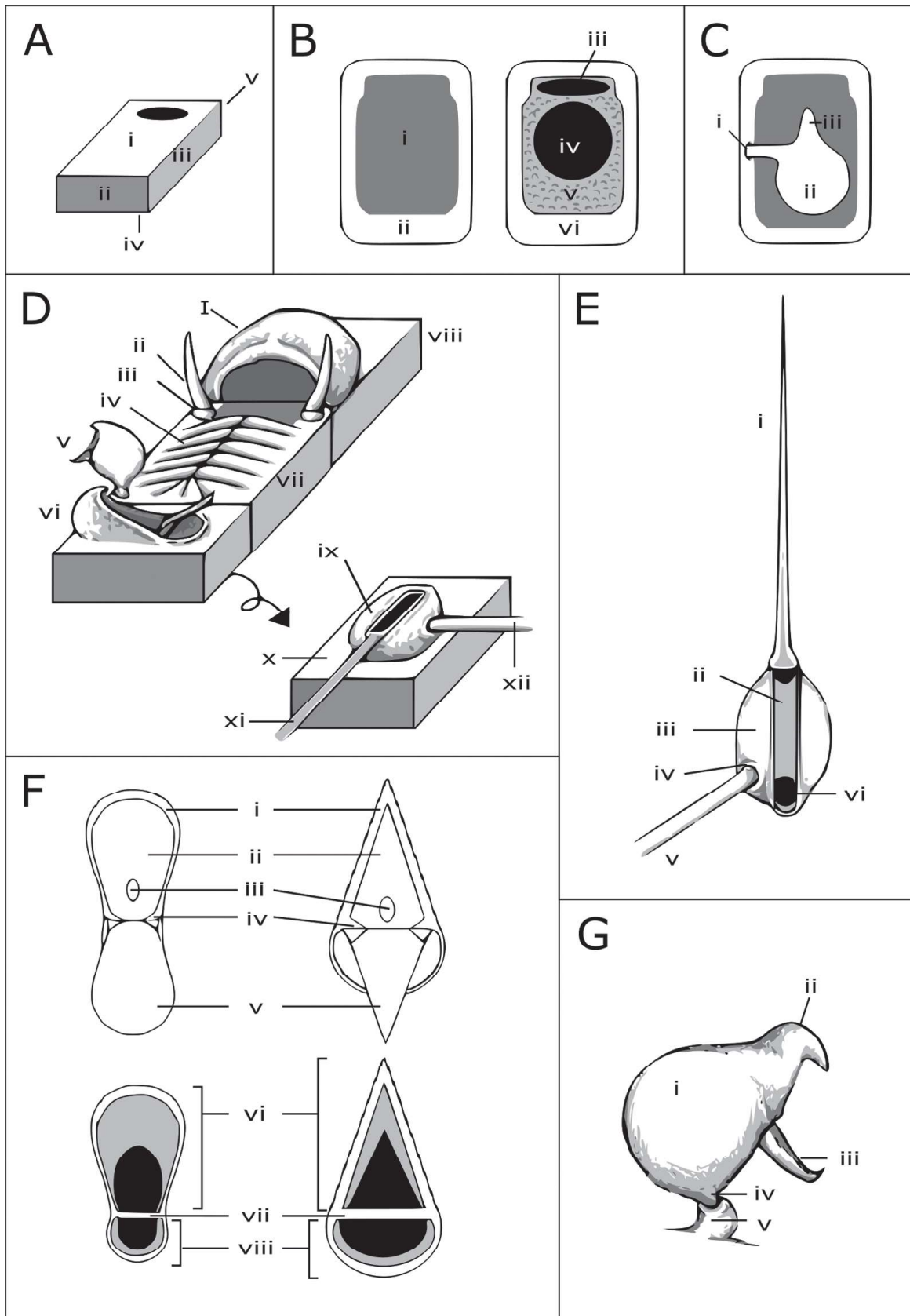
If colonies are monocormidial, then the arrangement of primary modules into higher-level structures is fairly simple (Figure 2D-F). If the colony is nonbranching then the secondary module is the entire colony, while in branching colonies, the secondary modules are branches that repeat within the tertiary structure of the colony.⁶² Colonies with multiple types of cormidia have more complicated secondary structures (Figure 2G-I). In *C. tubulifera*, each cormidial phase can be considered a secondary module that may (phases 2 and 3) or may not (phase 1) repeat within the tertiary structure.⁶³ Other polycormidial colonies have multiple cormidial types interspersed throughout the colony without any visible pattern (e.g. *Chaperiopsis rubida* (Hincks 1881), pers. obs.). The pattern may not be the order of budding, but the relative abundance of each cormidium present in the secondary structure, which may change between branches.

These explorations of definitions may seem overly rigorous, but understanding and defining the modular construction of bryozoans allows us to examine responses to selection pressures at different levels of organization. Selection pressures at the level of the primary module (zooid/cormidium) can be different from those at the level of secondary and tertiary modules (colony level).⁶⁴ A non-modular organism might be caught between two selective pressures, but the modular nature of bryozoans allows them to tailor their zooid-level and colony-level responses separately.⁶⁵ Changing the composition of submodules within cormidia does not restrict the form a colony can take, and vice-versa. This is particularly evident when examining predation. Attacks by zooid-level predators (which damage a single zooid), allow the colony to persist, while attacks by colony-level predators, which damage large parts of the colony or the entire colony, tend to result in colony death.⁶⁶ Although epibionts can be deterred by zooid-level defenses (such as avicularia^{67, 68}, spines⁶⁹ and ovicells⁷⁰) these are not effective against larger predators, which typically consume bryozoans as “bycatch” while pursuing epibionts or the bryozoan’s substratum. Instead, bryozoans avoid large predators through changes in their colony form.⁷¹

1.4 Usage

The classification is organized based on the types of polymorphs present in cheilostomes (avicularia, vibracula, spines, scuta, ooecia, and kenozooids including rhizoids). For accessibility, the traits (characters) are organized by the position, shape, size, etc. of those polymorphs. Trait levels (character states) are listed under each trait. If a trait is ordinal or binary then each trait level is listed with a number in parentheses, which is how they should be recorded for statistical analyses. When necessary, the polymorphs, traits and trait-levels are described and illustrated.

To use this classification to its full extent, specimens should be examined under an



SEM. Most characters within the system are minute and may be indistinguishable or unmeasurable by light microscopy (though one character, the internal channels of scuta, does require a light microscope to classify). The user must decide whether to bleach specimens: cuticularly joined structures (spines, mandibles, etc.) will fall off if bleached, but some structures do require bleaching to classify them (lumen pores in spines). Since the system is nonhierarchical, users can handpick those characteristics that are most pertinent to their research. As mentioned previously, this classification system has a wide range of applications: taxonomy; examining complexity and modularity; determining trait-environment relationships; building a database of cheilostome polymorphs; etc.

2. Classification of polymorphs

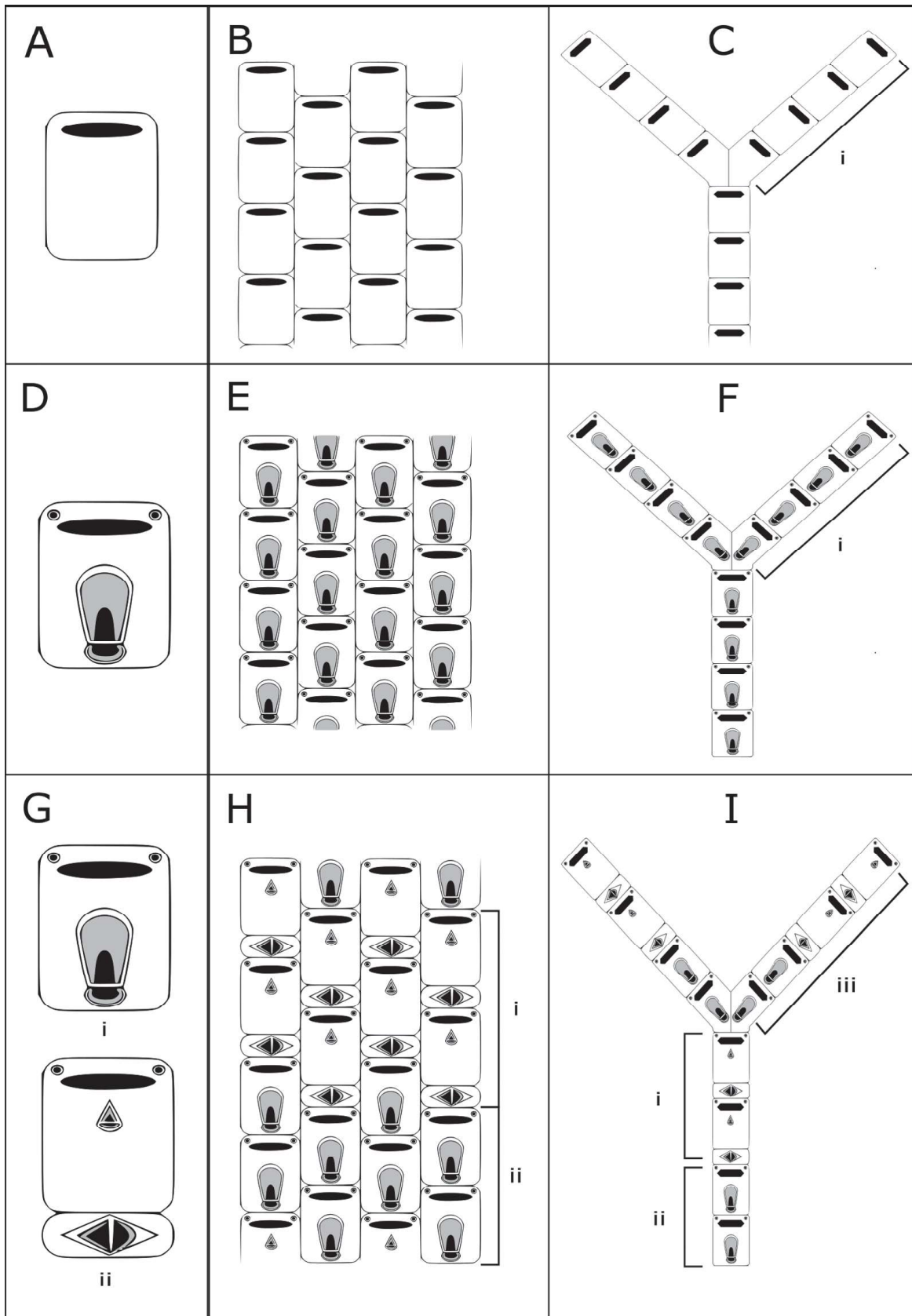
2.1 AVICULARIA

Avicularia are transformed zooids in which the operculum is modified into a mandible and the polypide is reduced to a vestige with a ciliary tuft.^{72,73,74} When the vestige is stimulated, either mechanically or chemically, the mandible is snapped shut against the palatal surface of the rostrum via adductor muscles (see Figure 1F-G for anatomical descriptions).^{75,76}

Type: A general classification of avicularia based on their size and budding pattern (Figure 3A).

- *Vicarious (0)*: Distally or distolaterally budded. Generally equal in size to autozooids and replacing them in the budding sequence. May retain functional polypide and gut. May retain functional polypide and gut (as in *Steginoporella*⁷⁷, *Crassimarginatella*⁷⁸, and *Wilbertopora*⁷⁹).
- *Interzooidal (1)*: Distally budded. Smaller than autozooids and occur in-between them (i.e., their basal walls touch the substratum in encrusting forms).
- *Adventitious (2)*: Budded on frontal, lateral and/or basal walls of autozooid. Smaller than autozooids and occurring upon them (i.e., their basal walls do not touch the substrate in encrusting forms).

Figure 1. Zooid and polymorph anatomy. **A. Zooid walls** i. frontal, ii. ventral, iii. lateral, iv. basal, v. dorsal; **B. Frontral surface** i. membranous frontal wall, ii. calcified gymnocyst, iii. orifice, iv. opesia, v. cryptocyst, vi. calcified gymnocyst; **C. Scutum anatomy** i. articulated base, ii. proximal lobe, iii. distal lobe; **D. Hypoetheical cormidium** i-vii have the frontal wall facing up, while ix-xii have the basal wall facing up i. ooecium of ovicell, ii. spine, iii. spine base, iv. costa of a cribrimorph shield, which lack spine bases, v. adventitious bird's head avicularium, vi. interzooidal sessile avicularium, vii. ovicelled maternal zooid, viii. distal daughter zooid, ix. vibraculum, x. basal wall, xii. vibraculum mandible, xii. rhizoid; **E. Vibracula anatomy** i. mandible, also referred to as a "seta", ii. palatal surface, iii. vibracular chamber, iv. rhizoid pore, v. rhizoid, vi. foramen in palatal surface; **F. Avicularia anatomy** i-v show a relaxed avicularia while vi-viii show skeleterized avicularia, i.rostrum, ii. palatal surface, iii. orifice, where the reduced polypide/ciliary tuft protrudes from, iv. hinge, v. mandible, vi. rostrum, vii. pivot bar, viii. opesia; **G. Bird's head avicularium** i. cystid, ii. rostrum, iii. mandible, iv. peduncle, v. peduncle cushion, which is formed by the autozooid.



Position: The location of the avicularium on its zooid, which is most pertinent for adventitious forms.

- **Zooid Wall:** The budding site of the avicularium, based on the orientation in Mukai *et al.* (1997).⁸⁰ Interzooidal and vicarious avicularia are generally considered dorsally budded.

- *Frontal:* Zooid wall containing orifice (ventral wall)
- *Lateral:* Transverse wall perpendicular to the direction of growth and the frontal wall
- *Distal:* Transverse wall facing the direction of growth
- *Proximal:* Transverse wall facing away from the direction of growth
- *Basal:* Zooid wall opposite frontal wall (dorsal wall)

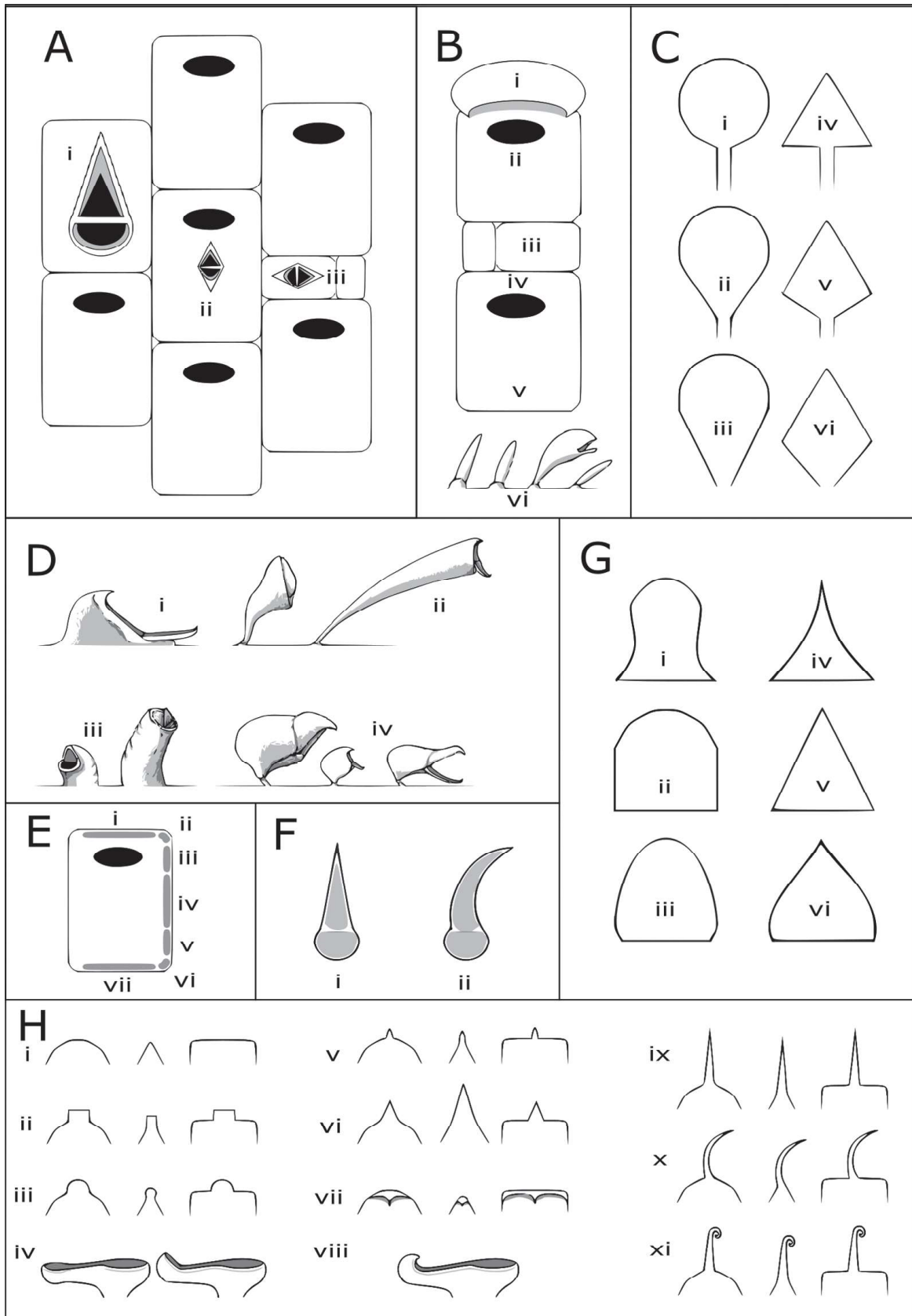
- **Relative Location:** Location relative to other morphological features of the parent autozooid (Figure 3B).

- *Hyper-oral:* Located next to orifice, distally
- *Sub-oral:* Located next to orifice, proximally
- *Spine substitute:* Replaces spine in series
- *Ovicell:* Associated with the ooecium of the ovicell
- *Gymnocyst:* Located on gymnocyst, but not near any distinctive morphological features
- *Between zooids:* For vicarious and interzooidal avicularia.

- **Distal-proximal location:** Location on a modified distal-proximal axis (Figure 3E).

- *Mid-distal (0):* Located at distal end of zooid, on or close to its vertical midline (hyper-oral)
- *Distal Corner (1):* Located on one distal corner of zooid (typically hyper-oral)
- *Distolateral (2):* Located below (proximal to) distal corners of zooid (typically in-line with or below orifice)

Figure 2. **Modularity and cormidial examples.** A-C are monomorphic bryozoans, D-F show polymorphic, monocormidial bryozoans, and H-G are polymorphic, polycormidial bryozoans. **A.** The primary module of a monomorphic bryozoan that consists of a single autozooid. **B.** A nonbranching monomorphic colony: here the entire colony is a secondary module. **C.** A branching monomorphic colony: here each branch is a secondary module (i), and the entire colony is a tertiary module. **D.** The primary module of a monocormidial bryozoan: a cormidium. The cormidium in this case possesses four submodules; an autozooid, two spines, and an adventitious avicularium. **E.** A nonbranching monocormidial colony: here the entire colony is a secondary module. **F.** A branching monocormidial colony: here a branch is a secondary module (i) and the entire colony is a tertiary module. **G.** The primary modules of a polycormidial colony: two cormidia (i and ii). Cormidium i. is the same as in D above and cormidium ii. has five submodules; an autozooid, two spines, an adventitious avicularium, and an interzooidal avicularium. **H.** A nonbranching polycormidial colony: here each cormidial band (i and ii) is a separate secondary module, while the entire colony is a tertiary module. **I.** A branching polycormidial colony: each cormidial band (i and ii) is a secondary module, each branch (iii) is a tertiary module, and the whole colony is a fourth-level module.



- *Mid-Lateral (3)*: Located near horizontal midline of zooid. Also used for vicarious and interzooidal avicularia which are located in the center of their own zooids.
- *Proximolateral (4)*: Located between horizontal midline and proximal end of zooid.
- *Proximal Corner (5)*: Located on one proximal corner of zooid
- *Mid-Proximal (6)*: Located at proximal end of zooid, on or close to its vertical midline

Rostral Direction: The direction the tip of the rostrum is pointing along the parent zooid's distal-proximal axis. This is the direction in which the mandible closes. If the direction is variable, then select the direction most rostra are facing in respect to their own zooids.

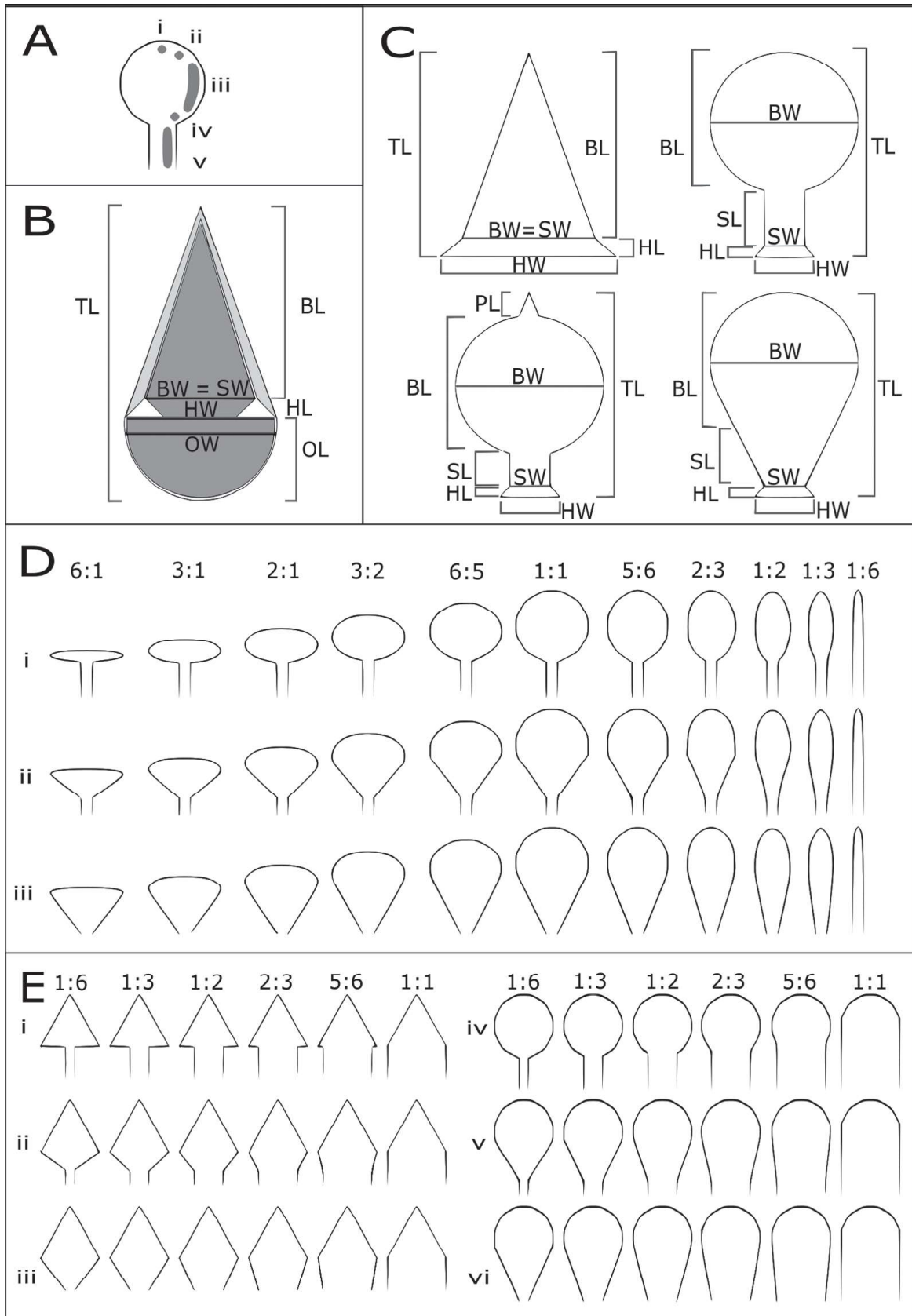
- Distal (0)
- Distolateral (1)
- Lateral (2)
- Proximolateral (3)
- Proximal (4)

Variable Direction: The variability of direction of the rostrum in relation to the zooid's distal-proximal axis.

- *Non-variable (0)*: Direction of the rostrum is constant in relation to the zooid's distal-proximal axis for all aviculiferous zooids within the colony.
- *Variable (1)*: Direction of the rostrum is unconstrained and can face in a variety of directions within the colony.

Attachment: The form of the cystid and manner of attachment of the adventitious avicularium to its parent zooid (Figure 3D). Vicarious and interzooidal avicularia are sessile, but adventitious avicularia have a greater diversity of attachment types. The peduncular cushion, which is formed by the parent zooid,⁸¹ is an important distinguishing

Figure 3. Avicularium classification. Where appropriate, historical morphological terms are given in brackets and quotes alongside those present in this classification system. A. Avicularia types i. vicarious, ii. adventitious, iii. interzooidal; B. Relative location i. ovicell, ii. sub-oral, iii. between zooids, iv. hyper-oral, v. gymnocyst; C. Tapering i. rounded, abrupt tapering ("spathulate"), ii. rounded, partial tapering ("spathulate"), iii. rounded, full tapering ("spathulate"), iv. pointed, abrupt tapering ("deltoid" or "triangular"), v. pointed, partial tapering ("trullate"), vi. pointed, full tapering ("rhomboid"); D. Attachments i. sessile, ii. tubular, iii. columnar, iv. bird's-head; E. Location i. mid-distal, ii. distal corner, iii. distolateral, iv. lateral, v. proximolateral, vi. proximal corner, vii. mid-proximal; F. Curvature i. straight, ii. curved; G. Concavity i. rounded, concave ("spathulate"), ii. rounded, straight ("linguiform"), iii. rounded, convex ("ovate", iv. pointed, concave, v. pointed, straight ("deltoid" or "triangular"), vi. pointed, convex; H. Projections i. none, ii. oblong, iii. auriculate, iv. auriculate rostrum side view, v. mucronate, vi. cuspidate, vii. hooked, viii. hooked rostrum side view, ix. aristate, x. falcate, xi. cirrhose.



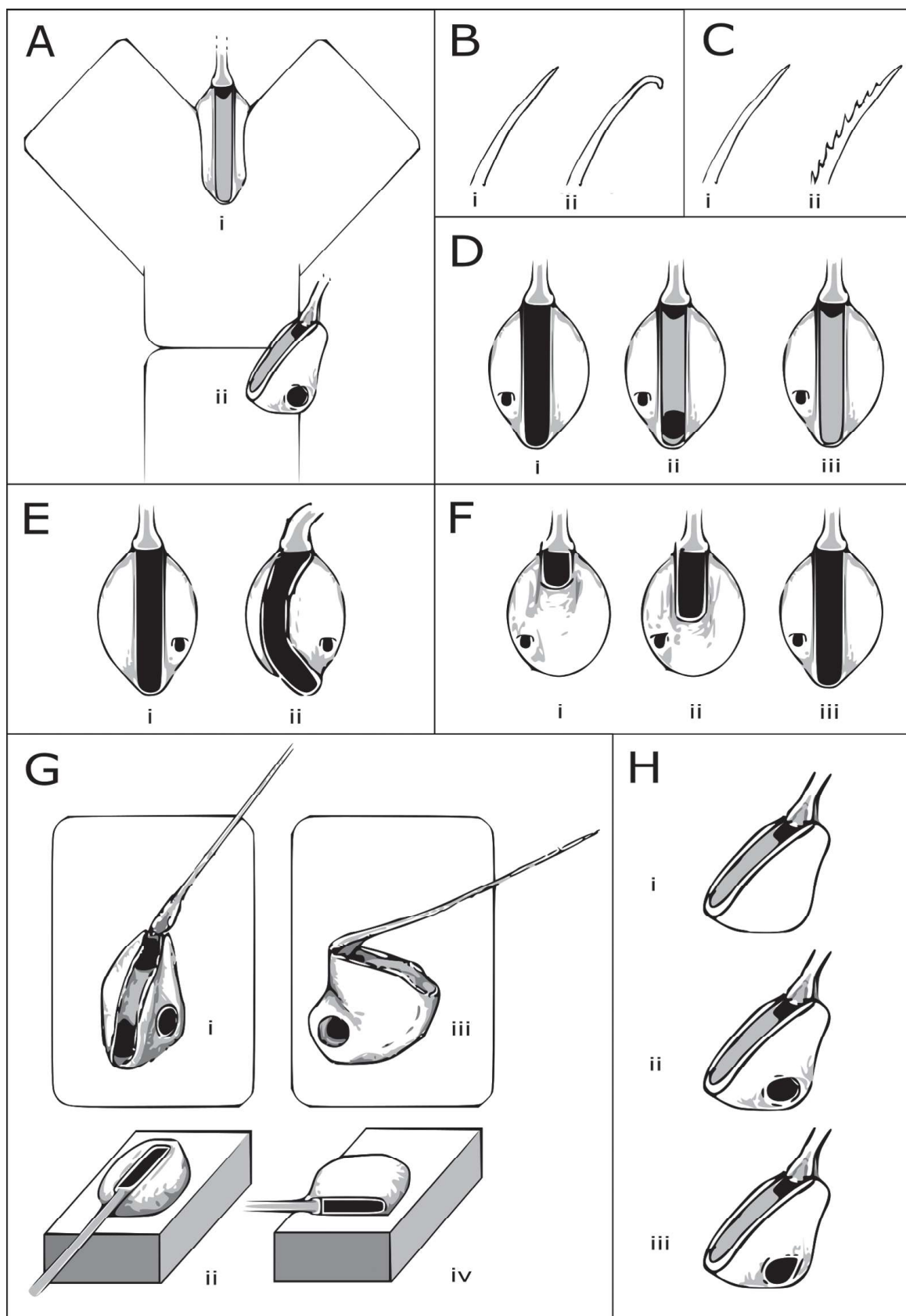
characteristic.

- *Sessile*: Avicularium is broad-based on parental zooid, and secondary calcification may result in an opesia flush with the frontal wall. Rostrum may be flush with the frontal wall or tilted upwards.
- *Columnar*: Opesia and rostrum are elevated from the frontal wall of its zooid. Avicularium is supported on a thick stalk that does not have a peduncular pad/cushion. The stalk may be tapered toward the point of attachment.
- *Tubular*: Opesia and rostrum are elevated from the frontal or frontolateral wall of the zooid. The avicularium is supported on a stalk that tapers from rostrum and opesia toward the peduncular pad/cushion.
- *Bird's head*: Opesia and rostrum are elevated from the frontal wall of its zooid. The bulbous body of the avicularium is supported on a thin stalk with a peduncular pad/cushion. This form allows the avicularium to “nod”.

Additional information on the avicularian cystid is given with three descriptors:

- **Spikes**: Spine-like protrusions (spikes) on the avicularian cystid. This typically occurs on columnar avicularia.
 - *None*: No spikes are present on cystid of the avicularium
 - *Simple*: Spikes are unbranched
 - *Branching*: Spikes are branched
 - *Mixed*: Unbranched and branching spikes are present on cystid of the avicularium
- **Spike Abundance**: The number of spikes on the cystid of the avicularium.
- **Elevation**: The distance between surface of the opesia and the frontal surface of the zooid. If the avicularium is crowning an ovicell, then the elevation is measured from the surface of the ovicell to the end of the avicularium.
 - *None (0)*: Face of the avicularium is flush with the zooid's frontal surface (embedded by secondary calcification).
 - *Short (1)*: Elevation is less than or equal to $\frac{1}{4}$ the length of the gymnocyst, but not flush with the frontal surface ($0 < x \leq \frac{1}{4}$)
 - *Intermediate (2)*: Elevation is between $\frac{1}{4}$ and $\frac{1}{2}$ the length of the gymnocyst ($\frac{1}{4} < x \leq \frac{1}{2}$)

Figure 4. Avicularium classification continued. A. Projection locations i. mid-disal, ii. distal corner, iii. lateral, iv. bulb-stem, v. stem; B. Rostrum measurements TL. total length, HL. hinge length, HW. hinge width, SL. stem length, SW. stem width, BL. bulb length, BW. bulb width, PL. projection length, OL. opesia length, OW. opesia width; C. Mandible measurements; D. Bulb width: bulb height i. abrupt tapering, ii. partial tapering, iii. full tapering. E. Stem width: bulb width i. pointed, abrupt tapering, ii. pointed, partial tapering, iii. pointed, full tapering, iv. rounded, abrupt tapering, v. rounded, partial tapering, vi. rounded, full tapering. D and E both show a range of potential mandible and rostral morphologies, which may not all be realized in nature.



- *Long (3)*: Elevation is between $\frac{1}{2}$ and 1x the length of the gymnocyst. ($\frac{1}{2} < x \leq 1$)
- *Very Long (4)*: Elevation is greater than 1x the length of the gymnocyst. ($1 < x$)

Mandible and Rostral Shape: The shape of the rostrum and the mandible. These should be described separately since the rostrum shape does not always match the shape of the mandible.

- **Overall Shape:** The general shape of the mandible/rostrum including the stem, bulb, and all projections.

- *Pointed (0)*: Mandible /rostrum narrows to a point or multiple points
- *Rounded (1)*: Mandible /rostrum widens and is blunt

- **Curvature:** Bending of the overall shape of the mandible/rostrum in the horizontal plane (x-y axis) (Figure 1F).

- *Straight (0)*: Mandible/rostrum is not bent
- *Curved (1)*: Mandible/rostrum is bent laterally

- **Proximal Tapering:** The transition between the stem and the bulb (Figure 3C)

- *No stem (0)*
- *Abrupt (1)*: There is a sharp change from the stem to the bulb
- *Partial (2)*: The transition from the bulb to the stem is smoother, but the lower part of the stem is still straight and distinct (parallel edges)
- *Full (3)*: The stem widens at the base and is difficult to distinguish from the base.

- **Bulb Outline:** The roundness of the mandible/rostrum edges relative to each other in the horizontal (x-y) plane, not including the hinge (Figure 3G).

- *Concave (0)*: The edges curve inwards
- *Straight (1)*: The edges are straight.
- *Convex (2)*: The edges curve outwards

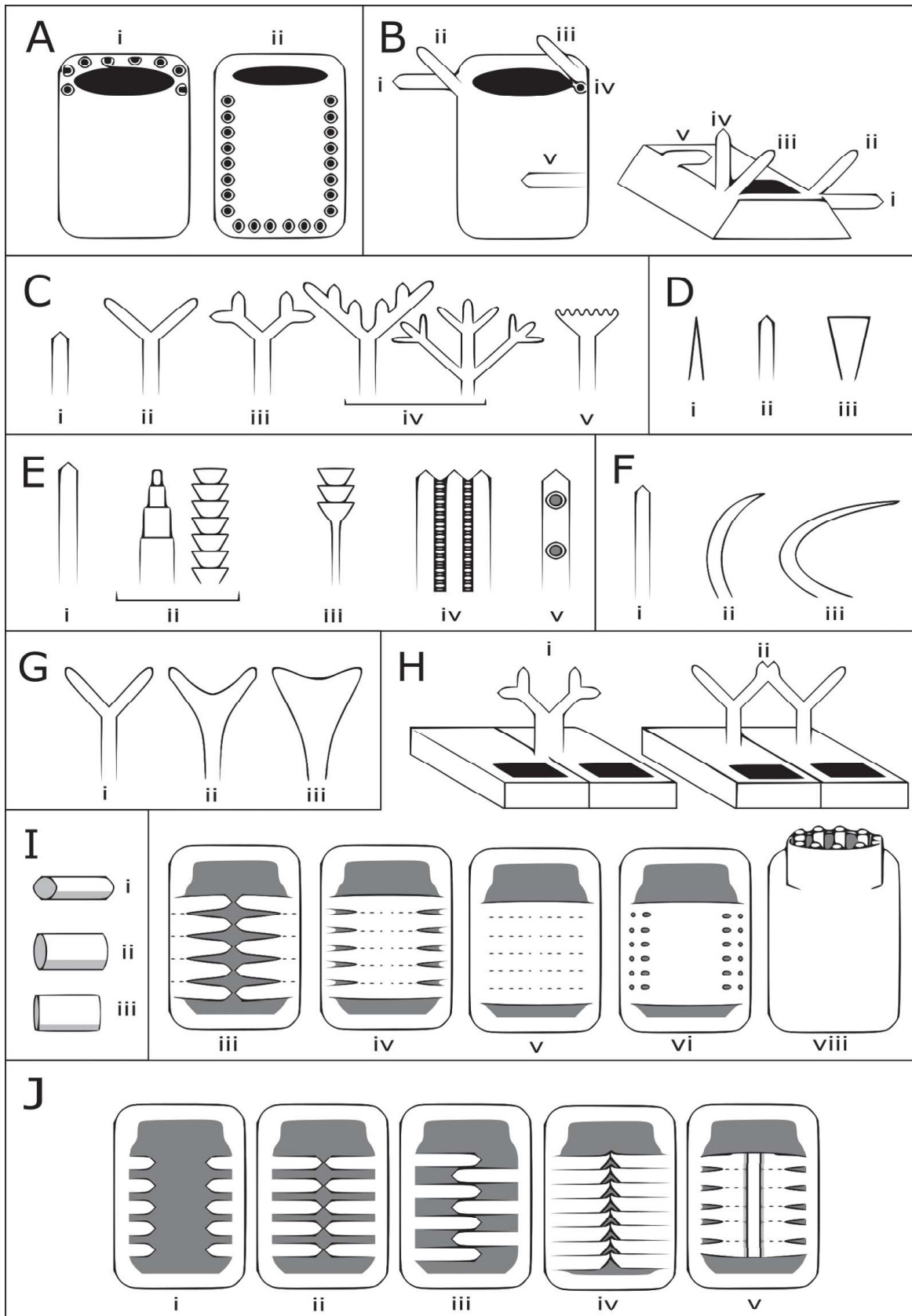
- **Bulb Shape:** The shape of the bulb (the main part of the mandible/rostrum, sometimes separated from the hinge by a stem), ignoring any projections.

- *Pointed*: Bulb is widest at base and narrows to a point
- *Rounded*: Bulb is widest at midpoint and rounds towards tip

- **Bulb Tip:** The shape of the tip of the bulb (not including projections). This usually matches the bulb shape, but cases occur where the tip is different from the overall bulb shape.

- *Pointed*: Tip narrows to a sharp point
- *Rounded*: Tip is smooth and rounded off
- *Truncated*: Tip is flat

Figure 5. Vibraculum classification. A. Vibraculum position i. axial, ii. non-axial; B. Vibraculum mandible tip i. straight, ii. hooked; C. Vibraculum mandible surface i. smooth, ii. barbate; D. Palatal surface i. absent, ii. present, with foramen, iii. present, no foramen; E. Setal groove curvature i. straight, ii. curved; F. Setal groove length i. short, ii. intermediate, iii. long; G. Setal groove location i. oblique, basal view of zooid, ii. oblique, isometric view of zooid, iii. transverse, basal view of zooid, iv. transverse, isometric view of zooid; H. Rhizoid pore i. absent, ii. circular, iii. ovate.



- **Projection Shapes:** Irregularities or projections on the mandible/rostrum that complicate its shape (Figure 3H).

- *None:* No projections present
- *Oblong:* Small rectangular projection
- *Auriculate:* Small rounded projection. If a mandible is hooked, the rostrum may have an auriculate projection to sheath the hooked portion of the mandible
- *Mucronate:* Abrupt, short point
- *Cuspidate:* Slightly tapered, short point
- *Hooked:* Pointed projection curving inwards. If a mandible is hooked, the rostrum may have a larger, hollow, hooked projection to hold the hook of the mandible.
- *Aristate:* Long, thin, and pointed projection
- *Falcate:* Long, thin, and curved projection ending in a point
- *Cirrrose:* Long, thin projection that ends in spiral

- **Projection Location(s):** The location of the projection on the mandible/rostrum (Figure 4A). This should influence function – a hook at the tip of a mandible/rostrum should be more effective than a lateral one.

- *None:* Mandible/rostrum does not possess projections
- *Bulb (Mid-distal):* Located on tip of bulb
- *Bulb (Distal corner):* Located on distal corner of bulb
- *Bulb (Lateral):* Located on the side of bulb
- *Bulb-Stem:* Located at the transition between bulb and stem
- *Stem:* The projection is located on the stem

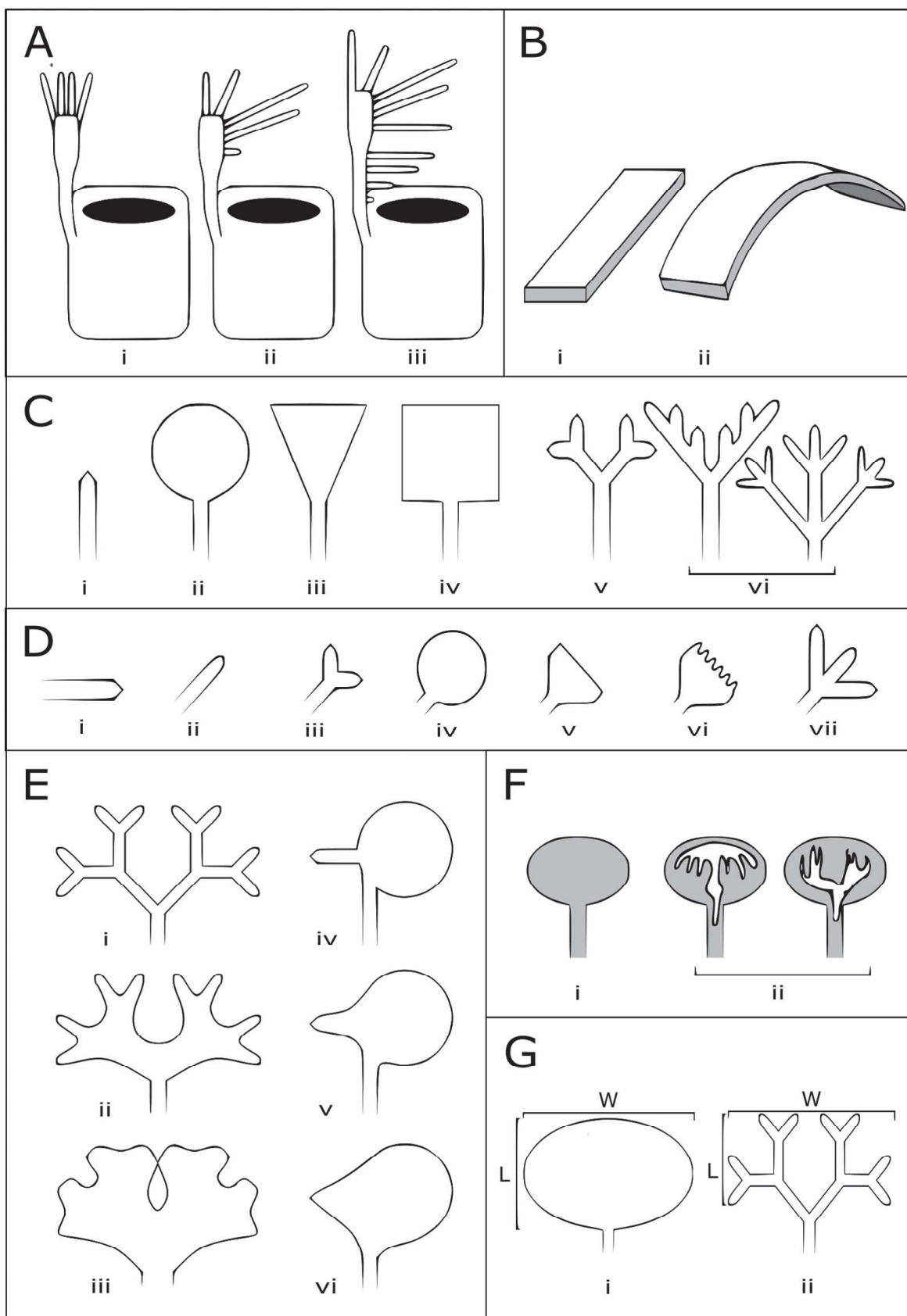
- **Teeth:** Very small, short, spine-like projections that are repeated along the edges of the mandible/rostrum.

- *None:* No teeth present
- *Sharp:* Teeth strongly pointed
- *Blunt:* Teeth resemble scalloped edges and can be round or slightly pointed.

- **Teeth Location:** The location of teeth on the mandible/rostrum

- *None:* The mandible/rostrum does not possess teeth
- Teeth are present on the
 - *Bulb*
 - *Bulb-stem:* Bulb-stem transition

Figure 6. **Spine classification** **A. Spine locations** i. oral, ii. opesial, see Figure 1e for other locations: circles represent the location of spine bases; **B. Angle** i. flat (outer), ii. obtuse, iii. acute, iv. erect, v. flat (inner); **C. Branching** i. unbranched, ii. forked, iii. bifid, iv. cervicorn, v. tines; **D. Spine thickness** i. tapering, ii. consistent, iii. flaring; **E. Spine construction** i. simple, ii. antenniform, iii. stalked antenniform, iv. pinnate, v. lumen pores; **F. Spine curvature** i. none, ii. slight, iii. strong; **G. Spine distinctness** i. distinct, ii. intermediate, iii. indistinct; **H. Spine fusion** i. lateral neighbors, ii. medial neighbors, iii. lateral, iv. medial, v. complete, vi. lacunae, vii. peristome; **I. Spine compression** i. cylindrical, ii. somewhat flattened, iii. flattened; **J. Midline interaction** i. separate, ii. meet, iii. interdigitate, iv. push, v. carina



- *Stem*
- *Rachis*: Central ridge of avicularium
- *Projection*
- *Entire*: Whole perimeter of the mandible/rostrum has teeth

Shape Measurements: Much of the variation in shape can be explained with simple ratios, particularly bulb width: bulb length, and stem width: bulb width (Figure 4B-E).

- **Total Length:** From the base of the mandible/rostrum to the tip, including any projections
- **Hinge Length:** Distance from the mandible/rostrum's base until the slope changes
- **Hinge Width:** The widest part of the mandible/rostrum's base
- **Stem Length:** Distance from the change in slope to the bottom of the bulb
- **Stem Width:** width after a change in slope of the mandible/rostrum
- **Bulb Length:** The length of the bulb, not including projections. If the mandible/rostrum is rounded and possesses a strongly tapered stem, measure from the midpoint of the bulb to its tip and multiply this value by 2 to get the total length.
- **Bulb Width:** The maximum width of the bulb
- **Projection Length:** Only measured for projections at the tip of the bulb
- **Opesia Length:** The maximum length of the opesia
- **Opesia Width:** The maximum width of the opesia

2.2 VIBRACULA

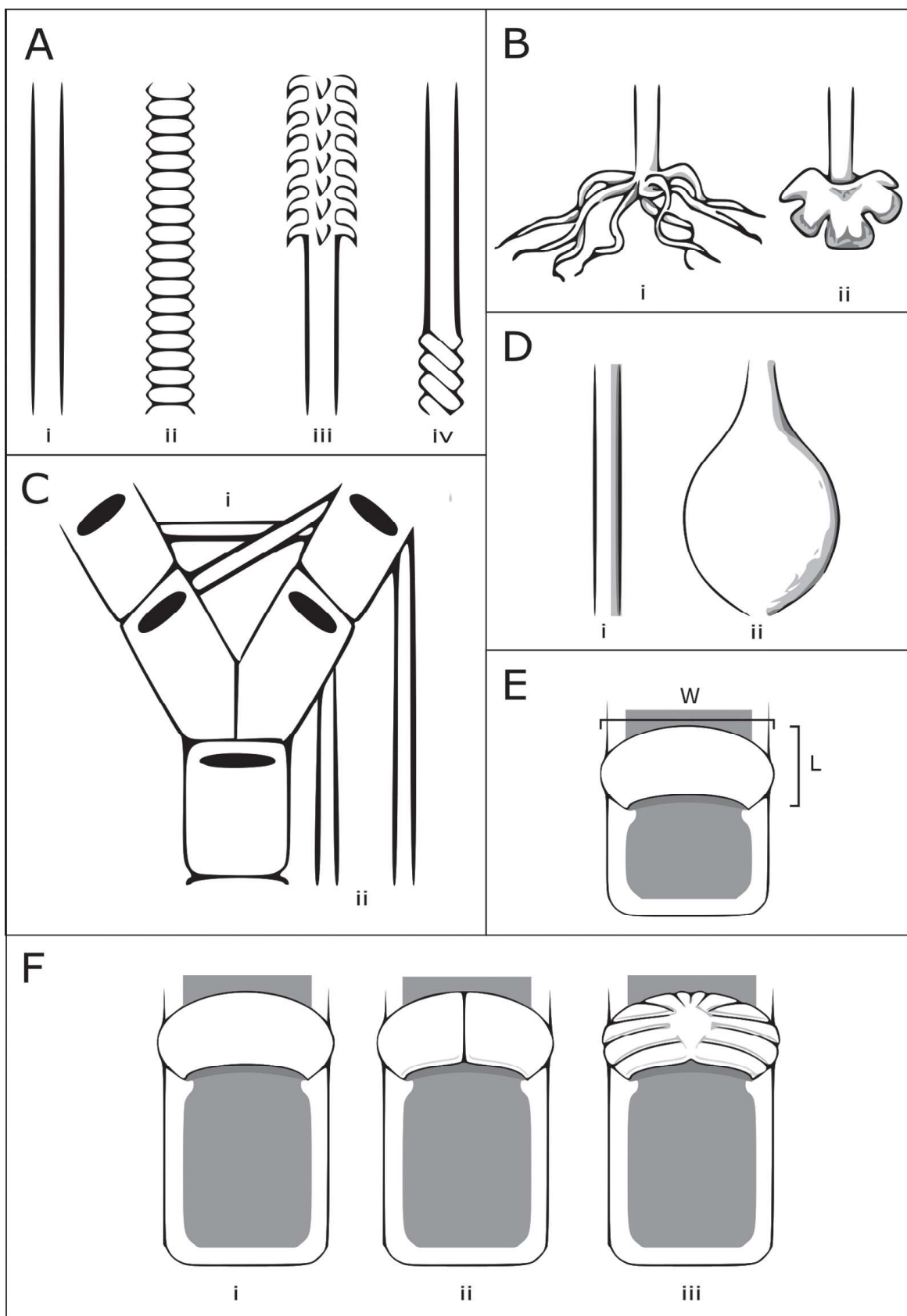
Vibracula are adventitious avicularia with extreme morphology. The mandible/rostrum is very long, and their hinge structure allows the mandible/rostrum to rotate over the surface of the colony (unlike avicularia which can only swing in one plane^{82,83}). (Figure 1E for anatomical descriptions.)

Location:

- *Zooid Wall*:

- Frontal
- Lateral
- Distal

Figure 7. Spine and scutum classification **A. Spiniferous process** *i. bulb tip, ii. bulb only, iii. entire*; **B. Scutum cross-section** *i. flat, ii. curved*; **C. Scutum overall shape** *i. spike, ii. round, iii. fan, iv. oblong, v. bifid, vi. cervicorn*; **D. Scutum lobe types** *i. spike (h), ii. spike (a), iii. forked, iv. round, v. truncated, vi. tines, vii. trifid*; **E. Scutum distinctness** *i. branching, distinct, ii. branching, intermediate, iii. branching, indistinct, iv. mixed lobes, distinct, v. mixed lobes, intermediate, vi. mixed lobes, indistinct*; **F. Scutum internal channels** *i. absent, ii. present*; **G) Scutum measurements** *i. length (L). and width (W). of an unbranching scutum, ii. length and width of a branching scutum.*



- Proximal
- Basal
- **Direction:** The direction the vibraculum faces in relation to the distal-proximal axis of the zooid
 - Distal (0)
 - Distolateral (1)
 - Lateral (2)
 - Proximolateral (3)
 - Proximal (4)
- **Position:** (Figure 5A)
 - *Non-axial (0)*: Vibraculum does not occur at a branch node
 - *Axial (1)*: Vibraculum occurs at a branch node

Mandible structure: Unlike avicularia, vibracula have much less variation in their mandible structure (also referred to as setae).

- **Mandible Length:**
 - *Short (0)*: Mandible shorter than the gymnocyst length of an autozooid
 - *Intermediate (1)*: Mandible between 1 and 2 zooids long
 - *Long (2)*: Mandible longer than 2 zooids
- **Mandible Tip:** (Figure 5B).
 - *Straight (0)*: Mandible tip is uncurved
 - *Hooked (1)*: Mandible tip curls into a hook
- **Mandible Surface:** (Figure 5C).
 - *Smooth (0)*: Mandible surface entire
 - *Barbate (1)*: Mandible surface covered in small, pointed protrusions

General Structure:

- **Palatal Surface:** The palate is the surface where the mandible rests in the rostrum and is contained in the setal groove (Figure 5D).
 - *Absent (0)*: No palatal surface present
 - *Present, with foramen (1)*: Palatal surface present with a small hole (foramen) near the distal end
 - *Present, no foramen (2)*: Palatal surface present and lacks a hole at the distal end
- **Setal Groove Curvature:** (Figure 5E).
 - *Straight (0)*
 - *Curved (1)*
- **Setal Groove Length:** Length of setal groove relative to body of vibraculum (Figure 3F). Regardless of setal groove length, the mandible will always be longer.

Figure 8. Rhizoid and ovicell classification. A. Rhizoid surface i. smooth, ii. ringed, iii. hooked, iv. coiled at origin; B. Rhizoid tip i. fimbriate, ii. holdfast; C. Connections i. branches, ii. substratum; D. Rhizoid thickness i. consistent, ii. dilated; E. Ovicell measurements l. length, w. width; f. Ooecium structure i. entire, ii. bilobed, iii. multilobed.

- *Short (0)*: Groove length less than $\frac{1}{4}$ the length of vibraculum
- *Intermediate (1)*: Groove length between $\frac{1}{4}$ and $\frac{1}{2}$ the length of vibraculum
- *Long (2)*: Groove length is between $\frac{1}{2}$ and 1x the length of vibraculum
- **Setal Groove Location:** (Figure 5G).
 - *Transverse (0)*: Setal groove/palatal surface is perpendicular to the zooid wall
 - *Oblique (1)*: Setal groove/palatal surface is parallel to the zooid wall
- **Rhizoid Pore:** Vibracula often have pores that rhizoids bud from (Figure 5H).
 - *Absent (0)*: No rhizoid pore present
 - *Circular (1)*: Rhizoid pore circular in shape
 - *Ovate (2)*: Rhizoid pore egg-shaped

2.3 SPINES

“Spine” is a non-technical term that is used here to refer to both derived kenozooids (= “spinozooids” of Silén⁸⁴) and body-wall projections from the parent zooid (spiniiform non-zooidal adventitious modules).^{85,86} Both structures are typically hollow, have varying degrees of calcification, and possess an inner lining of epithelial cells.^{87,88,89} With one exception, (spinozooids in *Belluopora*) the lumen of both spine types is confluent with the zooidal coelom and lacks a pore-cell complex.^{90,91,92} It is important to note that not all spines may be homologous, and it may not be valid to compare spines in different locations (i.e. spines that lack “topographical correspondence” as described in Vieira *et al.* 2014).⁹³ (Figure 1E for anatomical descriptions).

Number of Spines: the level of spination a colony possesses

- **Total Number of spines:** The total number of spines on the zooid
- **Number of spines:** The number of spines of a specific type. This can be used to separate groups of spines with that exhibit different traits.
- **Paired:** Symmetry in spine number and position location across the midline of the bryozoan. Spines are typically paired, though irregularities can occur.
 - No (0)
 - Yes (1)
- **Location:** the general location of spines on the zooid’s distal-proximal axis (Figure 3E, and Figure 6A).
 - Mid-distal
 - Distal corner
 - Distolateral
 - Orificial: Spines surrounding the orifice (not including sub-oral spines)
 - Sub-oral
 - Lateral
 - Proximolateral
 - Proximal corners
 - Mid-proximal

- Opesia: spines surrounding the opesia (not including sub-oral spines)
- Distal Wall
- Outer distal corner: Edge between the dorsal and lateral wall
- Distal (Lateral wall): Distal end of a lateral wall
- Lateral (Lateral wall): Near middle of a lateral wall
- Proximal (Lateral wall): Proximal end of a lateral wall
- Proximal Wall
- **Direction:** The direction a spine is pointing along its zooid's distal-proximal axis.
 - *Abfrontal*: Spine perpendicular to the frontal surface, its tip facing directly upwards
 - Distal
 - Distolateral
 - Lateral
 - Proximolateral
 - Proximal
- **Angle:** The angle of the spine in relation to the zooid surface (Figure 6B).
 - *Flat (inner) (0)*: Spine parallel to frontal surface of the zooid, its tip facing midline
 - *Acute (1)*: Spine angled above frontal surface ($<90^\circ$), pointing towards midline
 - *Erect (2)*: Spine perpendicular to frontal surface of the zooid
 - *Obtuse (3)*: Spine angled above frontal surface of the zooid ($>90^\circ$), pointing away from midline
 - *Flat (outer) (4)*: Spine parallel to the frontal surface of the zooid, its tip facing away from midline

Structure: The shape, size, and growth patterns of spines.

- **Branching:** The growth/division pattern of spine tips (Figure 6C).
 - *Unbranched*: Simple spine with single tip
 - *Forked*: Spine branches into two sections
 - *Bifid*: Each branch bifurcates into two sections that are similar in length, capable of further bifurcation
 - *Cervicorn*: Irregular branching, with branches either undergoing trifurcation or antler-like growth
 - *Tines*: Spine widens and possesses multiple small tips in a single row
- **Branch Distinctness:** The clarity of a spine's branching structure, or the sharpness of the division between spines (Figure 6G).
 - *Distinct (0)*: Each branch node clearly visible and terminal branch ends have similar width to earlier ones
 - *Intermediate (1)*: Branch tips clear, but branch thickness obscures earlier nodes
 - *Indistinct (2)*: Branching structure strongly obscured by branch thickness, branch tips hard to distinguish

- **Curvature:** The extent of curvature in a spine (Figure 6F).
 - *None (0):* Spines are completely straight
 - *Slight (1):* Spines are slightly curved
 - *Strong (2):* Spines are strongly curved
- **Compression:** The difference between the major and minor axes of the spine cross-section (Figure 6I).
 - *Cylindrical (0):* Spine cross-section is close to circular (major axis \approx minor axis)
 - *Somewhat flattened (1):* Spine cross-section is elliptical (major axis $>$ minor axis)
 - *Flattened (2):* Spine cross-section narrowly elliptical (major axis \gg minor axis)
- **Proximity:** The amount of space between neighboring spine bases.
 - *Sparse (0):* Spines spaced more than two spine bases apart
 - *Intermediate (1):* Spines spaced one spine base apart
 - *Appressed (2):* Spine bases touch their neighbors
- **Construction:** The growth structure of the spine (Figure 6E).
 - *Simple:* Spines relatively smooth with continuous structure
 - *Antenniform:* Spine made of regularly repeating segments, either of similar size or decreasing in size towards the tip. A simple spine can be mistaken for an antenniform one due to regeneration after breakage, which produces a similar looking structure. However, regenerated spines do not repeat regularly and the breaks between their segments typically appear ragged.
 - *Stalked Antenniform:* Antenniform structure on a thin, simple stalk
 - *Pinnate:* Spine connected to its neighbors via short, thin, lateral “struts”
 - *Lumen pores:* Holes in the spine calcification sealed by a cuticular plug, only seen after bleaching. The hole is in the spine itself, unlike lacunae, which are holes between spines. Also called pelmata or pelmatidia
- **Spine Tip:** The structure of the spine’s terminal end.
 - *Pointed:* Spine ends in a sharp point and is calcified
 - *Rounded:* Spine tip is rounded and is calcified
 - *Truncated:* Spine tip is flat and calcified
 - *Membrane:* Spine tip is flat, covered by a small membrane
- **Basal Joint:** The point of attachment between a spine and the zooidal surface.
 - *Absent (0):* Base unjointed or calcified
 - *Present (1):* Base has cuticular articulation
- **Thickness:**
 - *Tapering (0):* Spine tapers to a thinner width
 - *Consistent (1):* Spine width stays relatively constant along its length
 - *Flaring (2):* Spine increases in width along its length
- **Length:**
 - *Short (0):* Spine length less than $\frac{1}{4}$ the length of the gymnocyst
 - *Intermediate (1):* Spine length is between $\frac{1}{4}$ and $\frac{1}{2}$ the length of the gymnocyst
 - *Long (2):* Spine length is between $\frac{1}{2}$ and $1\times$ the length of the gymnocyst
 - *Very long (3):* Spine length greater than the length of the gymnocyst

- Base Width:

- *Thin (0)*: Base width is less than $\frac{1}{8}$ the width of the gymnocyst
- *Intermediate (1)*: Base width of the spine base is about $\frac{1}{8}$ the width of the gymnocyst
- *Thick (2)*: Base width is about $\frac{1}{5}$ the width
- *Very thick (3)*: Base width is greater than $\frac{1}{5}$ the width

Fusion: Spines often calcify together, either with spines on the same zooid or neighboring ones.

- Fusion: (Figure 6H)

- *None*: No fusion between spines
- *Lateral*: Spines fuse with their adjacent neighbors along their sides
- *Medial*: Spines fuse at their tips with the spines across from them
- *Lateral neighbors*: Spines of one zooid fuse laterally with those of a neighboring zooid
- *Medial neighbors*: Spines of one zooid fuse medially with those of a neighboring zooid
- *Complete*: Spines fuse with their adjacent neighbors and the spines across from them, forming a continuous surface
- *Lacunae*: Spines fuse to form a complete structure, except for small holes between spines
- *Peristome*: Spines laterally fuse with their neighbors around the orifice

- Midline Interaction: Spines situated opposite each other may interact at the midline of the zooid. This can also be applied to spines medially fused with their neighbors (Figure 6J).

- *Separate*: Opposite spines do not approach each other
- *Meet*: The tips of opposite spines touch each other on the midline
- *Interdigitate*: Opposite spines mesh with each other
- *Push*: The tips of opposite spines grow into each other, pushing their tips upward
- *Carina*: When spines are medially fused, additional raised calcification occurs over the spine tips

Spiniferous Process: Occasionally, spines are borne away from the zooid itself on a long extension, which can host multiple spines (Figure 7A).

- *None (0)*: No spiniferous process present
- *Bulb tip (1)*: Spines only present on the tip of spiniferous process
- *Bulb only (2)*: Spines only present on the bulbous end of process but not along the thinner extending “arm”
- *Entire process (3)*: Spines present along the length of process

Zooid: The number and type of spines can change depending on what kind of zooid they originate from, with the ancestrula typically more spinose and armored than later zooids.

- Autozooid
- Ancestrula
- Ovicelled zooid

2.4 SCUTA

Scuta are modified spines that are large, shield-like, and lie flat over the opesia. They are unpaired and do not exist within a spine-series. See Figure 1C for anatomical descriptions.

Scutum Shape:

- **Overall Shape:** The entire shape of the scutum, regardless of its individual lobe shapes (Figure 7C).

- *Spike:* Scutum thin and spine-like
- *Round:* Scutum terminates in a large rounded structure
- *Fan:* Scutum widest at the tip
- *Oblong:* Scutum terminates in a rectangular structure
- *Bifid:* Scutum branches, undergoing roughly equal bifurcation at each node
- *Cervicorn:* Scutum branches, undergoing trifurcation or antler-like growth

- **Lobe Types:** This trait is applied to the distal, central and proximal lobes of the scutum (Figure 7D). The default is two lobes (distal and proximal), though there may be cases where all 3 are present, or where only the central lobe is present. To prevent differences in classification, the simplest lobe type should be used. For example, instead of describing a scutum as having 1 central lobe that is trifid, the scutum should be described as having 3 lobes, each with a spike.

- *None:* No lobe present
- *Spike (H):* Horizontal (perpendicular to the stem of the scutum), thin, spine-like lobe
- *Spike (A):* Angled, thin, spine-like lobe
- *Forked:* Bifurcated spine-like lobe
- *Round:* Circular lobe
- *Truncated:* Fan-like lobe that terminates in a blunt edge
- *Tines:* Fan-like lobe that terminates in a row of small spikes
- *Trifid:* Trifurcated spine-like lobe

- **Relative Lobe Size:** A comparison of the relative sizes of the proximal and distal lobes.

- *Reduced (0):* Proximal lobe is smaller than distal lobe
- *Symmetrical (1):* Proximal and distal lobes are roughly equal in size, or both are absent
- *Enlarged (2):* Proximal lobe is larger than distal lobe

Structure:

- **Distinctness:** This provides a measure of the permeability of the scutum, and the degree of lobe fusion (Figure 7E).
 - *Distinct (0)*: In branching scuta each branch node is clearly visible and the terminal branch ends have a similar width to earlier ones. In non-branching scuta, lobes are clearly defined and the transition between them is abrupt.
 - *Intermediate (1)*: In branching scuta branch tips are clear, but branch thickness obscures earlier nodes. In non-branching scuta the transition between lobes is gentle, but lobes can be distinguished.
 - *Indistinct (2)*: In branching scuta branching structure is strongly obscured by branch thickness, branch tips are hard to distinguish. In non-branching scuta lobes are difficult to distinguish and nearly appear as a single shape.
- **Cross-section:** (Figure 7B).
 - *Flat (0)*: Scutum uncurved and may be parallel with the opesia.
 - *Curved (1)*: Scutum arched over the opesia.
- **Internal Channels:** Some scuta possess internal channels, which are only visible under a light microscope (Figure 7F).
 - Absent (0)
 - Present (1)

Base: The scutum is a modified articulated spine, and it possesses a spine base.

- **Base Location:**

- Distal (0)
- Distolateral (1)
- Lateral (2)
- Proximolateral (3)
- Proximal (4)

- **Base Width:**

- *Thin (0)*: Base width less than $\frac{1}{8}$ the width of the gymnocyst
- *Intermediate (1)*: Base width is about $\frac{1}{8}$ the width of the gymnocyst
- *Thick (2)*: Base width about $\frac{1}{5}$ the width of the gymnocyst
- *Very thick (3)*: Base width greater than $\frac{1}{5}$ the width of the gymnocyst

Measurement: The size of the scutum (Figure 7G).

- *Length*: Maximum length of the scutum, not counting the stem
- *Width*: maximum width of the scutum
- *Percent cover of the opesia*: This measurement takes into account the holes present in branching structures and irregular lobe shapes. The part of the stem that covers the opesia is included in the measurement.

2.5 RHIZOIDS

Rhizoids, also referred to as rhizozooids, rootlets, and radicle fibers, are elongated kenozooids that extend to the substratum or other areas of the colony. They provide attachment to the substrate and colony support, and are common in flexible erect colonies (Silén, 1977).

Form: Some structures are similar in appearance and function to rhizoids, and these are distinguished here.

Prop (0): Extensions of the basal calcified wall. Colony supported on the substrate by several short, cylindrical structures that lack holdfasts or fimbriated structures (see rhizoid tip).

- *Rhizoid (1):* Colony anchored to the substrate or other branches by long, hollow, cylindrical kenozooids that may terminate in a holdfast or a fimbriated structure. Variable calcification.

Origin: It is common for rhizoids to originate from the basal wall or a vibracular chamber. They rarely arise from a frontal wall.

- Lateral wall
- Basal wall
- Dorsal wall
- Ventral wall
- Frontal wall
- *Vibracular chamber:* The rhizoid emerges from a pore in a vibraculum

Rhizoid Surface: (Figure 8A).

- *Smooth:* Rhizoid surface is unblemished
- *Ringed:* Rhizoid resembles a string of close-set, short cylindrical beads of similar lengths.
- *Hooked:* Parts of rhizoid surface covered in backwards-facing hooks
- *Coiled (base):* Rhizoid is coiled at the origin

Rhizoid Tip: The terminal end of the rhizoid anchors the bryozoan, and different types may be found on different substrates (Figure 8B).

- *Single:* Rhizoid ends abruptly or tapers.
- *Fimbriate:* Rhizoid tip splits into many thin hair-like ends
- *Holdfast:* Rhizoid ends in a thicker, sometimes spikey structure

Connections: Rhizoids can either anchor the colony to the substrate or can connect branches to each other (Figure 8C).

- *Substrate (0)*

- Branches (1)

Thickness: Rhizoids are typically slender, but some can be swollen into a bulbous structure (Figure 8D).

- *Consistent (0)*: Rhizoid has a continuous thickness
- *Dilated (1)*: Rhizoid possesses are swollen sections

2.6 SKELETAL CHAMBERS FOR EMBRYONIC INCUBATION

This part of the classification system follows that of Ostrovsky *et al.* (2009)⁹⁴ and Ostrovsky (2013).⁹⁵ Although it includes all brood chambers present in cheilostomes, the focus here is on the skeletal ones. The commonest type are exterior ovicells that consist of a calcified double-walled ooecium enclosing a brooding cavity. The ooecium is a body-wall outgrowth formed from the distal daughter zooid in most cases.^{96,97} Spines provided by the distal daughter evolved into ooecia through fusion or reduction in the number of spines, their flattening, loss of basal articulation, relocation of spine bases, in addition to other trends.⁹⁸ Since the ooecium originated evolutionarily from spines, it could be considered kenozooidal in nature. The maternal (egg-producing) zooid transfers the egg to the ovicell and closes it with a body-wall plug (ooecial vesicle) or autozooidal operculum, or both.

In some cheilostomes (Tendridae, Scrupariidae, Thalamoporellidae, Alysidiidae, and Belluloporidae), the skeletal parts of independently evolved ooecia-like structures do not rely on a distal daughter. Instead, they form from plate-like kenozooids, kenozooidal spines, or outgrowths of the maternal body-wall.

The variable presence of an ooecium-contributing distal daughter makes ovicells difficult to describe in cormidial terms. This is further complicated by the ability of the distal daughter to act as a maternal zooid to *its own* distal daughter. To simplify, only the maternal zooid will be considered as possessing an ovicell. Although this is an artificial cut-off, it is necessary to prevent cormidial overlap/ the double-counting of submodules, which would present difficulties in statistical analysis. The distal daughter zooid should only be included in the cormidium if it is a kenozooid or avicularium. Thus, a maternal zooid would be in a cormidium that consists of two submodules (autozooid + ovicell), while the distal daughter would be considered as a standard autozooid unless it possessed other polymorphs. If the distal daughter is not an autozooid, the cormidium would consist of three submodules (autozooid + ovicell + kenozooid/avicularium).

Embryo Incubation Chamber:

- External membranous sac (0)
- *Skeletal chamber (1)*: Includes ovicells and brood chambers formed by spines. These can external or internal
- Non-calcified internal brood sac (2)

- *Intracoelomic incubation (3)*: Occurs in specialized female zooids

Skeletal Chamber:

- *None (0)*
- *Acanthostegous (1)*: Adjacent mural spines (spines near the edge of the frontal wall) overarch an incubational space above the frontal wall (including frontal membrane).
- *Ooecia (2)*: Consists of a calcified double wall (ooecium) enclosing a brooding cavity that is plugged by a non-calcified portion of the maternal zooid's distal wall. The ooecium is phylogenetically derived from one or more spines of the distal daughter zooid.

Ovicell Immersion: This examines how much the brood cavity is sunken below the surface of the zooid. There is a trend in Recent bryozoa towards the immersion of the brood cavity in the frontal surface.^{99,100} Note that this refers to the brood cavity and not the ooecium itself, which is always an external structure.

- *Immersed (0)*: Brood cavity entirely below the surface of the zooid
- *Sub-immersed (1)*: Less than half of the brood cavity above the surface of the zooid
- *Prominent (2)*: More than half of the brood cavity above the surface of the zooid.

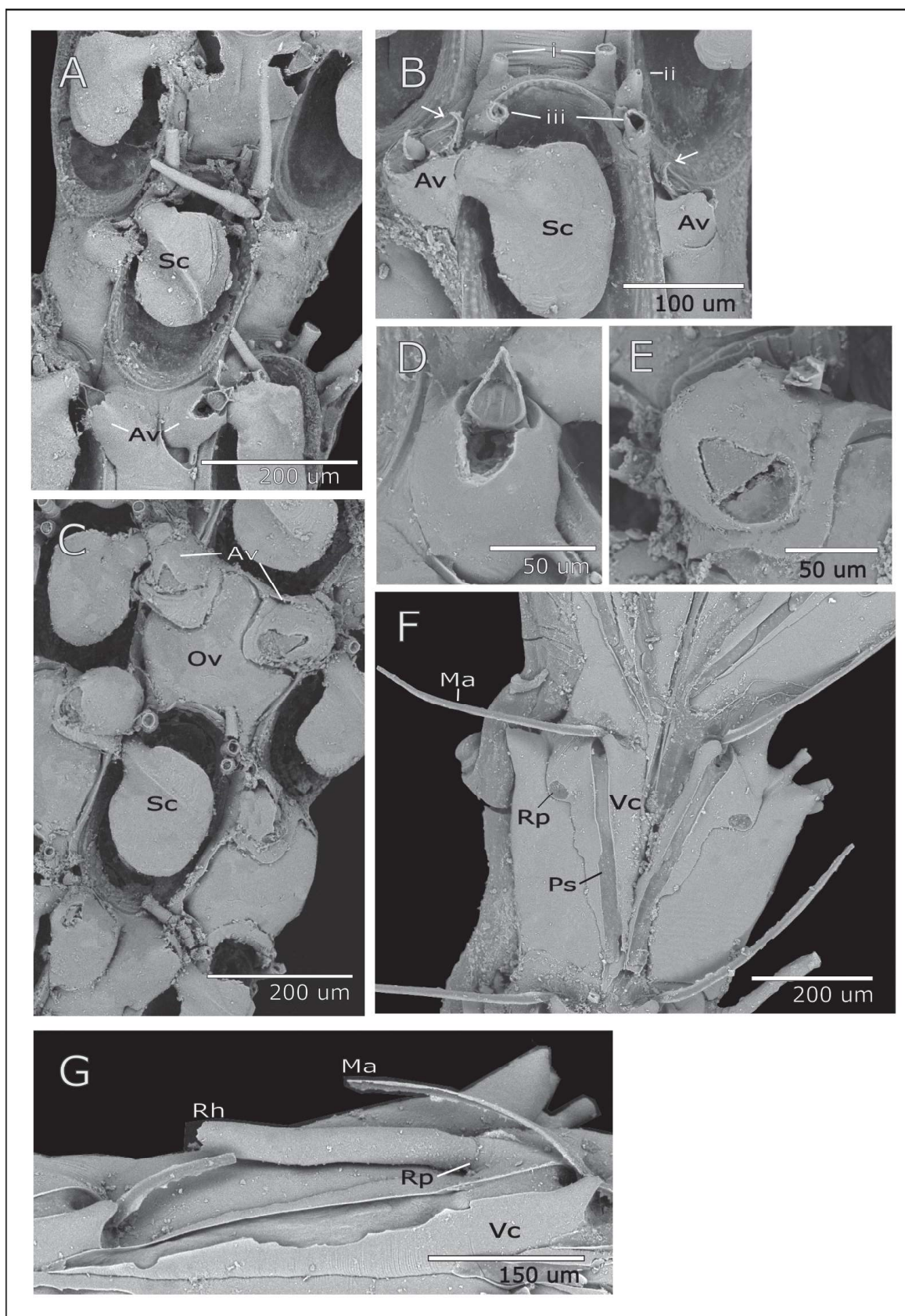
Ooecium Structure: This examines the number of parts that constitute the ooecium (Figure 8F). Note that this is not based on the number of parts that the ooecium was derived from evolutionarily, but the appearance of the derived structure.

- *Entire (0)*: Ooecium appears whole
- *Bilobed (1)*: Ooecium appears to be constructed from two parts/components, which are fused in *the middle*
- *Multilobed (2)*: Ooecium appears to be constructed from more than two parts/components

Daughter Zooid: The type of distal daughter zooid from which the ooecium originates.

- Autozooid (0)
- Avicularium (1)
- Kenozooid (2)

Figure 9. SEM images of Amastigia puysegurensis. These images have been digitally modified to remove fouling debris. "Sc" = Scutum, "Av" = Avicularium, "Ov" = Ovicell, "Ma" = mandible, "Ps" = Palatal surface / setal groove, "Vc" = Vibracular chamber, "Rp" = Rhizoid pore "Rh" = rhizoid **A.** Frontal view of non-ovicelled autozooids, showing some intact spines, scuta, and avicularia; **B.** Close-up of a non-ovicelled autozooid, showing three kinds of spines (i-iii, all broken off), a scutum, and two avicularia. The hooked projection on the avicularian mandibles can clearly be seen (arrows); **C.** Frontal view of ovicelled autozooids, showing scuta and avicularia; **D.** The gymnocystal avicularium of an autozooid; **E.** An avicularium crowning an ovicell; **F.** Basal side of a branch, showing paired vibracula. **G.** Vibraculum with a rhizoid emerging from the rhizoid pore.



Measurement: The shape of the ovicell is roughly described through length and width ratios (Figure 8E).

- *Length:* Maximum length of ovicell along distal-proximal axis
- *Width:* Maximum width of ovicell

3. Example Classification:

The scope of this classification may seem daunting to potential users, especially from a data organization viewpoint. To alleviate this, and to reduce any confusion about the use of the classification system itself, we have provided a full example. *Amastigia puysegurensis* Gordon, 1986 (Candidae) is a useful model species because it possesses each kind of polymorphism described in this classification. A specimen of *A. puysegurensis* from the NIWA Invertebrate Collection¹⁰¹ was imaged with a SEM (Figure 9).¹⁰² The classification is presented below (Table 2-7), with each trait in bold. In a normal data table each trait should be in a separate column, but the layout is condensed here to save space. Note that when multiple kinds of a certain polymorphism were present (e.g. avicularia, spines) each kind received its own classification.

Avicularium	Type	Zooid Wall	Relative Location	Location	Rostral Direction
<i>Avi</i>	2	Frontal	Gymnocyst	4	3
<i>Avi (ovi)</i>	2	Frontal	Ovicell	1	1
Avicularium	Variable Direction	Attachment	Spikes	Spike Abundance	Elevation
<i>Avi</i>	0	Columnar	None	0	1
<i>Avi (ovi)</i>	0	Columnar	None	0	1
Avicularium	Overall Shape (Rostrum)	Bulb Shape (Rostrum)	Curvature (Rostrum)	Tapering (Rostrum)	Bulb Outline (Rostrum)
<i>Avi</i>	0	0	0	0	1
<i>Avi (ovi)</i>	0	0	0	0	1
Avicularium	Bulb Tip (Rostrum)	Projection Shapes (Rostrum)	Projection Location(s) (Rostrum)	Teeth (Rostrum)	Teeth Location (Rostrum)
<i>Avi</i>	Pointed	None	None	None	None
<i>Avi (ovi)</i>	Rounded	None	None	None	None
Avicularium	Overall Shape (Mandible)	Bulb Shape (Mandible)	Curvature (Mandible)	Tapering (Mandible)	Bulb Outline (Mandible)
<i>Avi</i>	0	0	0	0	1
<i>Avi (ovi)</i>	0	0	0	0	1
Avicularium	Bulb Tip (Mandible)	Projection Shapes (Mandible)	Projection Location(s) (Mandible)	Teeth (Mandible)	Teeth Location (Mandible)
<i>Avi</i>	Pointed	Hooked	Bulb (mid-distal)	None	None
<i>Avi (ovi)</i>	Rounded	None	None	None	None

Table 2. Avicularium classification of *A. puysegurensis*, which has two kinds of avicularia. The first form (“*Avi*”, Figure 9A, B, and D) is present on the gymnocyst of autozooids and ovicelled zooids, either singly or as a pair. The second form (“*Avi (ovi)*”) crowns the ooecium and is present in pairs (Figure 9C and E).

Vibraculum	Zooid Wall	Direction	Position	Mandible Length	Mandible Tip	Mandible Surface
-	Basal	4	0	1	NA	0
Vibraculum	Palatal Surface	Setal Groove Curvature	Setal Groove Length	Setal Groove Location	Rhizoid Pore	
-	2	0	2	1	1	

Table 3. Vibraculum classification of *A. puysegurensis* (Figure 9F). Vibraculae on adjacent zooids are paired with each other.

Spines	Total Num.	Num. Spines	Paired	Location	Angle	Direction
Spine I	5	2	1	Distal corner	3	Distal
Spine II	5	1	0	Distolateral	3	Distal
Spine III	5	2	1	Distolateral	3	Distal
Spines	Branching	Branch distinctness	Curvature	Compression	Proximity	Construction
Spine I	unbranched	0	0	0	2	Simple
Spine II	unbranched	0	0	0	2	Simple
Spine III	unbranched	0	0	0	2	Simple
Spines	Spine Tip	Basal Joint	Thickness	Length	Base width	Fusion
Spine I	NA	1	0	NA	1	None
Spine II	NA	1	0	2	2	None
Spine III	NA	1	0	2	2	None
Spines	Midline Interaction	Spiniferous Process	Zooid			
Spine I	None	None	autozooid			
Spine II	None	None	autozooid			
Spine III	None	None	autozooid			

Table 4. Spine classification of *A. puysegurensis*. Spine numbers vary depending on whether the zooid is marginal, central or ovicelled. Here, the spines of a central, non-ovicelled zooid are examined. Since spines cannot safely be considered homologous without topographical correspondence,¹⁰³ the three spine positions (i, ii, iii in Figure 9B) are examined separately.

Scuta	Overall Shape	Distal Lobe Type	Central Lobe Type	Proximal Lobe Type	Relative Lobe Size
-	Round	Truncated	None	Round	2
Scuta	Distinctness	Cross Section	Internal Channels	Base Location	Base Thickness
-	2	0	NA	1	2

Table 5. Scutum classification of *A. puysegurensis* (Figure 9A-C). The side (left or right) the scutum arises from is variable.

Rhizoids	Form	Origin	Surface	Tip	Connects	Thickness
-	1	Vibracular chamber	Smooth	NA	0	0

Table 6. Rhizoid classification of *A. puysegurensis* (Figure 9G).

Skeletal Chambers for Embryonic Incubation	Embryo Incubation Chamber	Skeletal Chamber	Immersion	Distal Zooid	Ooecium Structure
-	1	2	2	autozooid	0

Table 7. Embryo incubation classification *A. puysegurensis* (Figure 9B).

4. Discussion

The cheilostome polymorphism classification system presented here provides comprehensive and standardized morphological terminology. This classification system is robust, and is capable of describing a vast range of forms in fossil and recent cheilostomes. For example, $\sim 8 \times 10^9$ possible avicularia can be described qualitatively by this classification, not counting avicularia that possess multiple projections or those with divergent mandible and rostral morphologies. The nonhierarchical structure facilitates statistical comparisons at different levels of detail and allows it to be used even when some traits are missing. This classification system is well suited for use in morphological studies, several of which are discussed below.

It is important to identify those aspects of complexity that can be measured by this classification in cheilostome bryozoans. Complexity, as defined by McShea (1996)¹⁰⁴, can be divided into four categories:

1. Nonhierarchical object complexity: The number of different parts at a specific spatial or temporal scale
2. Nonhierarchical process complexity: The number of different interactions at a specific spatial or temporal scale
3. Hierarchical object complexity: The number of levels of organization
4. Hierarchical process complexity: The number of levels in a causal hierarchy¹⁰⁵

Since this classification system is focused on morphology, it facilitates the investigation of hierarchical and nonhierarchical object complexity. In bryozoans, nonhierarchical object complexity can be examined at the scale of the submodule (the number of different projections on an avicularian mandible), the cormidium (the number of different submodules), or colony form (the number of different cormidia and paramodules).¹⁰⁶ These levels are fairly independent of each other: a colony may be monocormidial (low complexity), but the one cormidium it possesses could have many submodules (high complexity). Hierarchical object complexity considers all levels of modular organization together, independent of their internal complexity. By this metric, monomorphic nonbranching colonies are the least complex (two levels: zooid, colony; see Figure 2B), while polymorphic branching colonies are the most complex (four levels: submodule, cormidium, branch, colony; see Figure 2I).¹⁰⁷ The appropriateness of each measure of complexity depends on the questions being asked. However, general questions about whether complexity has increased in cheilostomes should consider hierarchical and nonhierarchical object complexity at all levels of modular organization to produce a more nuanced picture of complexity in evolution. These measures of complexity can then be used in conjunction with tests for active selection described in McShea (1994).¹⁰⁸

This classification system also provides a framework for comparing bryozoan polymorphism morphology, which would be highly useful in a trait-environment analysis. Although relationships between bryozoan polymorphism and the environment have been investigated before, these studies only considered the number of different polymorph types (polymorphism diversity) and found no relationship with environmental

gradients.^{109,110} Although Schopf (1973) described a latitudinal trend in avicularian mandible shape, where pointed mandibles were commoner in the tropics, this was not tested statistically.¹¹¹ This highlights a gap in the literature that should be addressed. Since the classification system presented here is comprehensive in nature, it provides a high level of morphological (and thus ecological) resolution, which is necessary when teasing apart potential trait-environment relationships. The statistical methods then used would depend on the level of analysis: average, community-level trends could be investigated using canonical correspondence analysis (CCA), while RLQ analysis could be used for species-level trends.¹¹² Once these trends are identified, they can be used to predict bryozoan species/community responses to environmental shifts (e.g. climate change), or to reconstruct the paleoenvironment from fossil traits.

The analyses described above would not be possible with a purely phylogenetic study. Phylogenetics would not be able to separate forms arising from the high phenotypic plasticity in bryozoans, or provide comparisons between convergently evolved morphologies. Descriptions of form are particularly important for polymorphism, since many have unknown or debated functions (especially avicularia^{113,114}).¹¹⁵ Although this classification system does not explore function, the breadth of morphologies described here may facilitate analysis of form and function. Even within a single type of polymorphism, the range of forms suggests specialization for several different functions - though whether this holds true remains to be seen. However, analyses of complexity and trait-environment relationships would both benefit from the inclusion of genetic data. Genome and morphological complexity could be examined concurrently¹¹⁶, and phylogenetic effects could be controlled for in trait-environment analysis.^{117,118} Using this classification system in tandem with phylogenetics should contribute to a fuller understanding of cheilostome evolution and ecology.

Finally, this classification system should be of use to taxonomists. Although describing polymorphs and cormidia with this system is less concise than typical taxonomic descriptions (e.g. “spatulate mandible” vs “rounded overall shape, bulb, and tip; abrupt tapering; not curved; convex; no projections; no teeth”) the terminology is less ambiguous and captures a greater morphological resolution. Taxonomists may also wish to focus on species-discriminating traits, which can be picked from this classification system using the VARSEDIG algorithm.¹¹⁹

Throughout all the potential analyses mentioned, the standardized terminology provided here will allow clear, valid comparisons to be drawn between different studies. This will, we hope, encourage and enhance future research on bryozoan polymorphism.

5. Author contributions

CRS examined specimens, wrote, illustrated, and tested the classification system. DPG and KGR supervised the project. DPG provided guidance on terminology and definitions, the scope of the classification, and bryozoan anatomy. KGR assisted in fine-tuning definitions and increased the clarity of the classification system.

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Notes

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Appendix 1. Selected Definitions

Appendaged autozoid: An autozoid that possesses non-zooidal adventitious modules.

Autozooidal polymorph: A zooid that retains a protrusible tentacle crown but differs from an autozoid in the form of its cystid, polypide, or both. This includes reproductive zooids, appendaged autozooids, and nanozooids.

Cormidial arrangement: The pattern of cormidia within a colony so as to comprise secondary or higher-level modules.

Cormidium: A “colony within a colony” that consists of a feeding zooid and associated polymorphs. The cormidium is capable of performing most functions vital for life. Multiple types of cormidia may be present within a colony.

Heterozoid: Zooids that lack a feeding polypide and are separated from the autozooidal coelom by a pore plate (e.g. avicularia, some reproductive zooids).

Module: Any unit within a colony, the components of which are more integrated with each other than those of other units.

Monocormidial: A bryozoan colony that only possesses one type of cormidium.

Monomorphic: A structure that is made of only one kind of unit. In bryozoans, it refers to a colony that consists only of autozooids.

Non-zooidal adventitious module: A projection of the zooidal body wall that is not separated from the coelom by a pore plate, but displays reduced integration and exhibits variation separate from the parent zooid. These are typically spines, such as the spinules of *Membranipora membranacea* Linnaeus, 1767 or the spines of *Electra pilosa* Linnaeus, 1767. The parent zooid hosting a non-zooidal adventitious module may be an autozoid or a heterozoid.

Paramodule: Heterozooids that exist and repeat outside of cormidia (e.g. kenozooids in an anchoring kenozooidal stalk).

Polycormidial: A bryozoan colony that possesses multiple types of cormidia

Polymorph: A zooid or adventitious module that exhibits discontinuous variation (separating them from astogenetic and ontogenetic zooidal changes).

Polymorphic: Pertaining to the discontinuous variation that is exhibited by *any* modular structure in a bryozoan colony, including autozooids, etc.

Primary module: The basic repeating unit of a modular structure. In bryozoans, the primary module is either the autozoid (in monomorphic colonies) or the cormidium (in polymorphic colonies).

Secondary module: A structure formed through the repetition of primary modules, e.g. a branch composed of repeating cormidia. In terms of modular organization, this term is synonymous with “secondary structure”.

Submodule: The component parts (autozooids, autozooidal polymorphs, and heterozooids) of a cormidium.

Tertiary module: A structure formed through the repetition of secondary modules. In terms of modular organization, this term is synonymous with “tertiary structure”.

Appendix 2

Avicularia	Trait	Levels	# Levels	Data Type	Preserved in Fossils?
General	Type	Vicarious (0) Interzooidal (1) Adventitious (2)	3	Ordinal	Yes
Position	Zooid Wall	Frontal Lateral Distal Proximal Basal	5	Factor	Yes
	Relative Location	Hyper-oral Sub-oral Spine substitute Ovicell Gymnocyst Between zooids	6	Factor	Yes
	Distal-Proximal Location	Mid-Distal (0) Distal Corner (1) Distolateral (2) Lateral (3) Proximolateral (4) Proximal Corner (5) Mid-Proximal (6)	7	Ordinal	Yes
Direction	Rostral Direction	Distal (0) Distolateral (1) Lateral (2) Proximolateral (3) Proximal (4)	5	Ordinal	Yes
	Variable Direction	Non-variable (0) Variable (1)	2	Binary	Yes
Attachment	Attachment	Sessile Columnar Tubular Bird's head	4	Factor	Yes
	Spikes	None Simple Branching Mixed	4	Factor	Sometimes
	Spike Abundance	NA	NA	Numeric	Uncertain
	Elevation	None (0) Short (1) Intermediate (2) Long (3) Very long (4)	5	Ordinal	Yes
Mandible/Rostral shape	Overall Shape	Pointed (0) Rounded (1)	2	Binary	Only Rostra
	Curvature	Straight (0) Curved (1)	2	Binary	Only Rostra
	Tapering	No stem (0) Abrupt (1) Partial (2) Full (3)	4	Ordinal	Only Rostra
	Bulb Outline	Concave (0) Straight (1) Convex (2)	3	Ordinal	Only Rostra
	Bulb Shape	Pointed (0) Rounded (1)	2	Binary	Only Rostra
	Bulb Tip	Pointed Rounded	3	Factor	Only Rostra

		Truncated			
	Projection Shapes	None Mucronate Cuspidate Aristate Falcate Cirrhose Hooked Auriculate Oblong	9	Factor	Only Rostra
	Projection Location(S)	None Bulb (mid-distal) Bulb (distal corner) Bulb (lateral) Bulb-stem Stem	6	Factor	Only Rostra
	Teeth	None Sharp Blunt	3	Factor	Only Rostra
	Teeth Location	None Bulb Bulb-stem Stem Rachis Projection Entire	7	Factor	Only Rostra
Shape Measurements	Total Length	NA	NA	Numeric	Only Rostra
	Hinge Length				
	Hinge Width				
	Stem Length				
	Stem Width				
	Bulb Length				
	Bulb Width				
	Projection Length				
	Opesia Length				
	Opesia Width				

Vibracula	Trait	Levels	# Levels	Data Type	Preserved in Fossils?
Location	Zooid Wall	Frontal Lateral Distal Proximal Basal	5	Factor	Yes
	Direction	Distal (0) Distolateral (1) Lateral (2) Proximolateral (3) Proximal (4)	5	Ordinal	Yes
	Position	Non-axial (0) Axial (1)	2	Binary	Yes
Mandible Structure	Mandible Length	Shorter (0) Intermediate (1) Long (2)	3	Ordinal	No
	Mandible Tip	Straight (0) Hooked (1)	2	Binary	No
	Mandible Surface	Smooth (0) Barbate (1)	2	Binary	No
General Structure	Palatal Surface	Absent (0) Present, with foramen (1)	3	Ordinal	Uncertain

		Present, no foramen (2)			
	Setal Groove Curvature	Straight (0) Curved (1)	2	Binary	Uncertain
	Setal Groove Length	Short (0) Intermediate (1) Long (2)	3	Ordinal	Uncertain
	Setal Groove Location	Transverse (0) Oblique (1)	2	Binary	Uncertain
	Rhizoid Pore	Absent (0) Circular (1) Ovate (2)	3	Ordinal	Uncertain

Spines	Trait	Levels	# Levels	Data Type	Preserved in Fossils?
Number of spines	Total Number of Spines	NA	NA	Numeric	Yes
	Number of Spines	NA	NA	Numeric	Yes
	Paired	No (0) Yes (1)	2	Binary	Yes
Location	Location	Mid-Distal Distal Corner Distolateral Orificial Sub-Oral Lateral Proximolateral Proximal Corners Mid-Proximal Opesial Distal Wall Outer Distal Corner Distal (Lateral wall) Lateral (Lateral Wall) Proximal (Lateral Wall) Proximal Wall	16	Factor	Yes
	Angle	Flat (inner) (0) Acute (1) Erect (2) Obtuse (3) Flat (Outer) (4)	5	Ordinal	Only costae
	Direction	Abfrontal Distal Distolateral Lateral Proximolateral Proximal	6	Factor	Only costae
Structure	Branching	Unbranched Forked Bifid Cervicorn Tines	5	Factor	Rarely
	Branch Distinctness	Distinct (0) Intermediate (1) Indistinct (2)	3	Ordinal	Rarely
	Curvature	None (0) Slight (1) Strong (2)	3	Ordinal	Rarely
	Compression	Cylindrical (0) Somewhat flattened (1)	3	Ordinal	Rarely

		Flattened (2)			
	Proximity	Sparse (0) Intermediate (1) Appressed (2)	3	Ordinal	Yes
	Construction	Simple Antenniform Stalked Antenniform Pinnate Lumen Pores	5	Factor	Rarely
	Spine Tip	Pointed Rounded Truncated Membrane	3	Factor	Rarely
	Basal Joint	Present (1) Absent (0)	2	Binary	Yes
	Thickness	Tapering (0) Consistent (1) Flaring (2)	3	Ordinal	Rarely
	Length	Short (0) Intermediate (1) Long (2) Very long (3)	4	Ordinal	Rarely
	Base width	Thin (0) Intermediate (1) Thick (2) Very thick (3)	4	Ordinal	Yes
Fusion	Fusion	None Lateral Medial Lateral neighbors Medial neighbors Complete Lacunae Peristome	8	Factor	For costae, potentially for other spines
	Midline Interaction	Separate Meet Interdigitate Push Carina	5	Factor	For costae, potentially for other spines
Other	Spiniferous Process	None (0) Bulb tip (1) Bulb Only (2) Entire Process (3)	4	Ordinal	Uncertain
Zooid	Zooid	Autozooid Ancestrula Ovicelled zooid	3	Factor	Yes

Scuta	Trait	Levels	# Levels	Data Type	Preserved in Fossils?
Shape	Overall Shape	Spike Round Fan Oblong Bifid Cervicorn	6	Factor	Uncertain
	Lobe Types	None Spike (H) Spike (A) Forked Round Truncated Tines	8	Factor	Uncertain

Structure		Trifid			
	Relative Lobe Size	Reduced (0) Symmetrical (1) Enlarged (2)	3	Ordinal	Uncertain
	Distinctness	Distinct (0) Intermediate (1) Indistinct (2)	3	Ordinal	Uncertain
	Cross-section	Flat (0) Curved (1)	2	Binary	Uncertain
Base	Internal Channels	Absent (0) Present (1)	2	Binary	No
	Base Location	Distal (0) Distolateral (1) Lateral (2) Proximolateral (3) Proximal (4)	5	Ordinal	Yes
	Base Thickness	Thin (0) Intermediate (1) Thick (2) Very Thick (3)	4	Ordinal	Yes
Measurement	Height	NA	NA	Numeric	Uncertain
	Width				
	Percent Cover				

Rhizoids	Trait	Levels	# Levels	Data Type	Preserved in Fossils?
General	Form	Prop (0) Rhizoid (1)	2	Binary	Uncertain
	Origin	Lateral wall Basal wall Dorsal wall Ventral wall Vibracular chamber	5	Factor	Uncertain
	Surface	Smooth Ringed Hooked Coiled (base)	4	Factor	Uncertain
	Tip	Single Fimbriate Hold-fast	3	Factor	Uncertain
	Connects	Substrate (0) Branches (1)	2	Binary	Uncertain
	Thickness	Consistent (0) Dilated (1)	3	Ordinal	Uncertain

Skeletal Chambers for Embryonic Incubation	Trait	Levels	# Levels	Data Type	Preserved in Fossils?
General	Embryo Incubation Chamber	External membranous sac (0) Skeletal chamber (1) Non-calcified internal brood sac (2) Intracoeelomic incubation (3)	4	Ordinal	For skeletal chambers

	Skeletal Chamber	None (0) Acanthostegous (1) Ooecia (2)	3	Ordinal	Yes
	Immersion	Immersed (0) Sub immersed (1) Prominent (2)	3	Ordinal	Yes
	Distal zooid	Autozooid Kenozooid Vicarious avicularium	3	Factor	Yes
	Ooecia Structure	Entire (0) Bilobed (1) Multilobed (2)	3	Ordinal	Yes
Measurement	Length	NA	NA	Numeric	Yes
	Width				

Appendix 2

Errata for:

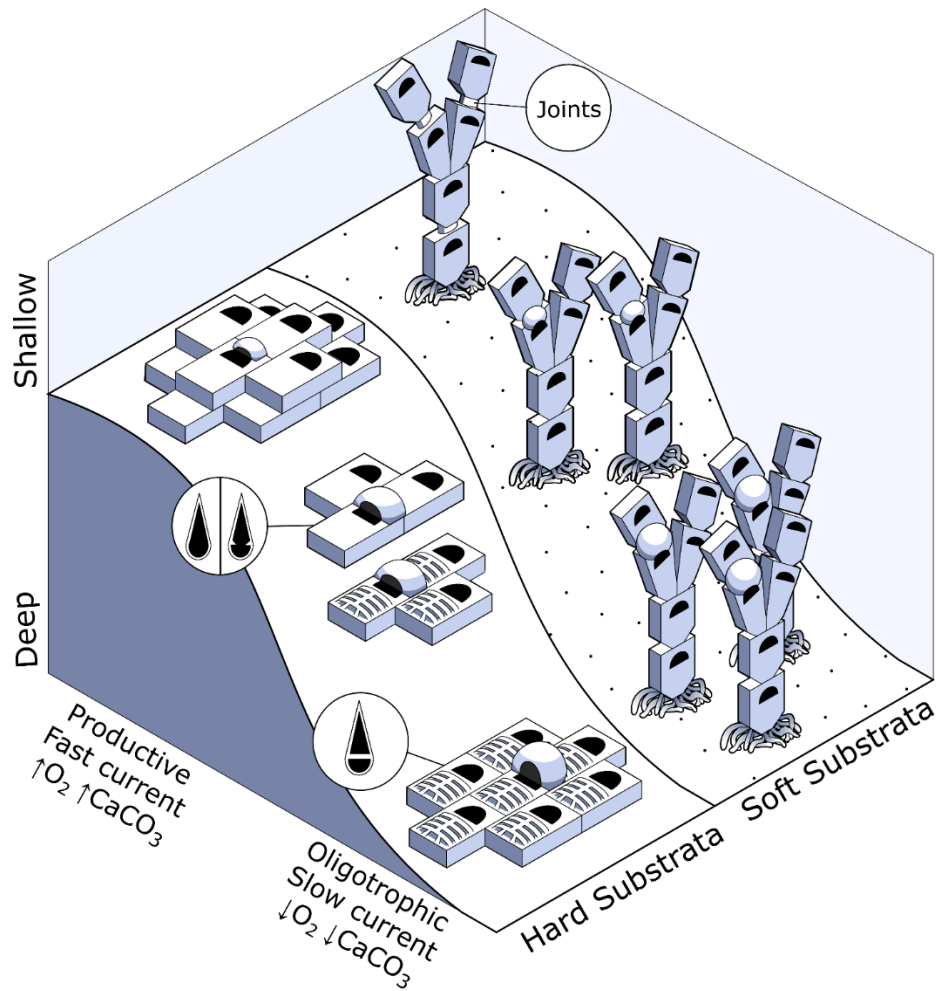
Chapter 3, Classification of cheilostome polymorphs. Page numbers area given as they appear in the thesis, while journal page numbers are given in parentheses.

Page	Original text	Corrected text
43 (87)	The system complements...	The system described here complements...
49 (93)	...relationships; building a database of cheilostome polymorphs; etc.	...relationships; and building a database of cheilostome polymorphs.
49 (93)	May retain functional polypide and gut. May retain functional polypide and gut (as in <i>Steginoporella</i> , <i>Crassimarginatella</i> , and <i>Wilbertopora</i>).	May retain functional polypide and gut (as in <i>Steginoporella</i> , <i>Crassimarginatella</i> , and <i>Wilbertopora</i>).
49 (93), Fig. 1 legend	B. Frontral surface <i>i. membranous...</i>	B. Frontal surface <i>i. membranous...</i>
49 (93), Fig. 1 legend	D. Hypoetheical cormidium...	D. Hypothetical cormidium...
55 (99), Fig. 4 legend	Projection locations <i>i. mid-disal...</i>	Projection locations <i>i. mid-distal...</i>
70 (114)	<i>Prop (0):...</i>	- <i>Prop (0):...</i>
75 (119), Table 5	unbranched... autozoid...	Unbranched... Autozoid...
80 (127), footnote 114	“Why bryozoans have avicularia- A review of the evidence.”	“Why bryozoans have avicularia—A review of the evidence.”

Chapter 4: Community assembly in a modular organism: the impact of environmental filtering on bryozoan colony form and polymorphism

Abstract

Understanding community assembly is a key goal in community ecology. Environmental filtering influences community assembly by excluding ill-adapted species, resulting in communities with similar functional traits. An RLQ (a four-way ordination) analysis incorporating spatial data was run on a dataset of 642 species of cheilostomes (Bryozoa) from 779 New Zealand sites, to investigate environmental filtering of colony form and zooid polymorphism. This revealed environmental filtering of colony form: encrusting-cemented taxa were predominant in shallow environments with hard substrata (<200 m), while erect-rooted taxa characterized deeper environments with soft substrata (>200 m). Furthermore, erect taxa found in shallow environments with high current speeds were typically jointed. Polymorphism also followed environmental gradients. External ovicells (brood chambers) were more common than immersed and internal ovicells in deeper, low oxygen water. This may reflect the oxygen needs of the embryo or increased predation intensity in shallow environments. Bryozoans with costae tended to be found in deeper water, while bryozoans with calcified frontal shields were found in shallow environments with a higher concentration of CaCO_3 . Avicularia did not appear to be related to environmental conditions, and changes in pivot structure with depth likely represent a phylogenetic signal. Factors influencing community assembly are somewhat partitioned by levels of organization, since colony form responds to environmental conditions, while the effects of evolutionary history, predation, and environmental conditions were not well-separated for zooid-level morphology. Finally, rootlets may have been a key innovation that allowed cementing taxa to escape hard substrata, potentially contributing to the cheilostome radiation.



Graphical Abstract: Both colony form and polymorphism in New Zealand cheilostomes change along environmental gradients. Encrusting taxa are limited to areas with hard substrata, while erect rooted forms dominate deep, soft-substratum habitats. Brood chambers became more prominent in deeper waters, potentially due to decreased oxygen availability or a decrease in predation. In erect taxa, colonies with flexible joints were common in areas with fast-current regimes due to their ability to reduce mechanical stress. Encrusters with Costae/Cribrimorph frontal shields tended to be found in deeper waters, potentially due to low CaCO_3 and predation. While pivot structure in avicularia appeared to change with productivity and variability in sea-surface temperature, this likely reflects a phylogenetic signal rather than a true trait-environment relationship.

1. Introduction:

A key goal in ecology is to understand the factors influencing community assembly. Community assembly can reflect evolutionary history and stochastic processes (dispersal and colonization), environmental conditions, and the influence of other organisms. Environmental filtering occurs when abiotic conditions restrict species from forming self-sustaining populations in a particular habitat [1,2]. Only taxa with a pre-existing tolerance to the environmental filter remain, forming a community of species with suites of similar functional traits. For example, ant communities under a high-temperature filter tend to have longer legs and increased heat tolerance [3]. Of course, traits not associated with the environmental filter can undergo character displacement [4]. Such trait-environment relationships can predict the success of invasive species [5], predict the community response to changing environmental conditions, reconstruct the paleoenvironment [6], suggest functions for obscure traits, and pinpoint “key innovations” that may have sparked species radiations.

However, environmental filters are not always strict. A weak filter (which simply decreases fitness) combined with biotic pressures can also produce communities of species with similar functional traits [7]. Identical community patterns may result from close phylogenetic relatedness among member taxa and stochastic processes (e.g. dispersal). Of course, community assembly may be simultaneously influenced by multiple processes [8,9], making it difficult to tease out the relative impacts of environmental conditions.

This is further complicated in highly integrated taxa, since groups of genetically or developmentally linked traits may all appear to follow the same environmental gradient – even when the environment directly impacts a single trait. Studying modular organisms allows the impact of certain factors (environmental, biological, stochastic) to be parceled out into biologically meaningful components (e.g. unique traits or levels of organization) [10]. This makes the highly modular Bryozoa an ideal taxon to study community assembly. Bryozoans are an understudied phylum of mostly marine, sessile, colonial invertebrates. They are an important fouling taxon, they provide “micro-reef” habitats, and their calcified skeletons make them vulnerable to ocean acidification [11]. Bryozoan colony form is thought to respond to environmental conditions [12], while polymorphism (presence of individuals specialized for certain tasks) is thought to respond to predation by small epibionts [13–15]. However, previous studies on bryozoan polymorphism were based on the number of polymorphs, rather than their morphology. These studies only focused on shallow-water reef communities (<25 m, <100 species), neglecting potential trends with depth and substratum type.

In this paper, I investigate environmental filtering of cheilostome bryozoan communities from New Zealand over a wide range of depths (0-7,400 m). The goal of this analysis is to identify those bryozoan traits that are linked to what environmental conditions (i.e. extract the shared structure between traits and the environment) and identify potential “key innovations”. I predict that colony-level trends will relate to large-scale environmental gradients such as substratum type and depth [12]. In contrast, polymorphism should exhibit no pattern with the environment – instead reflecting evolutionary history, dispersal, or biotic interactions [13,14].

1.1 Bryozoan Morphology: Bryozoan colonies are composed of iterated units (zooids), and non-polymorphic zooids are referred to as autozooids. Bryozoan polymorphism is reviewed in Schack et al. [16]. Briefly, avicularia are mandibulate polymorphs that typically exhibit a grasping function (though they may also function as cleaning or locomotory appendages). The term “spine” refers to a wide variety of structures hosted on the zooid wall that exhibit similar form and function (e.g. defense). Ovicells are reproductive structures with a calcified hood (ooecium) that protects developing embryos. Rootlets (also known as rhizoids) are simple attachment structures used to anchor the colony to the substratum.

2. Methods:

2.1 Occurrences and trait classification: Community data for New Zealand cheilostomes were acquired from various monographs [17–23], with positional and environmental data for species supplied by the National Institute of Water and Atmospheric Research (NIWA) Invertebrate Collection, funded by NIWA SSIF Marine Platform funding (Coasts & Oceans National Centre, Programme 2: Marine Biological Resources: Discovery and definition of the marine biota of New Zealand).

Specific NIWA voyages are acknowledged in Supplementary Data 1. Only samples with location data (latitude and longitude) were used, resulting in a total of 721 species from 953 sites. Forty species traits, encompassing both colony form and polymorphism, were classified from monographs (descriptions, drawings, and scanning electron micrographs) in addition to unpublished scanning electron micrographs (Data S1). If characters were not documented, they were assumed from genus-level descriptions. Colony-level traits were classified according to Hageman et al. [24], while polymorphs were classified according to Schack et al. [25] and Ostrovsky [26]. Unfortunately, polymorphisms were often inadequately described in the literature and classification had to be simplified to accommodate the available data (Data S1). Species ranges (endemic, native, or adventive) were taken from Gordon *et al.* [27], and undescribed species were assumed to be endemic. A complete list of species traits is provided in (Data S1).

Bryozoans can have multiple types of avicularia and spines [16]. To account for this intracolony variation, each level in variable traits (excluding pivot structure, spine branching, and maximum spine number) was converted into a separate binary variable. While this decouples traits in species with multiple types of avicularia or spines, it allows each trait level to be examined separately. Pivot structure was kept as an ordinal variable, and if a species possessed multiple pivot structures then only the most complete structure was recorded (i.e. a species with condyles and pivot bars would be recorded as just having pivot bars).

2.2 Environmental variables: The study area was confined to waters around New Zealand and southeastern Australia (20° S to 60° S, 143° E to 175° W). Location (longitude and latitude), date, and depth for each site were obtained from cruise reports from NIWA and other sources [17–23] (see Data S2). Distance from shore was calculated using a custom Mercator projection to preserve great-circle (orthodrome) distances between stations (function `omerc()` from R package `mapmisc` [28]). Stations with incorrect or missing depth data (Galathea station 649; NIWA stations I97, KAH1206/2, KAH1206/29, KAH1206/94, U1011, Z16144, Z2810) were corrected using a NIWA bathymetry.

Environmental variables and sources are given in Supplementary Data 2. Grain-size proportions (mud, sand, gravel), and the presence of hard-substratum “islands” [29] were included to provide a detailed picture of the substratum.

The standard deviations of satellite-derived sea surface temperature (SST) and surface chlorophyll-a were included as a measure of seasonality. One site in Doubtless Bay (NIWA station number P368) had a curiously high standard deviation in surface chlorophyll (9.00 mg chl-a m⁻³), likely caused by its proximity (<15 km) to the mouths of four rivers. This large concentration was corrected to that of a nearby site in Doubtless Bay (P641, 0.63 chl-a mg m⁻³).

Ocean chemistry, current speed, bottom stress, and benthic chlorophyll data were obtained from the New Zealand Regional Ocean Climatology model (NZROCS) (MacDonald 2019, pers. comm). This model couples a circulation model (Coastal and Regional Ocean Community model, CROCO [30]) and a biogeochemical model (Pelagic Interactions Scheme for Carbon and Ecosystem Studies model, PISCES [31]). The model was run for 98 time-steps (30 days each, ~8 years in total) and 30 depth intervals at each site. The Environmental conditions used in this paper represent the average value (across all time steps) at the depth interval closest to the sea floor. The NZROCS model has a resolution of 0.125 degrees, and the modeled sites were relatively close to the actual site locations (3.98 ± 1.57 km). However, the fidelity of

model depth locations was better for euphotic sites (169.25 ± 315.56 m, $n= 395$) than deeper ones (twilight, 310 ± 392.95 m, $n=243$; bathyal, 477.66 ± 430.74 m, $n=131$; abyssal, 376.70 ± 117.55 m, $n=6$), and was quite poor for sites below 6,000 m (2270.57 ± 983.55 m, $n=2$).

2.3 Data preparation: Rare species (with a single presence) were kept as long as they were not the sole member of their community. This reduces the influence of rare morphologies without removing them from the dataset entirely. In addition, sites with missing environmental data were excluded from the analysis. This resulted in a final community matrix of 642 species and 779 sites (Data S1).

Numeric environmental variables with a skewed distribution (distance from shore, depth, bottom stress, current speed, benthic chlorophyll, standard deviation of surface chlorophyll, and CaCO_3 concentration) were log transformed (base 10). Zeroes were present in depth, standard deviation of surface chlorophyll, and current speed so these variables were scaled by adding the minimum non-zero value to all values before log transforming. Proportional environmental variables (mud, gravel, sand) were logit transformed following Warton & Hui [32]. Since zeroes were present, the minimum non-zero value was also added to these variables before transformation. All environmental variables were then standardized by scaling values to have a range of 0-1.

To check collinearity among environmental variables, the variable inflation factors (VIFs) were examined (function `vif` in R package `car` [33]) and a non-metric multidimensional scaling (nMDS) analysis was run (function `metaMDS` in R package `vegan` [34]). Variables were removed from the dataset until all had a VIF less than three, resulting in a final suite of environmental variables (Table 1). Finally, a Moran's I test was performed to determine if environmental variables were spatially autocorrelated (function `gearymor` in R package `ade4` [35]).

Table 1. Environmental data used in the RLQs. Full list of environmental variables used in the nMDs provided in Supplementary Data S2.

Variable	Type	Units
Sea Surface Temperature (Standard deviation)	Numeric	°C
CaCO_3	Numeric	$\mu\text{mol} / \text{L}$
Benthic Chlorophyll	Numeric	$\text{mg chl} / \text{m}^3$
Surface Chlorophyll-a (Standard deviation)	Numeric	$\text{mg chl} / \text{m}^3$
Current Speed	Numeric	m/s
Depth	Numeric	m
Gravel	Numeric	%
Sand	Numeric	%
Hard Substrata	Binary	-

Plots were made with R packages ggplot2, cowplot, and ggridges [36–38].

2.4 RLQ analyses: The four-way RLQ was performed in R (version 3.5.2), following the methods of Pavoine et al. [8]. Despite recent efforts [39,40], our understanding of bryozoan phylogeny (particularly inter-family relationships) is not complete and could not be included in this analysis. This means that phylogenetic signals cannot yet be statistically separated from environmental trends.

Including a large number of variables in an ordination can artificially inflate the variance explained. To reduce the number of traits included in the analysis, the relationship between each trait and the environment was analyzed separately. For each trait, a permutation test was run to determine if the results of an RLQ (the sum of the eigenvalues) for the original dataset were significantly greater than one where species traits were randomized [8]. Only traits with a significant relationship with environmental variables were included in the final RLQ (Table 2).

Table 2. Traits used in each RLQ (all taxa, encrusting only, or erect only). Full trait levels are provided in supplementary Data S1.

Trait	Type (levels)	Description	All	Encrusting	Erect
Colony orientation	Categorical (6)	Whether the colony is encrusting, erect, or other	+		
Colony surface	Categorical (5)	Occupation of space (unilaminate, radial, etc.)	+	+	+
Colony attachment	Ordinal (4)	Attachment to substratum	+		
Arrangement of zooids	Ordinal (4)	Colony width in number of zooids (uniserial, macroserial, etc.)	+		
Joints	Ordinal (3)	Colony joint type			+
Branching	Binary	Presence of branching in colony		+	
Brood chamber immersion	Categorical (5)	Degree of brood chamber immersion (external, immersed, no brooding, etc.)	+	+	+
Ovicells	Binary	Presence of ovicells	+	+	+
Vicarious avicularia	Binary	Presence of large avicularia that take the place of an autozooid in the colony	+		+
Pivot structure	Ordinal (4)	Structure of the pivot bar, including whether the species lacks avicularia	+	+	
Costae	Binary	Presence of rib-like, unjointed spines	+	+	
Jointed Spines	Binary	Presence/absence of jointed spines	+		

It is possible that colony form could obscure relationships between polymorphism and the environment. To test this, separate RLQ analyses were performed for encrusting (302 species, 270 sites) and erect species (144 species, 215 sites), removing rare species as above. Permutation tests for individual traits were rerun and only significant traits were kept for each of the RLQs. Finally, global fourth-corner

permutation tests were performed on each dataset (all orientations, encrusting only, erect only) to determine if the selected traits had a significant relationship with either the environment or spatial structure of sites. The null hypothesis of this test assumes that species are randomly distributed across environmental conditions and space. Species occurrences are permuted while holding sites constant and a new RLQ analysis is run for each permutation. A p-value is simulated from the proportion of permutations that resulted in a higher variance explained (i.e. sum of eigenvalues) compared to the original RLQ.

3. Results:

3.1 nMDs: The nMDs of environmental variables resulted in low stress (0.061, $k=3$), indicated a good fit. The first axis of the nMDs contained variables related to depth, including most of the environmental variables modeled by NZROCs (Data S2). The second axis was related to sediment type and variability in surface chlorophyll, while the third axis was characterized by current speed, variation in SST, salinity, pCO_2 , and the concentration of O_2 .

All environmental variables were spatially autocorrelated ($p < 0.001$ in all cases). This may be due in part to the use of modeled/interpolated data and the resolution of the environmental grid cells.

3.2 Full RLQ: The results of RLQ indicate trends at the community-level (i.e. “average” trait expression of a particular community). While certain species may be exceptions within a community, the bryozoan communities as whole should reflect the results presented here. The first three axes of the full RLQ explained 89% of the total inertia (variation in species scores) (Fig. 1). Total inertia (0.005) and eigen values (< 0.003) were low. This is due to the use of exclusively qualitative traits, which allows multiple species to occupy the same location in “trait-space”. Despite this, global fourth-corner tests suggest that the trait-environment and trait-spatial relationships are significant (999 permutations, $p < 0.001$ in both cases).

The first axis explained 47.44% of the variation and was linked to substratum characteristics and productivity. Sites with high scores along this axis are characterized by the presence of hard substrata and gravel, less sand, low benthic chlorophyll, and low variability in surface chlorophyll-a (Fig. 1). Cemented, sheet-like encrusters with external ovicells and jointed spines tended to have higher scores on this axis (Fig. 2-3, S1).

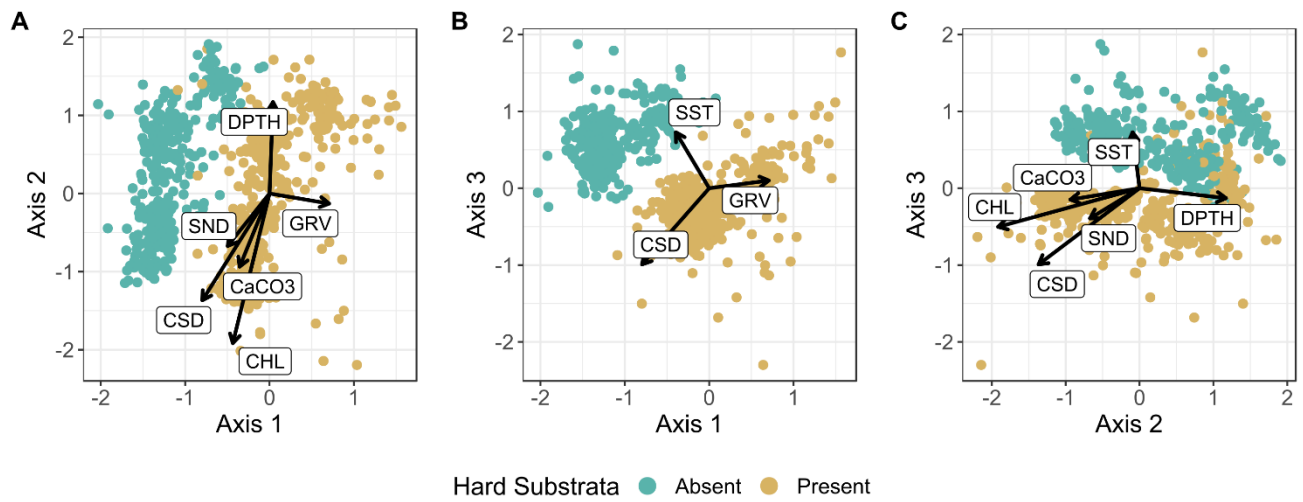


Figure 1. Biplots showing site scores along the first three axes of the full RLQ. Sites (points) are colored by the presence of hard substrata. Arrow length indicates the importance of environmental variables along each axis. For clarity, only environmental variables with a correlation of 0.3 or more on either axis were included. Abbreviations for environmental variables are as follows: CaCO₃, calcium carbonate concentration; CHL, benthic chlorophyll concentration; CSD, standard deviation of surface chlorophyll-a; DPTH, depth; GRV, percent gravel; SND, percent sand; SST, sea surface temperature.

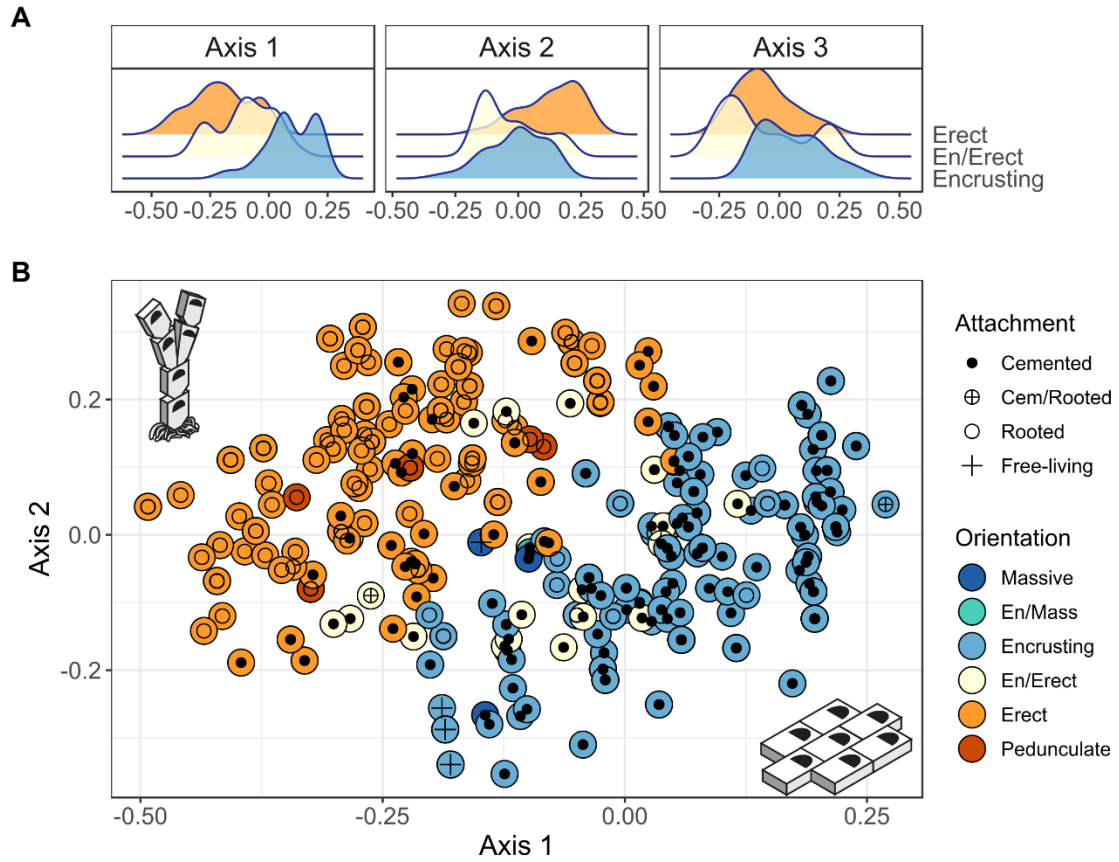


Figure 2. Colony orientation and attachment method for taxa in the full RLQ. A. Density plots showing the distribution of colony form along axes 1-3. B. Plot showing species scores along Axis 1 and Axis 2. Note that all species capable of both encrusting and massive colony forms (En/Mass) are obscured by regular encrusting species.

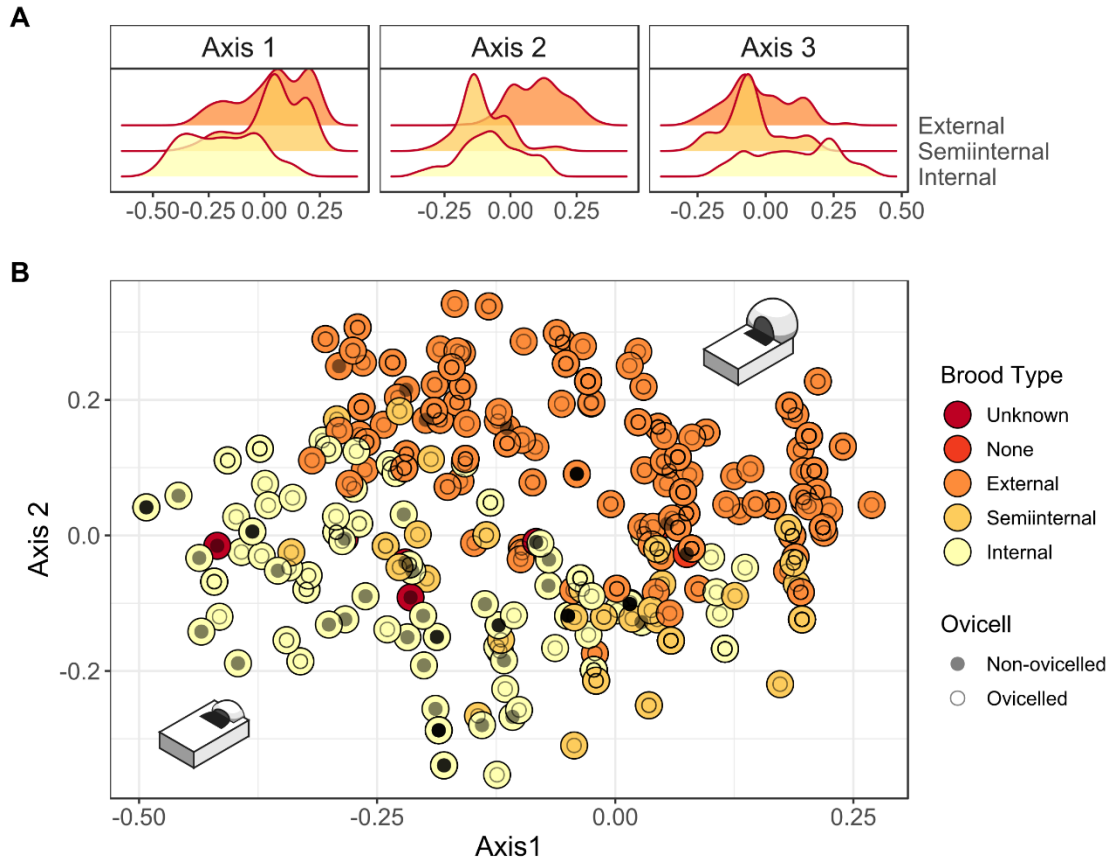


Figure 3. Degree of brood chamber immersion and presence of ovicells for taxa in the full RLQ. A. Density plots showing the distribution of brooding species along axes 1-3. B. Plot showing species scores along Axis 1 and Axis 2.

Axis 2 explained 31.48% of the variation and represents increasing depth, decreasing chlorophyll, and decreasing CaCO_3 (Fig. 1). This axis corresponds to the first axis of the nMDS (Data S2), linking it to increasing nutrients / dissolved inorganic carbon and decreasing temperature / salinity / oxygen / pH. Erect rooted taxa that lack avicularia score highly on this axis, while cemented encrusters that possess avicularia with complete pivotbars have low scores (Fig. 2, Fig. S2)

The third axis only explained 10.36% of the variation and relates to the presence of hard substrata and increasing variability in SST and surface chlorophyll (Fig. 1). External brooders with complete pivotbars have low scores on this axis, while internal brooders that lack avicularia have high scores (Fig. 2, Fig. S2).

3.3 Encrusting taxa: When encrusters were analyzed separately, colony attachment, arrangement of zooids, and the presence of vicarious avicularia were no longer significant traits. Like the full RLQ, total

inertia (0.004) and eigenvalues (<0.003) were low, but fourth-corner tests showed significant relationships among traits, the environment, and spatial structure ($p < 0.001$ in both cases).

Axis 1, which explained 62.24% of the variation, is the inverse of Axis 2 in the full RLQ and represents decreasing depth and nutrients (Fig. S3 D). However, surface variability and benthic concentration of chlorophyll are more important to Axis 1. Internal brooders capable of multilaminate growth and lacking costae have high scores on this axis (i.e. in shallow environments) (Fig. S4).

The second axis explained 24.83% of the variation and is characterized by decreasing variability in SST, increasing benthic chlorophyll and variability in surface chlorophyll (Fig. S3 D). Taxa lacking both ovicells and avicularia have low scores on this axis (Fig. S4).

3.4 Erect taxa: Only colony surface, degree of brood chamber immersion, joint type, and the presence of vicarious avicularia and ovicells had a significant relationship with the environment in the erect-only analysis. As expected, total inertia (0.026) and eigenvalues (<0.015) were low but relationships were significant ($p < 0.001$).

The first axis (explaining 57.60% of the variation) is nearly identical to Axis 1 in the analysis of encrusters. In contrast to the RLQ of encrusters, on this axis gravel increases along Axis 1 (decreasing depth and nutrients) (Fig. S3 A-B). Internal brooders with determinate cuticular joints and vicarious avicularia have high scores on this axis (Fig. S5).

Axis 2 explained 21.43% of the variation and is related to decreasing depth and SST variability, increasing gravel, current speed, and variability in surface chlorophyll (Fig. S3 A,C). Taxa with no joints have low scores on this axis, while taxa with determinate or indeterminate cuticular joints score highly (Fig. S5).

Axis 3 explained 10.95% of the variation and is related to increasing depth, current speed, and concentration of CaCO_3 (Fig. S3 B-C). Internal brooders lacking ovicells have low scores on this axis, while taxa with external or semi-internal ovicells score highly (Fig. S5).

4. Discussion

4.1 Colony form: Bryozoan colony form was primarily related to substratum type. Encrusting-cemented taxa were confined to habitats with hard substrata, while erect-rooted taxa have no such limitation. For example, many cheilostomes with rootlets attach to vertical rock walls and other hard substrata (e.g. *Bugula*). This may be an example of a strict environmental filter, where recruits of cementing species have complete mortality in a soft-substratum environment (with the exception of single-grain encrusters,

which were not present in this study). Therefore, rootlets may be a “key innovation” that freed bryozoan colonies from hard substrata and contributed to the radiation of cheilostomes.

It is likely that rootlets evolved during the cheilostome radiation, between the emergence of the oldest rigid-erect species (Late Albian [41]) and the oldest flexible-erect taxa (Cenomanian [42], 93.9-100 mya). However, rootlets evolved multiple times in cheilostomes, since they can be either kenozooidal, simple evaginations of the frontal wall, or extrazooidal [43]. Multiple reinvasions of soft-substratum environments by different cheilostome lineages suggest a series of diversifications, rather than a single burst.

Colony form is also related to depth: encrusting species dominate shallow-water communities (< 500 m), while deep-water communities contain more erect taxa (> 500 m). This confirms previous results from North Atlantic and Panamanian bryozoan communities [12,13], suggesting that this is a universal trend among cheilostomes. The shift from encrusting to erect taxa may be driven by decreasing disturbance with depth [12]. As depth increases, there is decreased current speed, bottom stress, and wave scour. In addition, there is a general reduction in the abundance of large grazers [44], which often consume bryozoan colonies as “bycatch” [45]. Erect taxa are more vulnerable to disturbance than encrusters since they extend into the water column. Indeed, erect taxa from shallow, fast-current habitats in this analysis tended to possess cuticular joints. Flexible joints allow erect colonies to withstand mechanical stress and ensure breakage occurs at the joint rather than the zooid-bearing internode [6]. In addition to disturbance, the relationship between colony form and depth may also be driven by increased sedimentation rates (due to slower currents) and reduced food availability. Erect growth provides greater feeding performance (clearance rate) than encrusting forms [46] and allows for gravitational shedding of sediment from subhorizontal surfaces [47] – making erect colonies well adapted for deep-water habitats.

In encrusting taxa, multilaminate growth was more common in shallow water environments. Multilaminate growth occurs when an encrusting colony has multiple layers of zooids from self-overgrowth or frontal budding [48] and together, these modifications increase the thickness of the colony edge. This provides a competitive advantage by making the colony difficult to overgrow [49–51]. Increased spatial competition in shallow, food-rich environments may drive the increase in multilaminate taxa. While current speed was unimportant, increased wave scour in shallow-water environments may also influence the presence of multilaminate encrusters. This trend may be linked to the increased presence of umbonuloid and lepralioid taxa in shallow water: these taxa possess hypostegal coeloms, which

facilitate the development of frontal budding and thus multilaminate growth [52]. However, multilaminate colonies can also result from self-overgrowth, which is not restricted to these taxa.

4.2 Polymorphism: Unlike previous studies [13,14], the RLQ analyses reveal a clear relationship between polymorphism and the environment. The most striking relationship is between the degree of brood chamber immersion into the autozoid and depth. For both encrusting and erect taxa, internal brooding and immersed ovicells are common in shallow-water communities, while external ovicells are common in deep-water. Deeper sites (most of which were between 200-2000 m) exhibited decreased oxygen concentrations (Data S2), suggesting that immersion of the brood chamber may be related to the availability of oxygen. Within the *Celleporella hyalina* species complex, the surface area of the ovicell is invariant to changes in oxygen concentration while the number of pseudopores in the ovicells increase in low oxygen conditions [53]. However, taxa that lack a plastic response to oxygen concentration (e.g. internal brooders lacking ovicells) may have high embryo mortality in oxygen-poor waters and be restricted from establishing populations.

The shift from external to internal brooding is a general evolutionary trend among Cheilostomes [26]. Internal brooding is less costly due to the reduced/absent calcified ooecium and provides greater protection for the developing embryo [26]. For example, some polychaete predators are able to suck out the embryo from external ovicells [54]. Internal brooding may also produce larger, more competitive larvae since it provides a larger cavity for embryo incubation [26]. Therefore, the ecological shift from external to internal brooding with decreasing depth may be driven by changes in predation and competition. Syllid polychaetes, which are most abundant and diverse in shallow environments with hard substrata [55], may be particularly impactful predators [45]. This would support previous work that suggested that zooid-level predation is the main driver of bryozoan polymorphism [13–15].

While the RLQ on encrusting and erect taxa suggests that spines are more common in areas with hard substrata, this simply reflects the higher prevalence of spines on encrusting species. This makes sense; since encrusters are more vulnerable to overgrowth competition than erect taxa, only encrusting species were found with more than ten spines (signifying heavier investment), and a significantly greater proportion of encrusters have jointed spines (two tailed z-test for proportions: $p < 0.001$, $n_{\text{erect}} = 65/211$, $n_{\text{encrusting}} = 187/385$).

When only encrusting taxa are considered, costal spines tend to be present in deep-water species rather than shallow-water species. Spines are typically thought of as defensive structures: they are an effective

deterrent against grazing nudibranchs [56], they can protect the frontal surface from abrasion [57], and many species produce spines to combat competitive overgrowth (despite not conferring a significant advantage [49,51,58]). Such spines may redirect or delay overgrowth, giving the losing colony a chance to reproduce or to grow into a spatial refuge. Costae are spines that lack a basal joint and are flattened over the frontal surface of the zooid (resembling ribs). This configuration of spines should defend against abrasion and predators that pierce the frontal membrane (like syllid polychaetes [59]). However, bryozoans have a variety of morphologies for protecting the frontal body wall. The cribrimorph frontal shield is composed of fused costae, and this structure is an evolutionary precursor for more-calcified frontal shields (gymnocystal, umbonuloid, and lepralioid) [60,61]. The more heavily calcified frontal shield morphologies of encrusters are more common in shallow environments (Fig. 4). This may be due to increased predation, wave-related abrasion, and calcium carbonate availability (Data S2) in shallow water. Such environmental factors should increase the benefit of producing a heavily calcified frontal shield. The depth-partitioning of different bryozoan taxa suggests a potential sequence of events where 1) cribrimorph morphology evolved (multiple times) under predation from small epibionts [15,61]; 2) continued predation pressure and abrasion led to the (repeated) evolution of taxa with calcified frontal walls; 3) such taxa out-competed most cribrimorphs in shallow habitats; 4) cribrimorphs survived in deeper refuges (200-2000 m) characterized by decreased predation and CaCO_3 availability. This series of events assumes that there are differences in predation between shallow and deep environments, and that ascophorans have a higher competitive ability than cribrimorphs (which may be inaccurate [51]). Syllid polychaetes, bryozoan-eating nudibranchs and Monodontin Trochidae gastropods are more common in shallow water [55,62–65]. However, bryozoan-eating pycnogonid genera can be found from 0-5000 m, and *Calliostoma* gastropods are found from 0-3000 m [66–69]. It is clear that more information on bryozoan predation is needed.

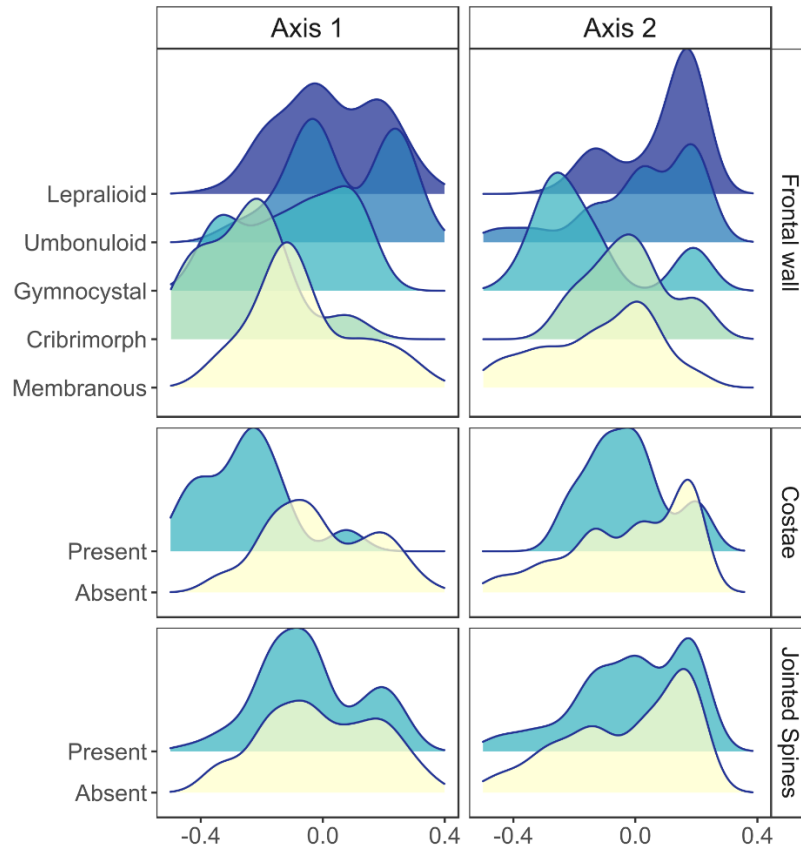


Figure 4. Density plots showing distribution of traits in encrusting taxa along the first two axes of the encrusting-only RLQ. Only presence of costae was included in the RLQ, and frontal wall morphology and the presence of jointed spines are just presented for comparison.

Most avicularian characteristics (including shape, size, and degree of integration) were not significantly related to the environment. However, in encrusting taxa, pivotbar structure appears to be related to seasonality. Encrusting taxa tend to lack avicularia in sites with high variability in SST and low benthic chlorophyll, while those encrusters in high-chlorophyll sites (high variability in surface chlorophyll but stable SST) tend to have avicularia with complete pivot bars. The absence of avicularia in encrusters appears to coincide with the absence of ovicells (Fig. S4), suggesting that monomorphic taxa are more suited for such food deserts. Bryozoans have an “energetically marginal” existence [70], and unnecessary or expensive polymorphs may be maladaptive where food is scarce. However, these monomorphic encrusters occur in the absence of hard substrata. These taxa (which include *Aetea*, *Electra*, and *Jellyella*, among others) probably encrust flexible, ephemeral substrata like eel grass and kelp. It makes sense that such short-lived taxa should invest in colony growth and swift reproduction rather than defensive structures like avicularia. Therefore, the presence/absence of avicularia is related to bryozoan life history

rather than environmental conditions. Any remaining pattern in pivot structure likely represents a phylogenetic signal: anascans tend to have condyles, while ascophorans tend to have pivotbars (with a few exceptions, like *Adeonellopsis*) [71].

Erect species with vicarious avicularia are more common in shallow-water environments (Fig. S5). However, any relationship between vicarious avicularia and depth is likely a phylogenetic signal. Of the ten erect taxa with vicarious avicularia, five were species in the genus *Cellaria*. This genus has avicularia with flexible joints that allow them to withstand mechanical stress from fast currents at shallower depths.

5. Conclusions:

Community assembly in cheilostome bryozoans is complex, and appears to be driven by environmental filtering, biotic interaction, and historical processes. 1) Colony form is strongly related to the environmental conditions: attachment mode (cemented vs rooted) is related to substratum type, while orientation (encrusting vs erect) and the presence of joints are related to a depth gradient (decreasing current speed and food availability, increasing sedimentation). 2) The degree of brood chamber immersion may reflect depth-related changes in oxygen concentration or predation. 3) Costae/cribrimorph frontal walls may relate to decreased CaCO_3 availability, wave-action, and predation with depth. 4) Morphology of avicularia was not related to environmental gradients and reflected potential phylogenetic signals. This suggests that morphology may be less important than potential chemical defenses [72] or plastic characters like the ratio of avicularia to feeding zooids. Finally, rootlets appear to be a key innovation that may have freed cementing taxa from hard substrata and contributed to the radiation of cheilostomes.

The pattern of bryozoan community assembly in New Zealand reflects a complex blend of evolutionary history, environmental conditions, and biotic factors (i.e. predation). These factors are somewhat partitioned by levels of organization, since colony form responds to environmental conditions, while the effects of evolutionary history, predation, and environmental conditions were not well-separated for zooid-level morphology. It must be stressed that trends found here are at the community-level (and exceptions certainly exist), and they need to be confirmed by phylogenetic analyses, in-situ environmental data, and information on the distribution of bryozoan predators in New Zealand.

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Appendix 3

Supplementary Information for:

Chapter 4: Community assembly in a modular organism: the impact of environmental filtering on bryozoan colony form and polymorphism

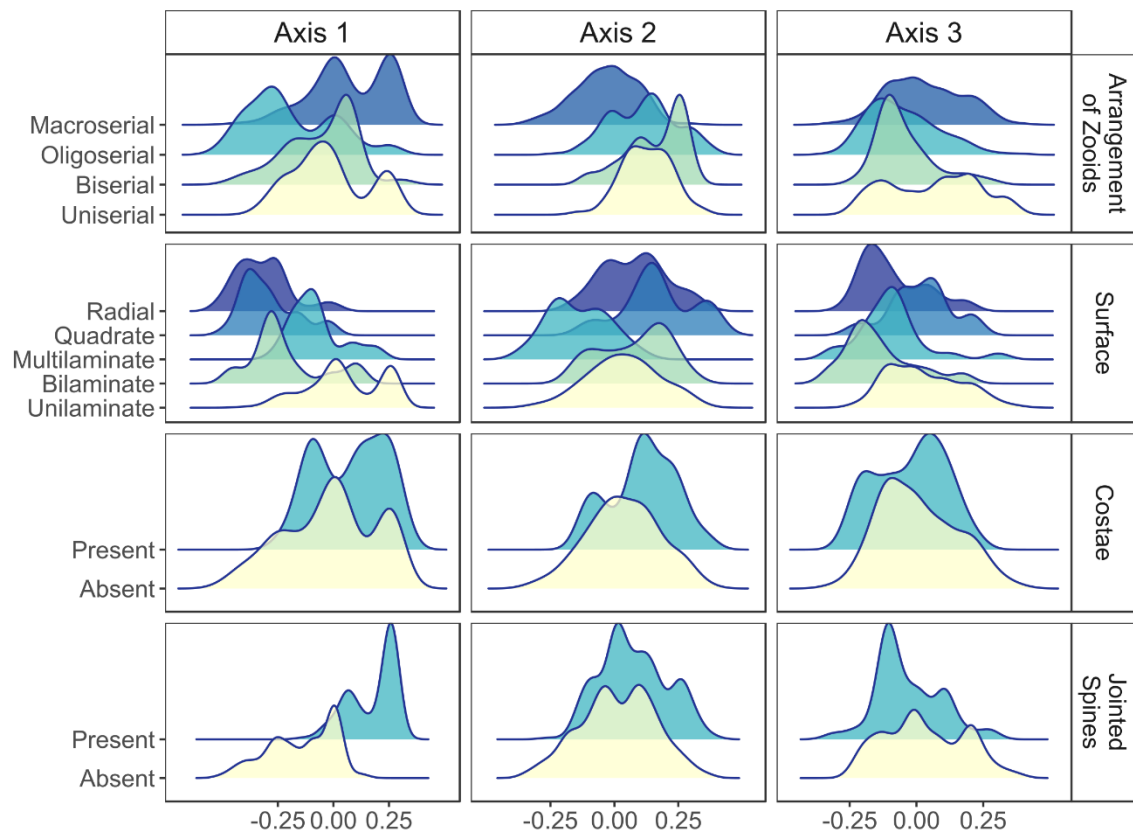


Figure S1. Density plots showing distribution of traits along axes 1-3 of the full RLQ.

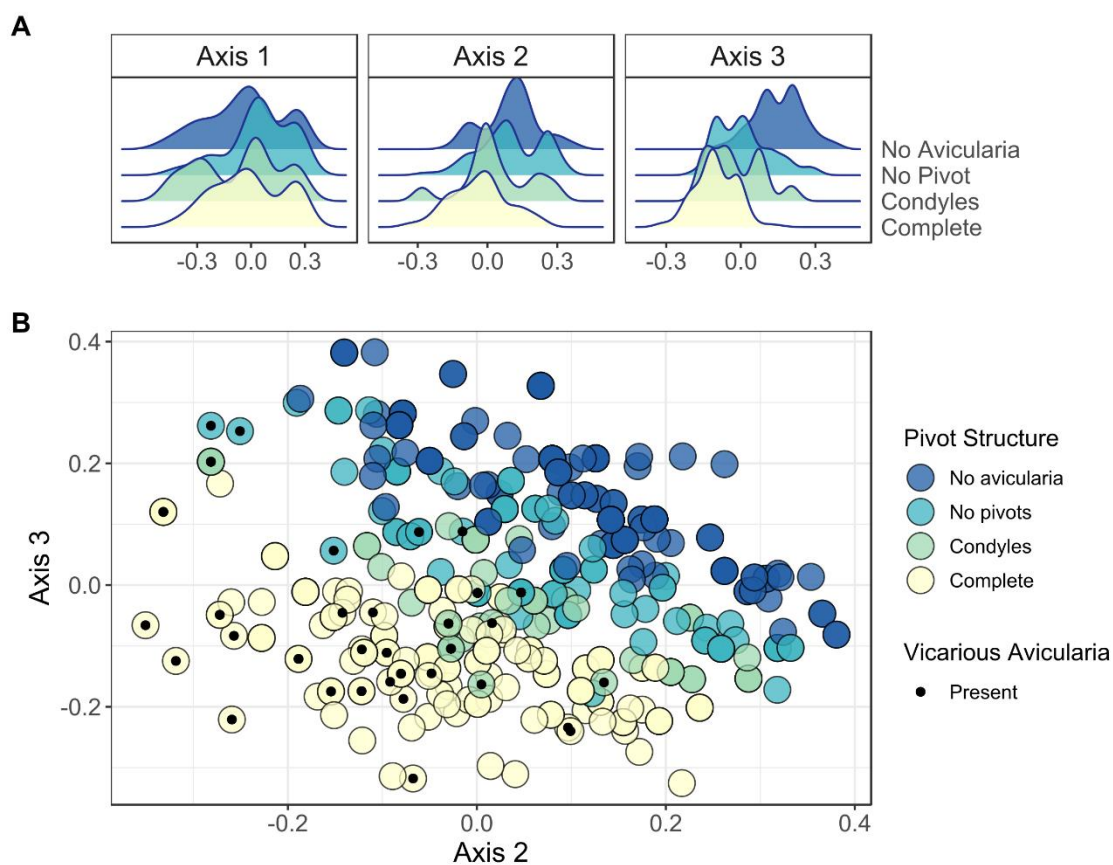


Figure S2. Pivotbar structure and the presence of vicarious avicularia for taxa in the full RLQ. A. Density plots showing the distribution of pivot structure along axes 1-3. B. Plot showing species scores along Axis 2 and Axis 3.

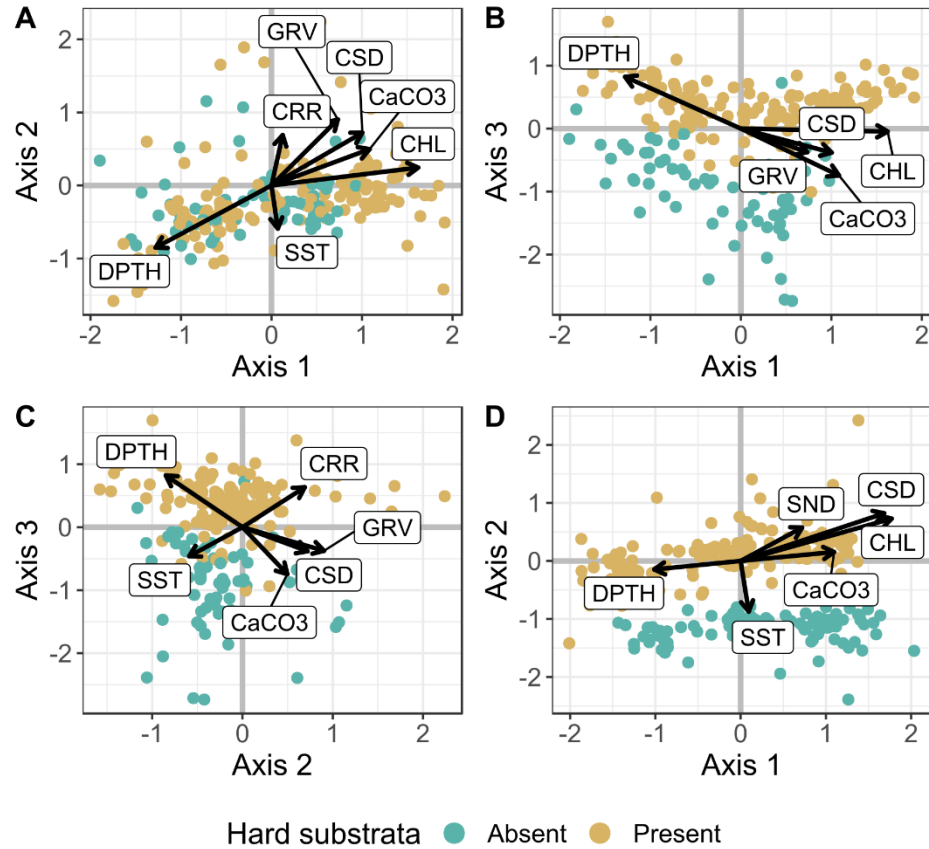


Figure S3. Biplots showing site scores for the erect-only RLQ (A-C) and the encrusting-only RLQ (D). Sites (points) are colored by the presence of hard substrata. Arrow length indicates the importance of environmental variables along each axis. For clarity, only environmental variables with a correlation of 0.3 or more on either axis were included. Abbreviations for environmental variables are as follows: CaCO₃, calcium carbonate concentration; CHL, benthic chlorophyll; CRR, current speed; CSD, standard deviation of surface chlorophyll-a; DPTH, depth; GRV, percent gravel; SND, percent sand; SST, sea surface temperature

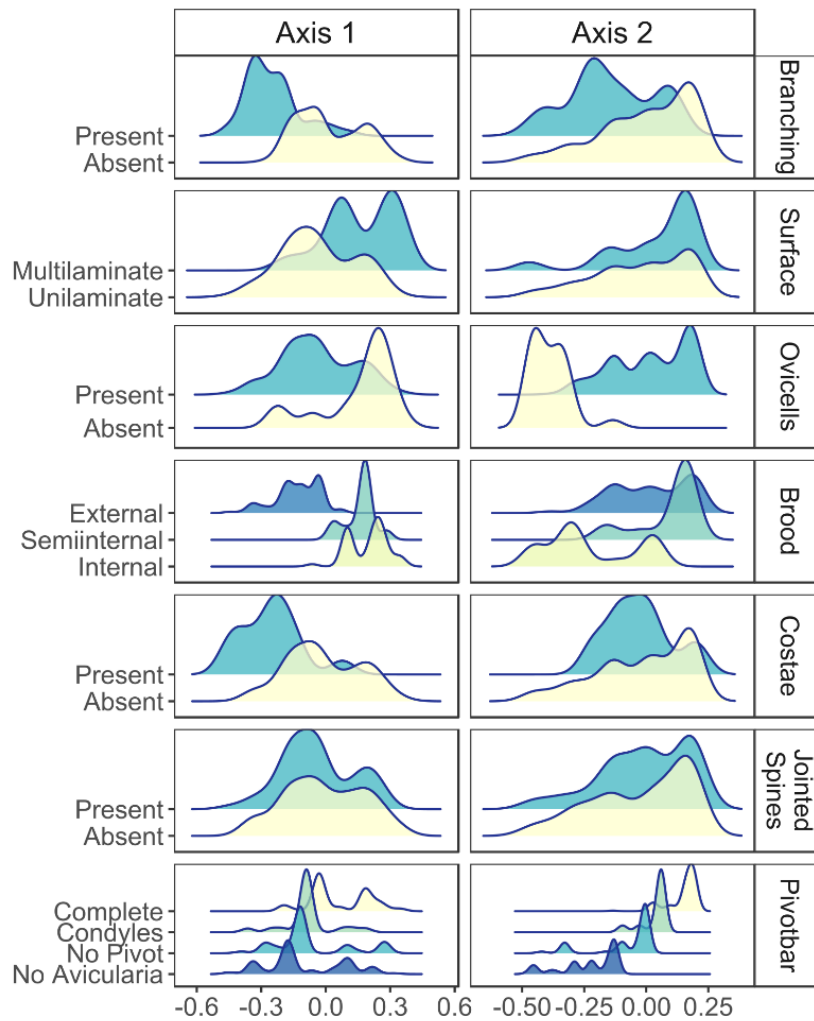


Figure S4. Density plots showing distribution of traits in encrusting taxa along the first two axes of the encrusting-only RLQ. Note that the presence of jointed spines was not included in the RLQ as a trait variable and is shown here only as comparison with costae.

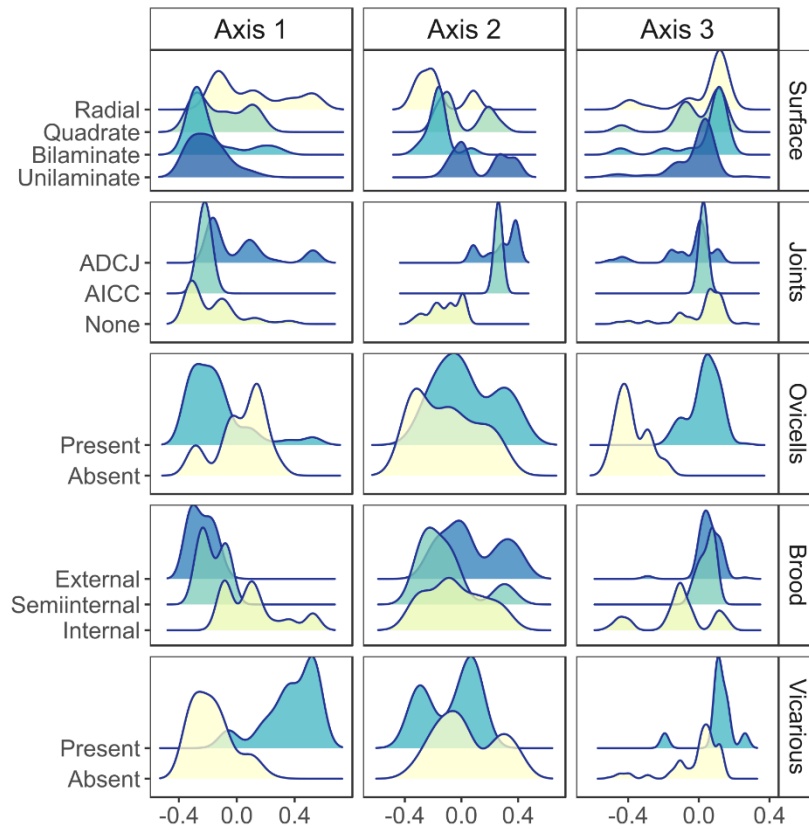


Figure S5. Density plots showing distribution of traits in erect taxa along the first three axes of the erect-only RLQ. Abbreviations for joints are as follows: AICC, articulated indeterminate cuticular connections; ADCJ, articulated determinate cuticular joints.

Table S1. Results of all three RLQ analyses, showing eigenvalues, and percent inertia (variance in species scores) explained, and simulated p-values from fourth-corner permutation tests.

Taxa set		<i>All Taxa</i>	<i>Erect Only</i>	<i>Encrusting Only</i>
# Species		642	144	302
# Sites		779	215	270
Total inertia		0.0052	0.0259	0.0040
Eigenvalues	Axis 1	0.0025	0.0149	0.0025
	Axis 2	0.0016	0.0055	0.0010
	Axis 3	0.0005	0.0028	0.0002
	Axis 4	0.0002	0.0010	0.0001
	Axis 5	0.0001	0.0008	0.0001
Projected inertia (%)	Axis 1	47.44	57.603	62.236
	Axis 2	31.48	21.431	24.831
	Axis 3	10.36	10.945	4.263
	Axis 4	3.14	3.976	3.781
	Axis 5	1.76	2.963	2.18
	Sum (1-3)	89.29	89.98	91.33
Permutation Test (p-value)	Environment	0.001	0.001	0.001
	Location	0.001	0.001	0.001

Data S1. Species data used in the analysis, including 1) description of traits; 2-3) sources for trait data; 4) species list with traits, including species scores on RLQ axes; 5) presence-absence of species at each station; 6) Results of monte-carlo permutation tests for each trait; 7) relationships of traits to RLQ axes.

Data S2. Environmental data used in the analysis, including 1) description of environmental variables; 2) data sources; 3) acknowledgements for specific NIWA cruises; 4) environmental conditions at each station, including site scores on RLQ axes; and 5) relationships of environmental variables to RLQ axes.

Chapter 5: Cheap labor and high evolvability: A lack of strong developmental constraints on the mandible shape of avicularia in New Zealand bryozoans

Abstract

Despite the diversity of life on earth, many morphologies have not been achieved. Morphology can be limited by a variety of constraints (developmental, historical, biomechanical) and comparing the distribution of realized forms in a theoretical form-space (i.e. “morphospace”) can highlight which constraints are at play and potential functions. If traits cluster around biomechanical optima, then morphology may be shaped by strong selective pressures. In contrast, a well-explored (filled) morphospace suggests weak constraints and high morphological evolvability. Here, constraints on morphospace exploration were examined for 125 cheilostome bryozoan species from New Zealand. The mandible morphospaces for avicularia (beak-like polymorphs) were visualized using Coordinate-Point Extended Eigenshape analysis. Mechanical advantage, moment of inertia, drag, peak force, and rotational work required to close the mandible were calculated for theoretical (n=47) and real mandibles (n=224) to identify biomechanical optima. The volume and surface area of the parcel of water passed through by the closing mandible (referred to as the “domain”) was also calculated. The theoretical morphospace of avicularia is well-explored, suggesting they are highly evolvable and have relaxed developmental constraints. However, there may be constraints within lineages. A well-developed fulcrum (complete pivot bar) may be an evolutionary pre/corequisite to evolving mandibles with extreme moments of inertia such as setose and highly spathulate forms. The most common mandible shape, triangular, represents a trade-off between maximizing domain size, minimizing energetic cost (force and construction material), and minimizing the potential for breakage. This suggests that they are well suited for catching epibionts, representing the first empirical evidence for avicularian function. Tendon length and mechanical advantage are limited by tendon width, which itself is constrained by the base width of the mandible. This explains the low mechanical advantage of setose mandibles and suggests that they are unable to grasp epibionts. The calories required to close the mandible of an avicularium (estimated from rotational work) are quite small (1.24×10^{-16} to 8.82×10^{-11} cal).

1. Introduction

Despite the stunning array of phenotypes present in both living and extinct faunas, many theoretical phenotypes are unrealized. Differences between theoretical and empirical morphospaces can be caused by sampling artefacts, evolutionary history, adaptive peaks, or biomechanical and developmental constraints [1,2]. Traits may have constrained morphospaces owing to coupling with other traits. For example, beak shape in raptorial birds is strongly constrained by braincase morphology [3]. Modular organisms may be able to occupy a larger proportion of the theoretical morphospace than unitary taxa. Modularity uncouples traits, allowing them to evolve separately. Foremost among modular organisms are the Bryozoa. These colonial, sessile suspension feeders are composed of individual units (zooids). Nutrient transduction between zooids allows feeding zooids (autozooids) to support specialized polymorphic zooids [4]. While polymorphic zooids are constrained by the same evolutionary starting point (an autozoid), a wide array of morphologies has arisen [4].

The most striking and enigmatic polymorph is the avicularium of cheilostome bryozoans. Darwin described these structures as “curiously [resembling] the head and beak of a vulture in miniature” that were able to “seize [a needle] so firmly that the [colony] branch can thus be shaken”[5]. Not all avicularia resemble bird heads. Nevertheless, there is a comparable diversity of “beak” shapes in avicularia, from spatulate forms resembling spoonbills, to bristle-like forms that mirror the beaks of sword-billed hummingbirds. As in birds, the extensive morphology of avicularia hints at a variety of functions. Colony defense (both mechanical and chemical), waste-water movement, cleaning, and locomotion have all been proposed [6]. Avicularia have a patchy record in terms of mechanical defense: they perform well against gammaridean amphipods [7] and nematodes (L. Vieira, pers. comm 2019), but poorly against pycnogonids [8] and nudibranchs [6]. It is unknown how efficient avicularia may be at performing other functions.

Avicularia are modified autozooids: the articulated mandible and adductor muscles are derived from the operculum and its occlusor muscles, respectively [9]. Unlike autozooids, avicularia have a severely reduced polypide and are unable to feed (with the exception of B-zooids)[4,10,11]. This means that investment in an avicularium has four potential costs: 1) the loss of a feeding autozoid, 2) cost of material construction, 3) cost of passive upkeep, 4) cost of operation. The loss of a feeding zooid can be avoided by switching from vicarious avicularia (which takes the place of a zooid in the colony) to interzooidal or adventitious avicularia (small structures that rest between or on the autozoid surface). The energetic costs associated with construction, upkeep, and operation are unknown, but the latter can be estimated.

Biomechanical analysis of avicularian mandibles can highlight potential trade-offs (e.g. force vs velocity), predict adaptive peaks and troughs for different functions, and describe impossible morphologies.

In this analysis I use geometric morphometrics and biomechanical analyses to determine the constraints on the morphological variation of avicularian mandibles in New Zealand cheilostomes. A patchy morphospace distribution indicates developmental, evolutionary, or biomechanical constraints. If the “patches” align with biomechanical optima then selection may constrain avicularian morphology. If instead the patches appear to be random, then variation in mandible structure may be constrained by lineage (i.e. a random walk through the morphospace, contingent on the morphology of the ancestral operculum and subsequent mutations), or developmental mechanisms (e.g. continuous genotype mapped to discontinuous phenotype) [2,12]. While patchy morphospace distributions are most common [1,2,12], other distributions are possible. For example, a thorough exploration of the theoretical morphospace would suggest weak developmental and evolutionary constraints. Such a distribution would imply that avicularian mandibles are highly evolvable, and differences in the frequency of certain morphologies may indicate directional selection if they align with biomechanical optima. Finally, I use biomechanical analysis of mandibles to better understand the function of avicularia and the energetic cost to the bryozoan colony.

2. Methods

2.1 Samples and image preparation

Bryozoan specimens were obtained from the National Institute of Water and Atmospheric Research (NIWA) Invertebrate Collection, a full list of specimens is available in the Supplementary Materials. The majority of sample were air dried following preservation in ethanol.

Avicularia were photographed using an Hitachi Tabletop 3000 Scanning Electron Microscope (SEM). Unbleached specimens were sputter-coated with gold-palladium to prevent charging. Avicularia were positioned so that the broad side of the mandible was perpendicular to the electron beam. Two-dimensional outlines of three-dimensional shapes are sensitive to viewing angle, so a constant angle ensures that outline comparisons are valid.

Five mandibles of each type of avicularium present in each specimen were photographed, although this was not always possible for rare avicularia. Mandibles were not photographed if they appeared strongly curled or shrunk, though some shrinkage was expected from air-dried specimens. If the specimen lacked mandibles, the rostrum was photographed instead. Rostra that diverge from mandible shape (as in

Bryopesanser pesanseris) and rostra with an open tip (which indicates that the mandible protrudes beyond the rostrum) were excluded from the analysis.

This resulted in photographs of 855 mandibles and rostra from 129 species (184 specimens). Each mandible was traced by hand in Inkscape using a Wacom tablet (Intuos4, model PTK-440) and exported as PNG images (Fig. 1A-D). To estimate error associated with tracing, the same photograph was traced at the start of each drawing session (resulting in 29 outlines).

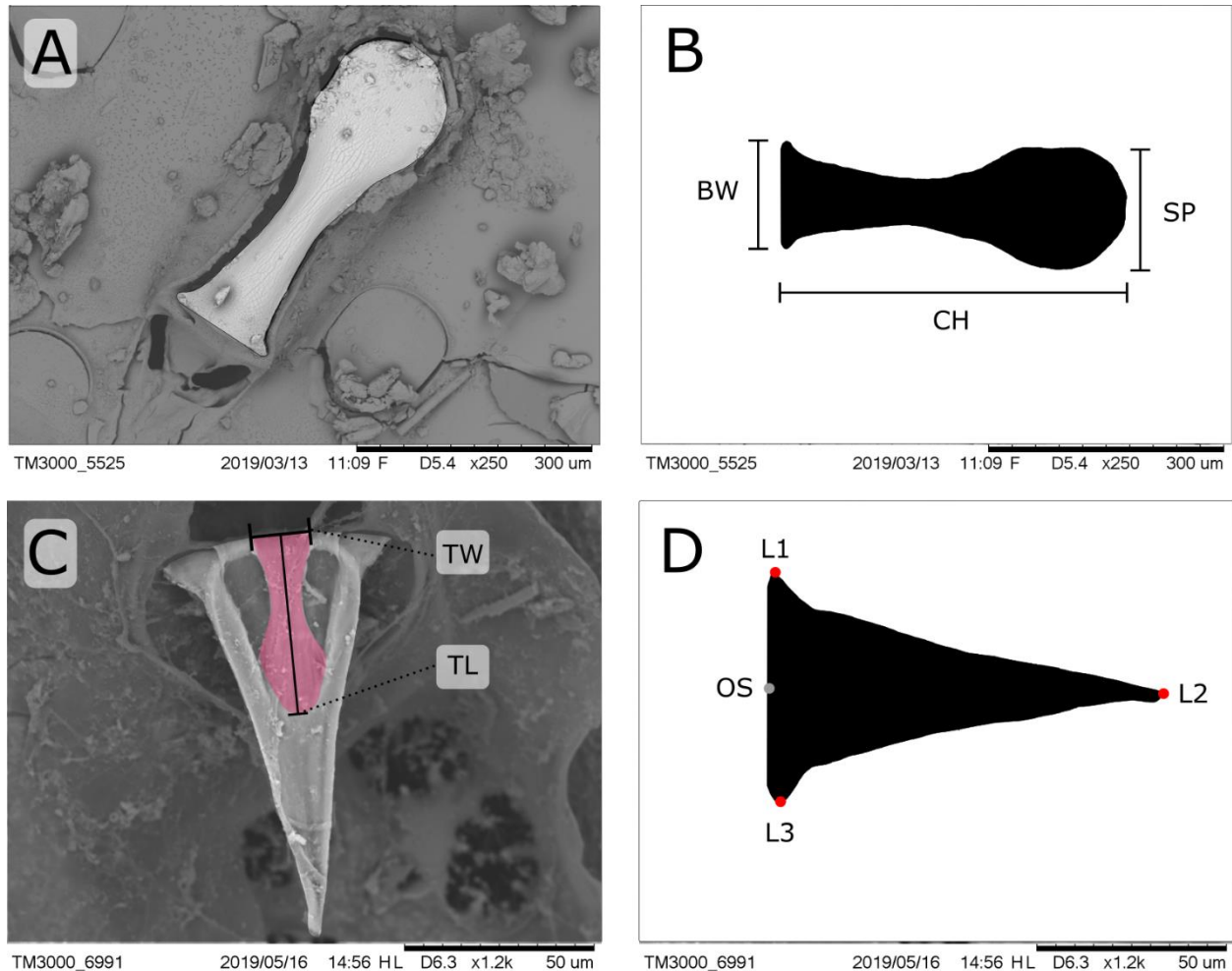


Figure 1: Image preparation, morphometrics, and landmarking of avicularia. SEM images of mandibles (A) were traced by hand, set to black, rotated so that mandible base was vertical, and placed on a white background while preserving the scale bar. Measurements (base width BW, maximum span SP, and chord length CH) were then taken on the traced mandible in ImageJ (B). The tendon (highlighted in pink) was measured on SEM images from the mandible base to the maximum point of attachment for tendon length (TL) and at the mandible base for tendon width (TW) (C). The position of landmarks (L1-L3) and the starting position for the digitized outline (OS) of the traced mandible are as shown (D). A-B show a vicarious avicularium of *Euthyroides jellyae*, while C-D show an adventitious avicularium of *Adeonellopsis sulcata*. Contrast is enhanced on A and C for visual clarity.

The mandible area, chord length (maximum length), span (maximum width), and base width of the PNG outlines were measured in ImageJ (Fig. 1B). Tendon lengths and widths were measured from the original SEM photographs (Fig. 1C), though tendons were only visible for 224 avicularia.

2.2 Geometric morphometrics

Coordinate-point extended eigenshape analysis (CP-EES) is a morphometric technique that provides an objective comparison between shapes. CP-EES constrains shape outlines using landmarks (which can represent evolutionarily or functionally homologous points) [13]. In other words, the landmarks divide the outline into sections that can be compared across shapes. The detail in these sections can then be “weighted” by the changing number of outline coordinates allowed. In CP-EES, outline coordinates and landmarks are aligned using Procrustes generalized least squares superimposition. Shape differences are then given as the variation from the mean shape along a series of eigenshape axes. A full description of CP-EES is given in MacLeod [13]. This analysis produces axes of shape variation, scores mandible shapes along each axis, and generates a theoretical morphospace.

Outlines and landmarks were digitized for all 884 PNGs in using tpsUtil and tpsDIG (version 1.40) software by F.J. Rohlf. Since avicularia are thought to have evolved multiple times, evolutionarily homologous landmarks cannot be chosen. Instead, three landmarks were chosen based on shape homology: L1, left corner of the mandible base; L2, mandible tip (the furthest part of the mandible along its center line); L3 right corner of the mandible base (Fig. 1D). Outline segments L1-L2 and L2-L3 had 250 coordinates each. Variation at the base of the mandible was not of interest, so only 50 coordinates were used for segment L3-L1. After shape digitization, CP-EES was performed using Mathematica notebooks provided by J. Kreiger [14].

2.3 Mechanical Advantage

Avicularia are closed by adductor muscles, which are attached to the mandible by either one or two tendons. The tendon of the adductor muscle is attached to the mandible in between the hinge (fulcrum) and the tip of the mandible (Fig. 1C). This construction is a third-order lever, which operates on the same principle as a hinged door. The mechanical advantage of a lever can be calculated by

$$MA = \frac{F_{out}}{F_{in}} = \frac{d_{in}}{d_{out}} \quad (1)$$

where F_{in} and F_{out} are the input (applied) and output (resulting) forces, and d_{in} and d_{out} are the distances from those forces to the fulcrum. In a third-order lever d_{in} is always less than d_{out} , so mechanical advantage is always less than 1 ($F_{out} < F_{in}$). The force needed to move the lever is minimized when d_{in} is close to d_{out} (which is why door handles are placed at the edge of the door, rather than close to the hinge). Mechanical advantage was calculated using equation (1) for the 224 mandibles where tendon length could be measured. This represents the ideal mechanical advantage, which does not account for differences in deformation of the fulcrum or friction between the mandible and the fulcrum. Multiplying peak input force (applied force at the point of tendon attachment) by mechanical advantage provides an estimate of the ideal output force (at the tip of the mandible).

2.4 Moment of inertia

The moment of inertia is the resistance of an object to rotational motion. This property depends on the distribution of mass perpendicular to the axis of rotation. The more mass there is farther from the axis of rotation, the greater the moment of inertia. Therefore, a spatulate mandible will have a greater moment of inertia than a triangular mandible of the same area and mass distribution. The moment of inertia for each mandible about its axis of rotation was approximated with the formula:

$$\rho_m h \sum_{i=1}^n A_i r_i^2 + A_{i+1} r_{i+1}^2 + \dots A_n r_n^2 \quad (2)$$

Where ρ_{man} is the density of the mandible, h is the thickness, A_i is the area of a thin slice i , and r_i is the distance of the slice from the axis of rotation, and n is the total number of slices used in the approximation. In this analysis 100 slices were used to reduce computing time.

To simplify calculations, density and thickness were assumed to be constant throughout the mandible (though this is unlikely to be the case for most taxa). The density of avicularian mandibles is unknown, though they are strongly cuticularized (i.e. a proteinaceous mucopolysaccharide with chitin fibrils) [15,16]. In phylactolaemate bryozoans, chitin is similar in structure (α -chitin) and surface morphology to insect chitin, though it has a crystalline index (ratio of crystalline to amorphous matter) that is closer to that of decapods [17]. Low-density decapod cuticle is around 1.39-1.43 g/cm³ (uncalcified, near the density of pure α -chitin: 1.400 -1.425 g/cm³), while high-density decapod cuticle ranges from 2.06-2.34 g/cm³ (high calcification, closer to the density of pure calcite: 2.71 g/cm³) [18]. Insect cuticle has a similar density to uncalcified decapod cuticle (1-1.4 g/cm³) [19,20]. While the operculum can be calcified in some taxa [15], calcified avicularian mandibles have not yet been reported. Therefore, the density of avicularian

mandibles was assumed to be 1.4 g/cm³. While the operculum can be calcified in some taxa [15], calcified avicularian mandibles have only been reported in the B-zooids of *Macropora* and *Monoporella exsculpta* [21].

2.5 Torque, drag, and work

Torque is the rotational equivalent of force, and can be calculated from in two ways:

$$\tau_{net} = I\alpha \quad (3)$$

$$\tau = F * r * (\sin \theta) \quad (4)$$

Where I is the moment of inertia (kgm²), α is the angular acceleration (radians/second²), and t is the time (seconds), F is the force, r is the distance from the applied force to the axis of rotation, and θ is the angle in radians between the applied force and the mandible. Only force applied perpendicularly to the axis of rotation generates torque (which is why you cannot open a door by pushing on its edge). To calculate the torque generated by the adductor muscles (applied torque), drag torque (which is negative) is subtracted from the net torque. This assumes that gravitational torque acts parallel to the axis of rotation (and is thus equal to zero) and that the acceleration reaction is nominal.

Displacement, angular velocity and angular acceleration need to be estimated in order to calculate net torque and drag. The maximum displacement of a living avicularium is 90° (1.57 radians)—any greater than that and the tendon wraps around the hinge [7]. However, displacement angle does not change linearly with time (i.e. angular velocity is not constant). Rotational motion in ant mandibles, copepod legs, and brachiopod shells follows a logistic curve [22–24], which can be described with the equation:

$$\varphi = \frac{b}{(1 + e^{(-k(x-c))})} \quad (5)$$

Where b is the maximum displacement in radians (1.57), x is the time, φ is the displacement at time x , c is the center of the curve, and k is the steepness of the curve. Equations for velocity and angular acceleration can then be found by taking the first and second derivatives of Equation 5, respectively:

$$\omega = \frac{kbe^{(-k(x-c))}}{(1 + e^{(-k(x-c))})^2} \quad (6)$$

$$\alpha = \frac{2bk^2(e^{(-k(x-c))})^2}{(1 + e^{(-k(x-c))})^3} - \frac{k^2be^{(-k(x-c))}}{(1 + e^{(-k(x-c))})} \quad (7)$$

Where ω is the angular velocity (rad/s) and α is the angular acceleration (rad/s²). Retraction of the bryozoan tentacle crown takes 60-80 milliseconds [25], so avicularia may take a similar time to close. This results in low angular velocities (44.07- 58.75 rad/s; Table 1), and it is possible that avicularia close considerably faster. The P5 legs of the copepod *Cylcops scutifer* have a peak angular velocity between 480-610 rad/s and are ~200 μ m long [26] (equal in size to some of the larger avicularia). To achieve peak angular velocity ~480 rad/s, an avicularium would have to snap shut in under 8 milliseconds (Table 1). Both slow (60-80 msec) and fast (8 msec) closing speeds were included in this analysis to compare the relative energetic cost associated with each.

Table 1: Estimated constants (c & k) for equations 5-7 and resulting values for peak angular velocity (ω) and angular acceleration (α). Note that c is also the time of peak angular velocity.

Time (s)	c	k	ω (rads/s)	α (rads/s ²)
60×10^{-3}	30×10^{-3}	149.6218	58.7563	3,383.741
80×10^{-3}	40×10^{-3}	112.2163	44.0672	1,903.353
8×10^{-3}	4×10^{-3}	1122.163	440.672	190,331.2

Drag is influenced by the size and shape of avicularia. For linear motion, the force owing to drag can be calculated using the equation

$$F_{drag} = \frac{1}{2} \rho v^2 * C_d * A \quad (8)$$

Where ρ is the density of the fluid, v is the velocity, C_d is the coefficient of drag, and A is the area. In rotational motion, velocity of each point of the mandible becomes the cross-product of the distance from the axis of rotation (r) and the angular velocity (ω). Since the fluid motion is perpendicular to the mandible surface, the cross product is simply $r * \omega$ (i.e. the arc length traveled by each slice during mandible rotation). The change in velocity is integrated over the surface of the mandible to find drag force:

$$F_{drag} = \frac{1}{2} \rho * C_d \int_{r=0}^r (r\omega)^2 * A dr \quad (9)$$

where ω is the angular velocity in radians/second and r_0 is the maximum distance from the axis of rotation (i.e. total length of the mandible). As with moment of inertia, force can be approximated by

$$F_{drag} = \frac{1}{2} \rho * C_d \sum_{i=0}^n (r_i \omega)^2 * A_i + \dots (r_n \omega)^2 * A_n \quad (10)$$

Where A_i is the area of a thin slice i , and r_i is the distance of the slice from the axis of rotation, C_{di} is the coefficient of drag, and n is the total number of slices used in the approximation (in this case, $n = 100$).

Drag due to torque is calculated by substituting Equation 4 into Equation 9. Since drag is perpendicular to the surface of the mandible, $\sin(\theta)$ becomes 1, giving the equation

$$\tau_{drag} = \frac{1}{2} \rho * C_d \int_{r=0}^r (r\omega)^2 * A * r dr \quad (11)$$

The resulting drag torque can then be subtracted from net torque (Equation 4) to calculate applied torque. Note that drag torque operates in the opposite direction to the applied torque and will be the opposite sign.

The coefficient of drag is determined by shape, angle of incidence, and Reynolds number (Re). Usually this property is calculated empirically from drag measurements. While shape is important for angles of incidence less than 90°, the shape of a flat plate normal to flow does not strongly influence the coefficient of drag [27,28]. Higher aspect ratios do increase the coefficient of drag, but these changes are gradual until an aspect ratio of ~1:10 [27,29]. Therefore, differences in the coefficient of drag owing to aspect ratio are assumed to be nominal. There is no formula for the coefficient of drag that encompasses all Reynolds numbers, so here we use equation 10.1 from Imai [30] for $Re \leq 5$, and equation 7.2 from Tamada & Miyagi [31] for $Re \geq 20$. A monotonic cubic spline was then used to determine the coefficient of drag for $5 < Re < 20$. The coefficient of drag depends on Reynolds number, which is found using the formula:

$$Re = \frac{\rho * (r_c \omega) * L}{\mu} \quad (12)$$

Where ρ is the density of the fluid, μ is dynamic viscosity of the fluid, L is the characteristic length (in this case, the total length of the mandible), and r_c is the distance from the mandible centroid to the axis of rotation. Angular velocity changes over time, so the Reynolds number, the coefficient of drag, and the torque due to drag also change over time.

To find the rotational work when torque is variable, torque must be integrated over the angular displacement:

$$W_{app} = \int_0^{\phi_{max}} \tau_{app} d\phi \quad (13)$$

Where ϕ is the displacement in radians. The rotational work applied by the adductor muscles represents the energetic cost of operating the mandible.

The force applied by the adductor muscles is also of interest. This can be calculated by:

$$F_{app} = \frac{\tau_{app}}{r_t \sin \theta} \quad (14)$$

Where r_t is the tendon length and θ is the angle between the tendon and the mandible (in radians). The angle θ increases from $<1^\circ$ to 90° as the mandible closes (i.e. as ϕ increases from 0° to 90°) (22) and is assumed to be equal to ϕ .

The applied force and torque calculated here do not account for shear stress or rolling friction of the mandible against the hinges. Rolling friction is likely greater for avicularia with complete pivot bars than it is for those with condyles, since pivot bars provide a greater area of contact with the mandible. However, surface area of pivot structures was not measured and the coefficient of friction for different pivot structures is unknown.

2.6 Water displacement

It is thought that some avicularia serve to direct water over the surface of the colony. In this case, the volume of water displaced by the movement of the mandible can be calculated as surface of rotation.

$$V = \int 2\pi r * h * dx \quad (12)$$

Essentially, each slice (parallel to the axis of rotation) is rotated around the axis of rotation 360° , at some radius r from the axis. To get the volume of the ring (or “shell”) the slice passes through, the height h and depth dx of the slice are multiplied by the circumference of the circle the slice travels ($2\pi r$). This can be approximated by taking a finite number of thin slices instead of integrating. Since the mandible does not actually rotate 360° , the volume is multiplied by the mandible gape rather than 2π :

$$V = \frac{\phi * \pi}{180} \sum_{i=1}^n 2\pi r_i * A_i + \dots 2\pi r_n * A_n \quad (13)$$

Where A_i is the area of thin slice parallel to the axis of rotation (defined as the height h multiplied the depth d), ϕ is the gape of the mandible in degrees, and n is the number of slices. This can then be divided by the total area to determine the water displacement per area.

2.7 Surface area of water parcel

The parcel of water that the avicularian mandible passes through can also be defined in terms of its surface area. The surface area to volume ratio of the water parcel may reflect the function of the avicularium. This can be calculated as

$$S = \int 2\pi(r_1 - r_2) * ds \quad (14)$$

In contrast to volume, surface area is estimated by taking slices *perpendicular* to the axis of rotation. These slices, when rotated 360°, form a frustrum (i.e. a cone without the tip). The frustrum is defined by the radii of its top and bottom circles (r_1 and r_2) and the length of the slant between the two (ds). The lateral surface area of each frustrum is summed to find the total surface area. This can be approximated with finite slices:

$$S = \frac{\varphi * \pi}{180} \sum_{i=0}^n \pi(r_{1i} - r_{2i}) * \sqrt{(r_{1i} - r_{2i})^2 + h^2} + \dots \pi(r_{1n} - r_{2n}) * \sqrt{(r_{1n} - r_{2n})^2 + h^2} \quad (15)$$

Where h is the height of each slice (constant for each mandible).

2.8 Data used in calculations

Moment of inertia was calculated for 244 avicularia mandibles and 47 mandible shapes generated by CP-EES. Surface area of the water parcel were only calculated for the 47 mandible shapes generated by CP-EES. Input force, drag, mechanical advantage, crushing force, volume of water displacement, and rotational work were calculated for 224 avicularia for closing speeds of 60 and 80 milliseconds. The density and dynamic viscosity for seawater at 0°C (1028 kg/m³ and 0.00188 kg/m-s, respectively) were used to calculate Reynolds number and drag. All calculations were performed in R ((version 3.5.2; Data S2-S11), and graphs were made using ggplot2 and cowplot [32,32].

































































3. Results

3.1 Morphospace of avicularia

The first eight eigenshape axes of the CP-EES explained 98.98% of the shape variation in avicularian mandibles (Table 2). Overall, the theoretical morphospace generated by the first two axes is well-explored by actual bryozoans (Fig. 2). Note that there is a small gap between heavily spathulate and moderately spathulate forms where no intermediate avicularia were found (Fig. 3). Mandibles with moderate aspect ratios (1:2) and relatively straight sides were the most common in the sample. Unexplored shapes were either biologically impossible (mandible edges cross over), low-aspect ratio triangles, or heavily bilobed.

While morphospace exploration was broad for the whole sample, setose and highly spathulate mandibles only appeared in species with complete pivot bars (Fig. 3). This supports a preliminary analysis that suggested that rounded mandibles are more common in taxa with complete pivot bars (two tailed z-test for proportions, $p < 0.001$, $n_{\text{pointed}} = 324$, $n_{\text{rounded}} = 250$ species).

Table 2: The first eight eigenshape axes from CP-EES, visualized at size points along each axis. Degree of variation from the mean shape (0) is illustrated by overlaying the other shapes along a particular axis.

Axis	Variance Explained	Shape variation								Interpretation
		-3	-2	-1	0	+1	+2	+3	Overlay	
ES1	78.59%									High to low aspect ratio
ES2	12.06%									Hastate to spatulate
ES3	2.65%									Degree of and direction of curvature
ES4	2.31%									Parallel and pointed to concave and rounded
ES5	1.61%									Covex and pointed to concave and broad
ES6	1.06%									Degree of base slant
ES7	0.35%									Concave and pointed to convex and bifurcated
ES8	0.35%									Sharp base transition to smooth transition

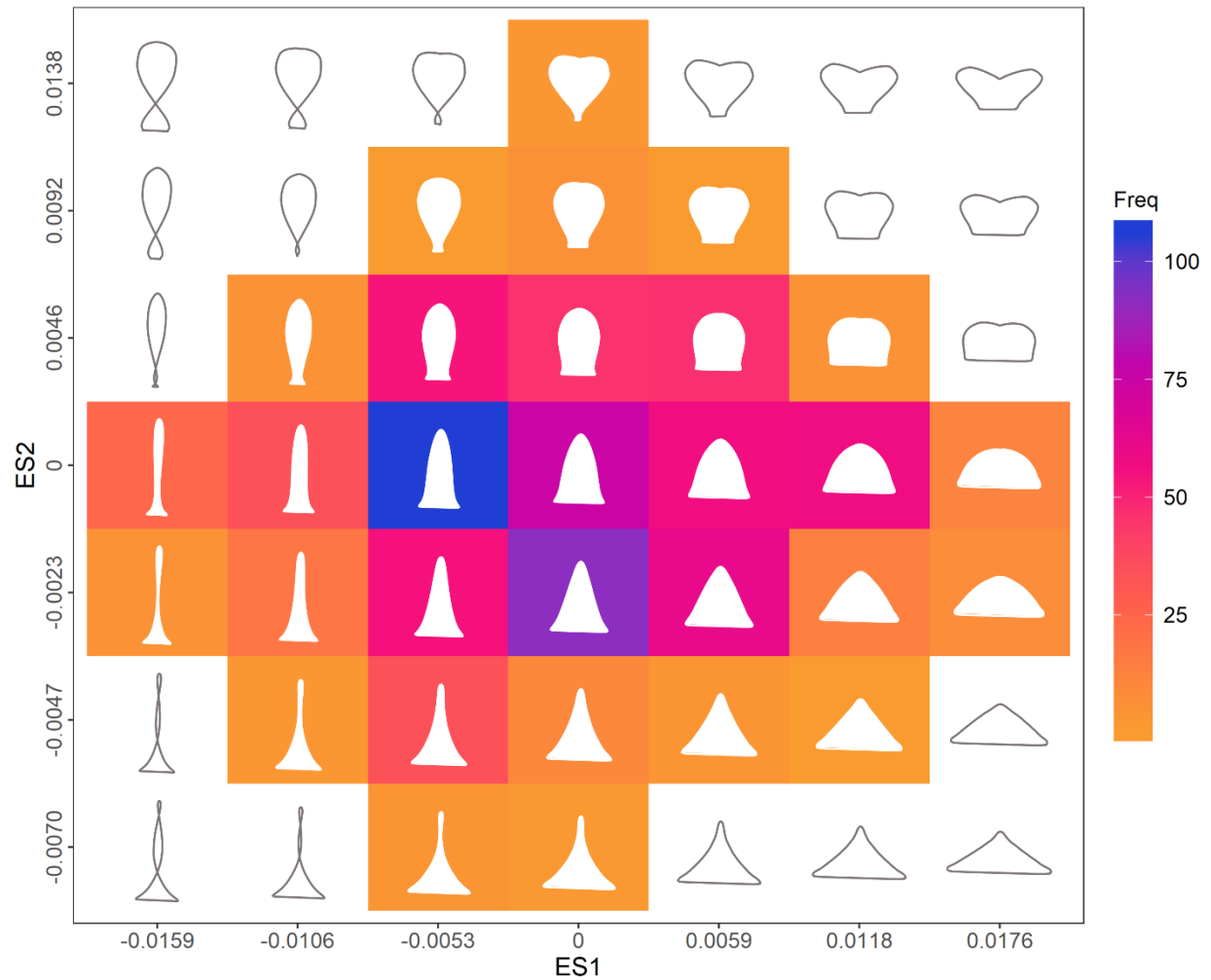


Figure 2: Heatmap of model shapes generated by the CP-EES along the first two eigenshape axes, showing the frequency of mandible shape in the sample (854 mandibles). Some shapes are biologically impossible (mandible edges cross over), while others were not found in the sample (e.g. bilobed mandibles). Note that the shape axes are not equally spaced. For example, there is more variation in the positive direction along ES2 than the negative direction.

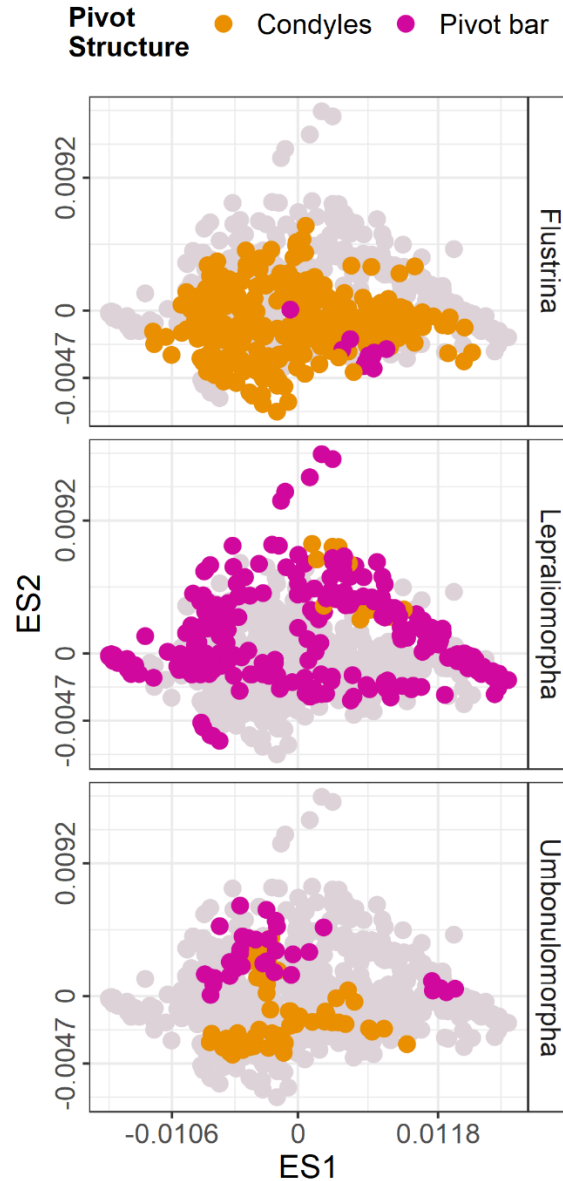


Figure 3. Pivot structures of three suborders (Flustrina, n= 395 avicularia; Lepraliomorpha, n=277; Umbonulomorpha, n=94) along the first two axes of the CP-EES. Grey circles show the extent of the full sample, including the suborders Acanthostega (n=46), Hippothoomorpha (n=6), and Thalamoporellina (n=7).

3.1.2 Tracing uncertainty

Tracing replications produced a standard deviation between 3.95 pixels (width) and 13.90 pixels (length), resulting in an uncertainty of 1-2%. This translates to an uncertainty of 2.07–7.28 μm for low resolution SEMs (low pixel: μm ratio) and 0.62–2.17 μm at high resolution. The majority of SEMs were at a high resolution (5.0 ± 3.45 pixels: $1\mu\text{m}$). The standard deviation along the first eight axes of CP-EES was below

7.4×10^{-5} for all axes, which is well below the range of axis values across the whole sample. These results indicate uncertainty associated with tracing is small enough to ignore.

3.2 Biomechanics

3.2.1 Moment of inertia

Comparing the inertia of mandible shapes generated by CP-EES reveals the relative cost of each shape. When area is constant, setose mandibles (high scoring on ES1) have the highest moment of inertia, but within shapes of equal aspect ratio (constant score on ES1) spathulate mandibles have the highest moment of inertia due to their distribution of area (Fig. 4). For example, triangular and hastate mandibles require 1.47-times and 2.02-times *less* force than a spathulate mandible of equal area. Closing a setose mandible requires 4.96-times *more* force than a spathulate mandible of equal area.

Moment of inertia scales faster with length than area (equation 2). Therefore, increasing the area of an elongate (setose) mandible results in a greater moment of inertia than the same increase in area for a squat (lunate) mandible (Fig. 5A). When the increase in length is held constant, squat mandibles have greater moments of inertia due to their large area (Fig. 5B). Changing mandible shape with increasing size (allometry) can reduce energetic cost, but isometric changes may be biologically or evolutionarily easier than allometric ones.

Perhaps unsurprisingly, lunate and setose mandibles only appear at certain length ranges (Fig.6). This likely reflects structural requirements: a tiny setose mandible would be quite fragile (minimum width), while a huge lunate mandible may take up too much space in the colony (maximum width). Highly spathulate mandibles are confined to a small range of lengths (190.56-217.17 μm), though moderately spathulate forms exhibit a much wider range (39.59-254.40 μm). Highly spathulate forms are typified by narrow base widths, so their truncated size range probably reflects a minimum base width. Mandibles with less extreme aspect ratios have a wide range of sizes. This suggests that isometric scaling of size does occur for most mandible shapes.

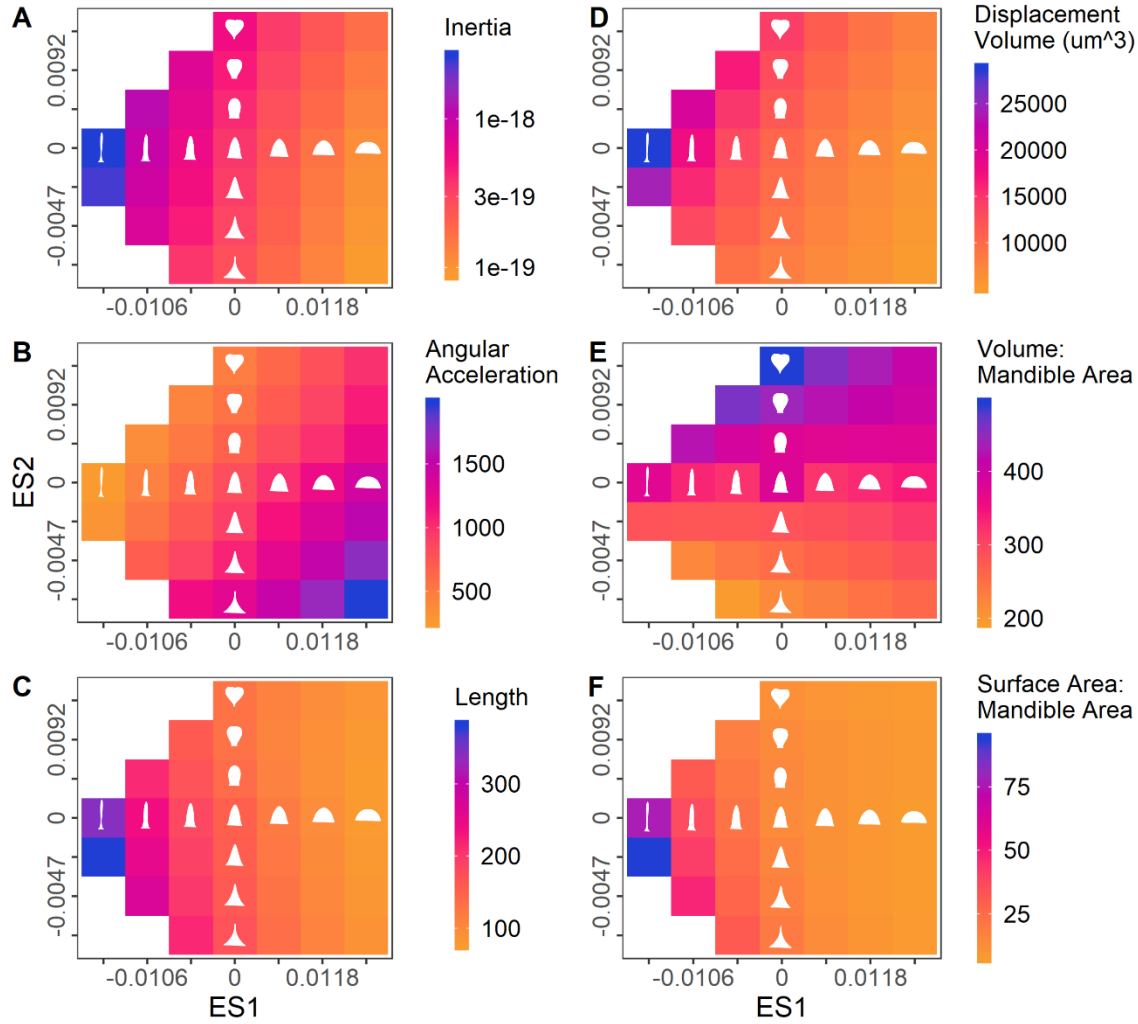


Figure 4: Adaptive landscape for computer-generated avicularian mandibles along the first two axes of the CP-EES. **A**, moment of inertia (kg m^2 ; log scale; mandible area = $1 \times 10^4 \mu\text{m}^2$), **B**, angular acceleration (rads s^{-2} ; mandible area = $1 \times 10^4 \mu\text{m}^2$) when the force is constant ($2 \times 10^{-12} \text{ N}$), **C**, length of each mandible (μm), **D**, volume of water displaced by the mandible (area = $500 \mu\text{m}^2$), **E**, ratio between displacement volume and mandible area (length = $500 \mu\text{m}$), **F**, ratio between surface area of the water parcel and mandible area (length = $500 \mu\text{m}$).

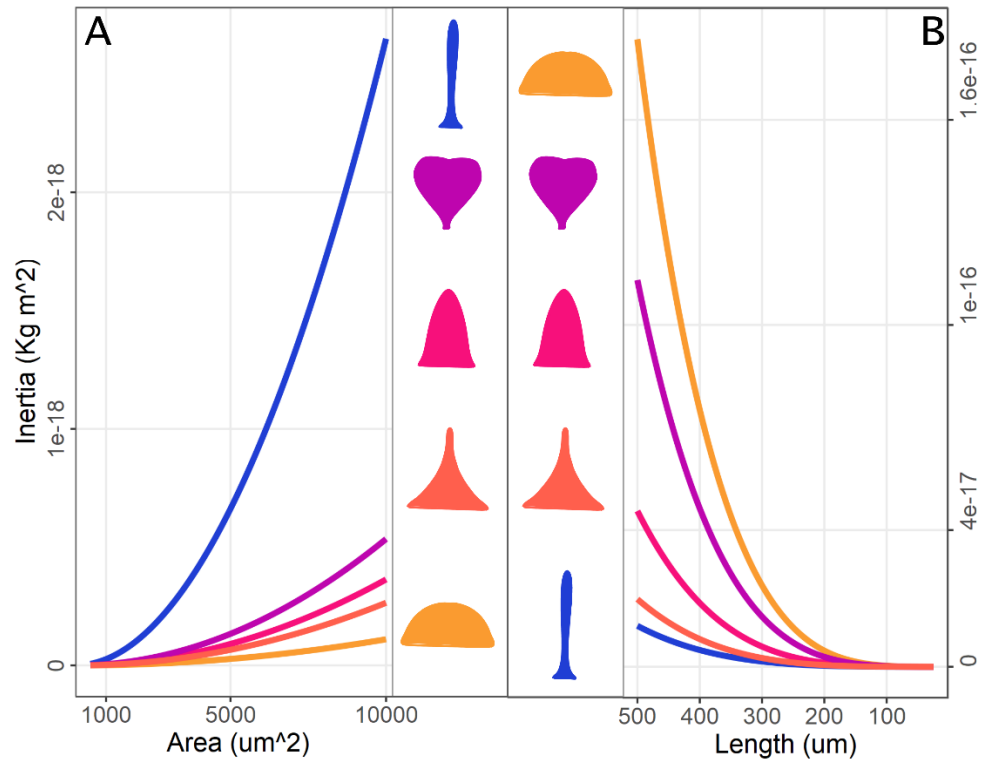


Figure 5: Isometric increases in mandible size and resulting moment of inertia when area (**A**) and length (**B**) are held constant between different mandible shapes (setose, spatulate, triangular, hastate, and lunate). Note that the x-axis of **B** is on a reversed scale.

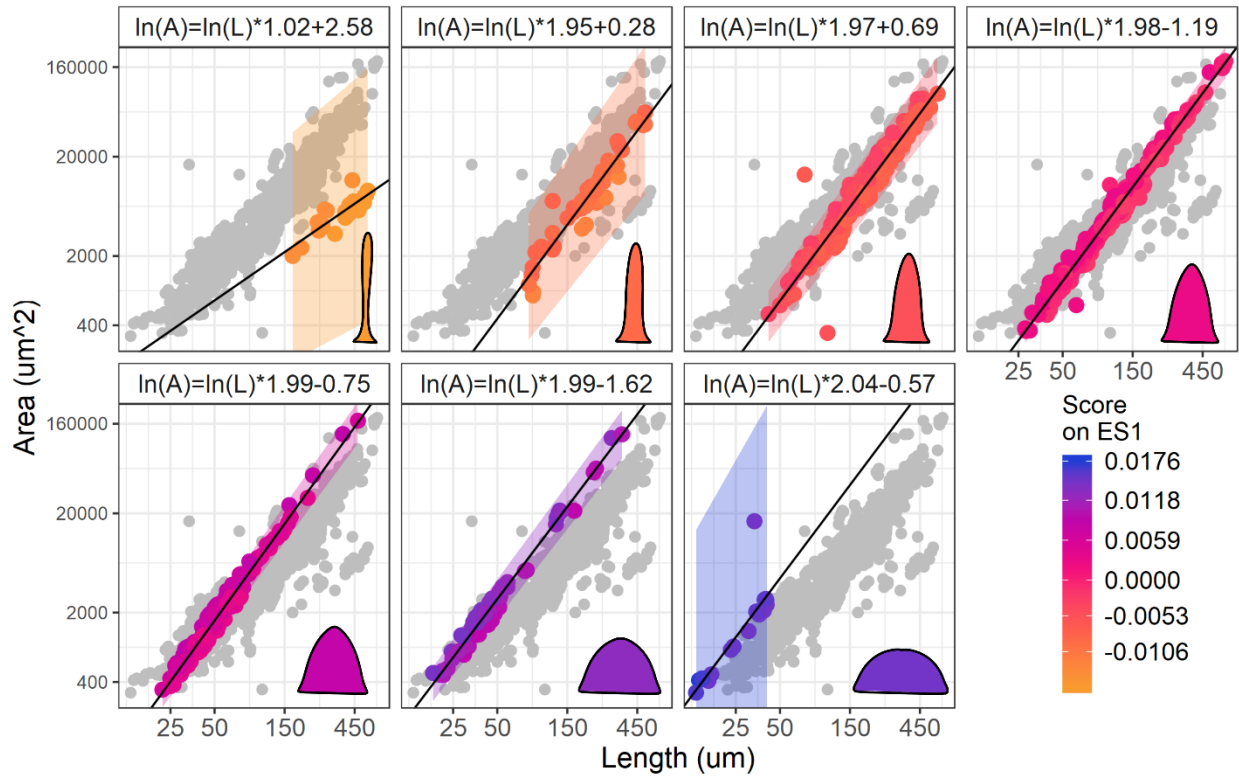


Figure 6: Relationships between mandible length and area, grouped by their scores on the first axis of CP-EES. Shaded polygons show 95% confidence intervals. Representative shapes along ES1 are provided in the bottom right of each plot.

Mandible curvature (CP-EES axis 3) does not affect moment of inertia as much as changing mandible shape. When mandible area is constant ($10000 \mu\text{m}^2$) there is little change in inertia along ES3 (3.43×10^{-19} to $3.68 \times 10^{-19} \text{ kgm}^2$), despite large changes along ES1 and ES2 (8.93×10^{-20} to $2.65 \times 10^{-18} \text{ kgm}^2$).

3.2.2 Water displacement and surface area

At constant area, setose mandibles displace the greatest volume of water (Fig. 4D). The ratio of displacement volume to mandible area is highest for spatulate mandibles, while the ratios for surface area to mandible area and surface area to volume are highest to setose mandibles (Fig. 4E-F).

3.2.3 Drag, torque, and force

Moment of inertia was miniscule for all mandibles, resulting in very low net torques (equation 3). This means that the force applied to the mandibles by the adductor muscles is used to overcome drag rather than mandible inertia (Fig. 7).

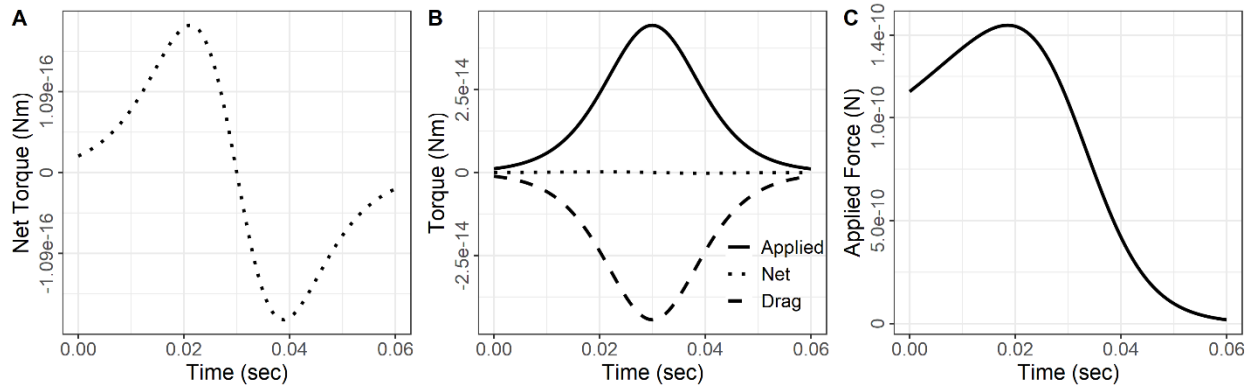


Figure 7: Torques and applied forces on a mandible of *Adeonellopsis* sp. with a length of 106.85 μm , assuming a total closing time of 60 msec. **A**, net torque (proportional to angular acceleration; equation 3) over time; **B**, comparison of net, applied, and drag torques over time (drag is proportional to velocity at low Re); **C**, force applied by the adductor muscles over time (which depends on the angle between the tendon and the mandible; equations 4 and 14). By convention, positive torque acts in the counterclockwise direction and negative torque acts in the clockwise direction.

The force needed to close the mandible of an avicularium spans five orders of magnitude across the sampled avicularia (Fig. 8). The peak force required to close a mandible is negatively correlated with ES1 (-0.61 , when force is log-transformed) and uncorrelated with ES2 (0.10). In contrast, mechanical advantage is positively correlated with ES1 (0.82). The difference between the peak input force (at the point of tendon attachment) and the ideal output force (at the mandible tip) can span an order of magnitude for setose forms (Fig. 8). This suggests that mandibles with a high-aspect ratio (e.g. setose forms) are more expensive to use than those with a low-aspect ratio (e.g. lunate).

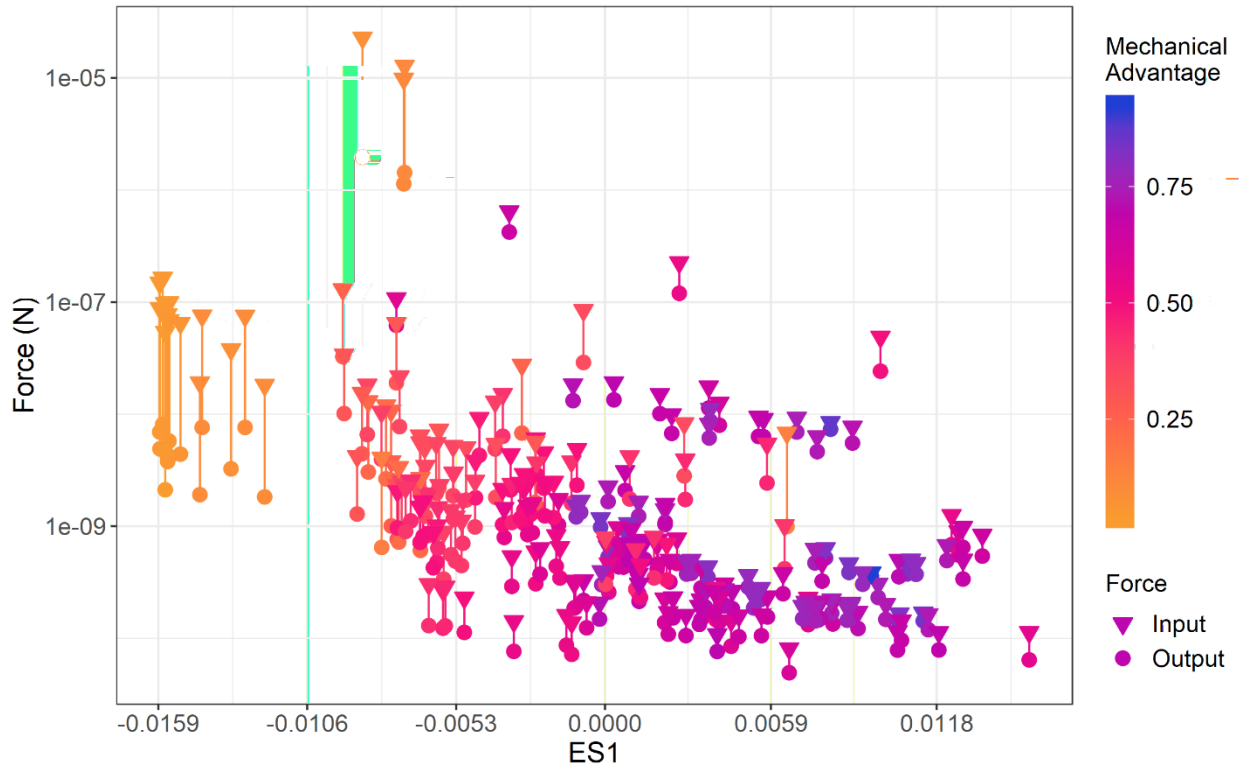


Figure 8: Comparison of the applied force (input at tendon attachment) and crushing forces (output at mandible tip) along the first axis of the CP-EES. Here, force is calculated assuming total closing time of 60 milliseconds.

High-aspect-ratio mandibles tend to be longer (Fig. 6), which may reflect a biological limit on tendon length. The longest tendon was 266.08 μm , recorded from a 502.40 μm long triangular vicarious avicularium (*Chaperiopsis* sp.). Setose mandibles in Microporellidae reached a similar length (553–169 μm), but their tendon lengths were all less than 43 μm . However, the tendon in *Chaperiopsis* had a maximum width of 157.80 μm , which was greater than the mandible widths of the setose avicularia in Microporellidae (90.32–55.60 μm). This suggests that the mechanical advantage of setose mandibles is limited by the ratio of tendon width: tendon length. In other words, a long, thin tendon may not withstand the forces needed to close the mandible. Tendon length is positively correlated with tendon width for taxa with a single tendon (0.74, $n=152$) and those with two tendons (0.84, $n=56$). Similarly, tendon length is positively related to the width of the mandible (single tendon: 0.83, $n=161$; double: 0.59, $n=60$). Therefore, base width limits mechanical advantage by constraining tendon width.

3.3 Energetic requirements

As with force, the energy needed to close the mandible of an avicularium spans a wide range (Fig. 9). Assuming a closing time of 60 milliseconds, the energy needed to close the mandible of an avicularium

ranges from 5.81×10^{-16} to 3.69×10^{-10} J (1.24×10^{-16} to 8.82×10^{-11} cal). The median cost is 2.89×10^{-14} J (6.92×10^{-15} cal), while the mean cost is $4.05 \times 10^{-12} \pm 3.11 \times 10^{-11}$ J ($9.67 \times 10^{-13} \pm 7.43 \times 10^{-12}$ cal).

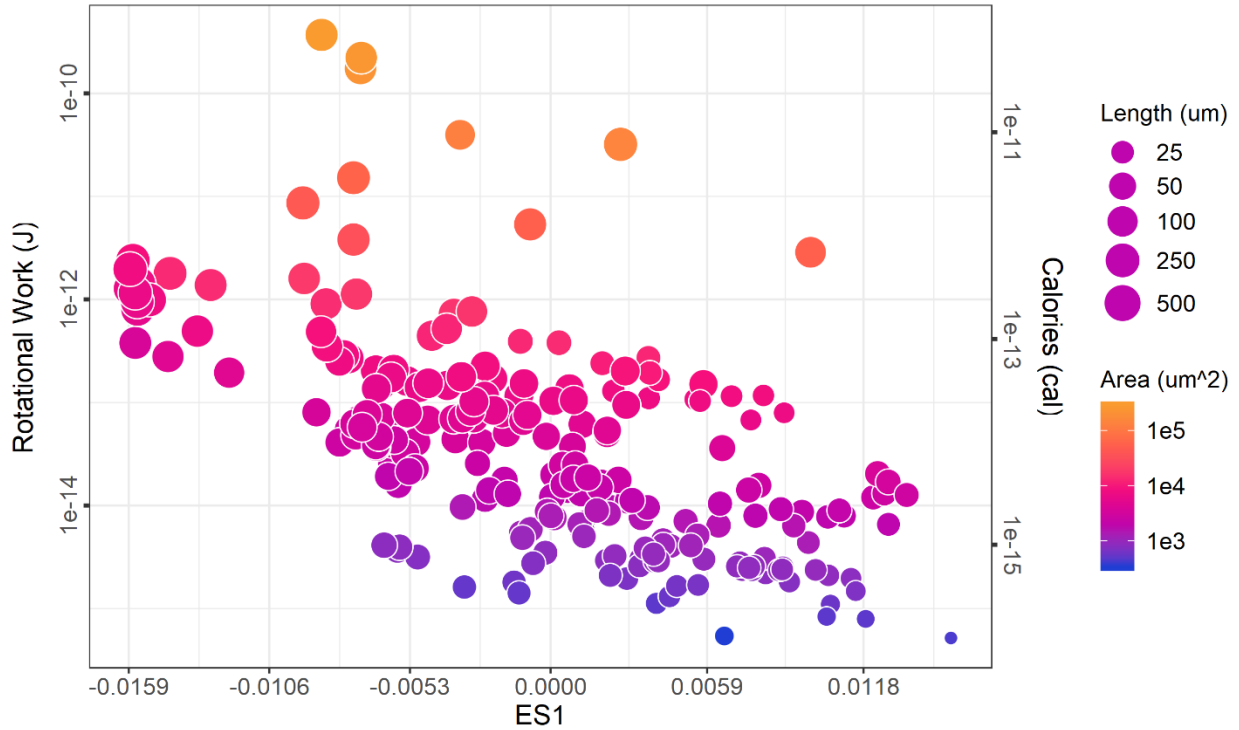


Figure 9. Energy required to close mandibles of avicularia (input torque + torque needed to overcome drag), shown along the first axis of CP-EES. Here, energy is calculated assuming a total closing time of 60 milliseconds. Note that the y-axis is on a log scale.

4. Discussion

4.1 Morphospace exploration

The avicularian morphospace generated by the first two axes of the CP-EES was well explored (Fig. 2). This suggests that the mandible shape of avicularia is highly evolvable across cheilostomes and lacks strong developmental constraints. Aside from impossible morphologies, low-aspect ratio triangles and bilobed mandibles were not realized for the sampled taxa. However, a variety of bi-, tri-, and tetrafurcated shapes have been reported in bryozoans (Fig. 10) [33–35]. Comparing these realized mandibles to the theoretical bilobed mandibles shows that bifurcation typically occurs in mandibles with higher aspect-ratios (comparing length to base width). This makes sense, since bifurcation extends the range (reach and breadth) of the avicularium while reducing the construction cost (total mandible area). In contrast, low-aspect-ratio bilobed forms barely extend the range and do not significantly reduce the mandible area. Both forms restrict the maximum tendon length to the depth of bifurcation, reducing mechanical

advantage. Therefore, it is unlikely that such deeply bifurcated, low-aspect-ratio mandibles would be adaptive (though they are not necessarily maladaptive).

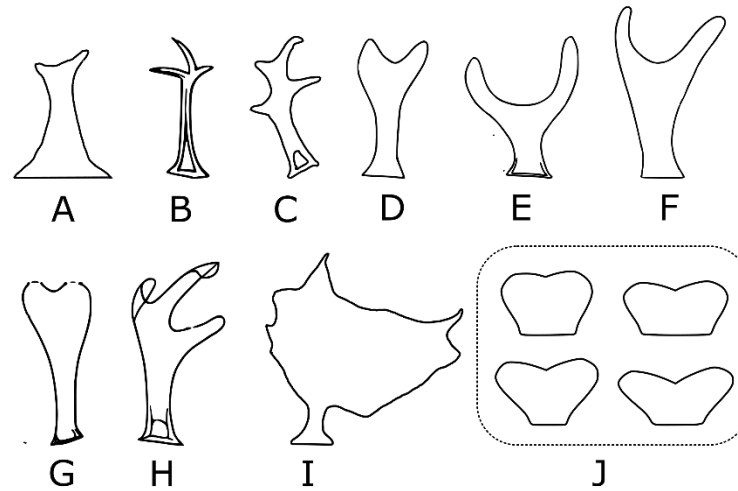


Figure 10. Comparison of bi/tri/tetrafurcated, bilobed, and fan-shaped mandibles (not to scale). **A** *Platypyxis* n. sp., **B** *Hippaliosina acutirostris*, **C** *Licornia longispinosa*, **D** *Iodictyum idmoneoides*, **E-F** *Reteporella suluensis*, **G** *Triphyllozoon hirsutum*, **H** *Triphyllozoon tubulatum*, **I** *Bryopesanser pesanseris*, **J** unexplored theoretical mandible shapes. Only **A** and **I** were present in the CP-EES. **B** reproduced from Fig. 148b in Canu & Bassler 1929; **C** reproduced from Fig. 29, Plate 25 in Harmer 1926; **D-F** reproduced from Figs. 26m, 10e, and 11b from Plate 38 in Harmer 1934; **G-H** reproduced from Figs. 4d and 10a from Plate 39 in Harmer 1934.

The absence of extremely low-aspect-ratio, pointed mandibles is worth noting. The “ancestral operculum” of most avicularia was probably a round, low-aspect-ratio shape [36,37] so it is unlikely that a random evolutionary walk would not have stumbled into this slightly pointier morphospace. Forms quite close to these low-aspect-ratio triangles have been realized, so it also seems unlikely that this gap in the morphospace is caused by developmental constraints. Instead, this gap may be due to differing selection pressures for pointy vs low-aspect-ratio mandibles (see section 4.2).

Finally, there is a small gap between highly spathulate (fan-like) mandibles of *Bryopesanser pesanseris* and those that are more moderately spathulate (Fig. 3). These intermediate morphologies are likely realized during the ontology of highly-spathulate mandibles, or may be present in unsampled taxa.

4.2 Form, function, and trade-offs

The differences in moment of inertia across the CP-EES shape axes suggest differences in function (Fig.4). To maximize speed of closure or minimize energetic cost, the mandible should be small with a low-aspect ratio. A prime example of this are lunate mandibles, which are typically between 25-50 μm in length. Such avicularia also tend to have a high mechanical advantage (0.74 ± 0.08). Such avicularia probably provide

a rapid, cheap response to stimulus from wandering epibionts. Indeed, high densities of these small lunate mandibles like those found in some species of Phidoloporidae could allow multiple avicularia to grasp a single epibiont [38]. Compared to other mandible shapes at the same length, lunate mandibles guard a parcel of water that has a high volume and a high surface area. This parcel of water is henceforth referred to as the “domain” of the avicularium. A large domain means that the avicularium is guarding more of the colony. The domain of the avicularium can be enhanced by increasing the length or area of the mandible. Domain size can also be increased by the “nodding” behavior of some pedunculate avicularia [7], though most avicularia lack this ability.

If the mandible is enlarged isometrically (retaining shape), lunate mandibles soon become unwieldy owing to their large total area. For example, a lunate mandible 500 μm long would be about 1000 μm wide – twice as wide as many autozooids. This favors allometric scaling (changing shape) as mandibles increase in size. However, the moment of inertia also increases with size. Shifting from a lunate mandible to a setose mandible at constant area provides a 4.84-times increase in domain volume and a 4.38-times increase in reach– at the cost of a 23.97-times increase in inertia. This trade-off between domain size and moment of inertia favors triangular mandibles with moderate aspect ratios. Hastate mandibles are particularly optimized. A shift from a lunate mandible to hastate mandible of the same area produces a 1.40-times increase in domain volume, with only a 2.40-times increase in inertia. Therefore, hastate mandibles could have evolved to maximize domain size and minimize the force needed to close the mandible.

However, triangular mandibles were more common than hastate mandibles in the sample (Fig. 2). While this may reflect sampling bias, it suggests that there may be additional constraints on mandible shape. The slender tip of a hastate mandible may be more prone to breakage than a simple triangular mandible. To maintain structural integrity, hastate mandibles might need to be thicker or more heavily cuticularized. Therefore, triangular mandibles are the result of three divergent constraints: maximizing domain size, minimizing energetic cost (force and construction material), and minimizing the potential for breakage. This provides support for the idea that such pointed mandibles are optimized for epibiont capture [39]. Prior discussions of mandible shape in relation to function have been speculative [6,39], and these results provide clear evidence of adaptation for colony defense.

Both spathulate and setose mandibles have a large domain size at the cost of a high moment of inertia. In order to minimize the energetic cost of closing the mandible the rotational velocity or angular acceleration must be reduced. For example, highly spathulate mandibles should close at least 1.91-times slower than

a triangular mandible of the same length. Similarly, a setose mandible should close 3.17-times slower than a triangular mandible of the same area. This likely means that setose and spatulate mandibles close too slowly to capture epibionts.

Setose mandibles are thought to be used for cleaning the colony [6,40]. While vibracula are able to swing over the colony surface and sweep off epibionts and detritus, the motion of setose avicularia examined in this analysis is limited to a single plane. Such mandibles may serve as sensory structures. Alternatively, setose mandibles may function like whips: startling epibionts off the colony through touch or sudden motion. Neither of these functions requires a strong crushing force (i.e. output force at the mandible tip), which allows these mandibles to reduce both base width and mechanical advantage. Setose mandibles maximize the domain surface area to mandible area ratio, as well as the ratio between domain surface area and domain volume. In other words, they reduce water displacement and material costs while maximizing the surface area of their domain.

Spatulate mandibles may be used to direct wastewater or chemical secretions over the surface of the colony, rather than serving as defense against epibionts [6,11,41]. In contrast to setose mandibles, spatulate mandibles maximize the volume of their domain while minimizing the mandible area (Fig. 4E). This allows spatulate mandibles to displace a large volume of water with minimum material cost.

Mandibles that represent intermediate morphologies may fulfill multiple functions. Colonies with a high division of labor of avicularia (e.g. Phidoloporidae) are likely more efficient, since they can optimize each type of avicularium for a particular function. Plasticity in mandible size/shape and budding plasticity (i.e. budding certain types only when signaled by external cues) would also help optimize the energy investment in bryozoan colonies.

The above discussion assumes that mandible shape should be well suited for the function(s) it performs. This may not necessarily be the case. For instance, the morphology of bird beaks does not strongly predict diet or feeding behavior (e.g. a beak well-suited for cracking seeds may instead be used to consume fruit) [42].

4.3 Pivot structure

Mandibles that require greater force to operate (setose, spatulate) are more common in taxa with complete pivot bars (Fig. 3). This suggests that a sturdier fulcrum may be necessary to withstand the increased forces applied. The movement of the mandible applies compressional stress to the fulcrum, and constructing the fulcrum out of calcite provides high compressional strength [7]. A complete, calcite pivot

bar may reduce compressional stress owing to its increased surface area (reducing force per unit area). Condyles should therefore experience greater force per area under an equal compressional stress, and may deform (reducing mechanical advantage) or break. On the other hand, increased surface area of complete pivot bars should generate more friction (reducing mechanical advantage) and increase the cost of construction. Pivot bars may then represent a trade-off between increasing compressional strength and reducing friction.

The results presented here suggest that pivot bars are an evolutionary prerequisite to developing highly spathulate and setose avicularia (not vibracula). However, sampling in this analysis was conducted to maximize the variation in mandible shape, rather than pivot structure or phylogenetic completeness. In this analysis, most avicularia with pivotbars were found in umbonulomorphs and lepraliomorphs (Fig. 3). This finding aligns with previous observations that condyles are common in anascans, while pivot bars are common in ascophorans [15]. Therefore, the relationship between pivotbar structure and mandible shape may instead reflect greater “evolvability” of lepraliomorph/umbonulomorph avicularia.

While mandible shape appears unconstrained when taxa are examined together, the consideration above highlights the potential for mandible shape to be constrained by phylogeny. Avicularia are thought to have evolved multiple times [36,37,43], and each lineage of avicularia may be subject to different developmental restrictions. A comparison of morphospaces between lineages, including morphology of the mandible and pivotbar, is warranted.







4.4 Energetic investment

The estimated caloric requirements to close the mandible of an avicularium are quite small (1.24×10^{-16} to 8.82×10^{-11} cal), and tend to be greater for larger avicularia (Fig. 8). To put this into perspective, a 500 ng rotifer provides enough energy ($\sim 8.81 \times 10^{-3}$ cal [44–46]) to close the mandibles of 9.98×10^7 expensive avicularia or 7.11×10^{13} cheap avicularia. This suggests that avicularia are energetically inexpensive. However, bryozoans are thought to live an “energetically marginal existence” and slight changes in energetic efficiency could have a large impact on their energy budget [47].

In colonies with multiple types of avicularia, more-expensive types are less common (Table 3). Large adventitious avicularia in *Chaperiopsis rubida* require 32.17x more calories than smaller forms and occur 5.49x less often. In contrast, large avicularia in *Hippomenella vellicata* are almost as common (~ 1.15 x less often) and only require 10.61x more calories. It is tempting to suggest that energetic cost could predict the degree of investment in certain avicularia. However, large avicularia in *Chaperiopsis splendida* appear

less often than those in *C. rubida*, despite having a similar energy ratio with smaller avicularia. The different abundances of avicularium types do not equalize their energetic cost, suggesting that even a limited number of large avicularia require significant investment. These comparisons represent a tentative first step and assume that different avicularia close at the same speed (which is likely not the case). The different occurrence rates within colonies may also reflect spatial/developmental limitations, construction costs, the influence of external forces, or division of labor between types of avicularia.

Table 3: Average characteristics of six avicularia. The number of colonies, total zooids, and mandibles measured is provided below each species name. Energy required represents the energy needed to close the mandible once and is calculated based on a closing time of 60 milliseconds. Abundance of avicularia from Schack, unpublished data.

Species	Shape	Mandible Silhouette	Area (μm^2)	Energy Required (cal)	#/ 100 zooids	Ratios (Small : Large)		
						Area	Energy	Abundance
<i>Hippomenella vellicata</i> (1, 72, 11)	Large lingulate		4676	3.48×10^{-14}	164	1:3.57	1:19.77	1.15:1
	Small lingulate		1309	1.76×10^{-15}	188			
<i>Chaperiopsis rubida</i> (6, 515, 15)	Large triangular		31777	1.71×10^{-12}	31	1:13.16	1: 403.48	5.49:1
	Small triangular		2414	4.24×10^{-15}	171			
<i>Chaperiopsis splendida</i> (14, 1299, 5)	Large triangular		18429	3.82×10^{-13}	15	1:7.01	1:69.27	11.64:1
	Small triangular		2628	5.51×10^{-15}	171			

4.5 A note on Reynolds numbers

At the angular velocities examined here, most avicularia ($< 180 \mu\text{m}$ long) have a Reynolds number below unity. At low Reynolds numbers, inertial forces and thus differences in moment of inertia become nominal [48]. Since shape differences and the distribution of area perpendicular to the axis of rotation still influences drag, our discussion of form and function still applies.

Many avicularia ($\leq 30 \mu\text{m}$ long) exist in Stokes' flow ($RE < 0.2$). In Stokes' flow, reciprocal motion cannot generate thrust [48]: avicularia operating in such conditions cannot move waste-water and must rely on diffusion to carry any chemical defenses. In addition, close proximity to walls (i.e. the colony surface) can greatly increase drag in Stokes' flow. To escape these conditions, it is possible that avicularia move at higher angular velocities than assumed here. At 440 rads s^{-2} (approximate speed of a $\sim 200 \mu\text{m}$ leg of

Cyclops scutifer [26]), only avicularia $< 10\ \mu\text{m}$ long would be in Stokes' flow. Naturally, increasing peak angular velocity raises the energetic cost (ranging from 1.24×10^{-15} to 3.93×10^{-9} cal).

4.6 Limitations and future directions

To simplify calculations, this analysis assumed that mandibles were flat plates of constant thickness and density. There are many exceptions to this, such as hooked projections at the tip of the mandible, serrations along the mandible edge, concavity of the mandible itself, and changing thickness/degree of cuticularization along the mandible [15,49]. To more accurately understand the biomechanics of avicularia, measurements need to be conducted on living bryozoan colonies using high-speed cameras. Empirical tests on mandible models and three-dimensional geometric morphometrics would also be informative. As mentioned previously, phylogeny should be included to elucidate evolutionary and developmental constraints in mandible shape.

Additionally, vibracula were absent from this analysis. The constraints on their biomechanics, and the evolutionary shift from avicularia to vibracula is worth investigation.

Conclusions

- Biomechanical analysis of morphospace provides the first quantitative evidence for functional specialization in avicularia
- The theoretical morphospace of mandible shape is well-explored, suggesting that mandible shape is highly evolvable across New Zealand cheilostomes and lack strong developmental constraints.
- Triangular mandibles represent a trade-off between maximizing domain size, minimizing energetic cost (force and construction material), and minimizing the potential for breakage. This implies that they are well-suited to catching epibionts.
- Tendon length and mechanical advantage are limited by tendon width, which itself is constrained by the base width of the mandible. This explains the low mechanical advantage of setose mandibles.
- Mandible shape may be developmentally constrained within different lineages. Complete pivot bars may be an evolutionary pre/corequisite to evolving mandibles with extreme moments of inertia such as setose and highly spatulate forms.
- Avicularia appear to be energetically inexpensive, ranging from 1.24×10^{-16} to 8.82×10^{-11} cal to close.

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Nomenclature

A	Area of the of the avicularian mandible (m).
A_i	Area of a thin slice of the avicularian mandible (m). The slice is parallel to the axis of rotation in equations 2,9, and 13 and perpendicular to the axis of rotation in equation 15.
a	Angular acceleration (rad/s ²)
C_d	Coefficient of drag (dimensionless)
F	Force (N)
F_{drag}	Force owing to drag (N)
γ	Euler's constant: 0.57721
h	Height of a thin slice of the mandible perpendicular to the axis of rotation (m)
I	Moment of inertia (kgm ²)
L	Characteristic length (square root of the total area) used to calculate Reynolds Number (m)
μ	Dynamic viscosity of seawater at 0°C (0.00188 kg/m·s)
n	Number of thin slices of the mandible (100), either parallel to the axis of rotation (equations 2,9,13) or perpendicular to the axis of rotation (equation 15)
φ	Gape of the mandible (radians, equation 5; degrees, equation 13,15)
ρ	Density of seawater at 0°C (1028 kg/m ³)
ρ_m	Density of the mandible, assumed to be equal to the density of α -chitin (1400 kg/m ³)
r	distance from the applied force to the axis of rotation (equation 4,6) (m)
r_i	Distance of a thin slice parallel to the axis of rotation to the axis of rotation (equations 8-9,11,13) (m)
r_1, r_2	Radii of a thin frustrum, equal to the length of a thin slice perpendicular to axis of rotation (equation 14-15)
R	Reynolds number (dimensionless)
S	Surface area of the parcel of water that the mandible of an avicularium passes through (m ²)
t	Total time required to close the mandible (s)
t_i	Time at constant angular acceleration of the mandible
t_f	Total time required to close the mandible (s)
τ	Torque (Nm)
θ	Angle between the applied force and the surface of the mandible, i.e. the angle between the tendon and the mandible (radians)
v	Translational velocity of the mandible (m/s)
V	Volume of water displaced (m ³)
ω	Rotational velocity of the mandible (rad/s)
W	Rotational work (J)

Appendix 4

Supplementary information for:

Chapter 5: Cheap labor and high evolvability: A lack of strong developmental constraints on mandible shape of avicularia in New Zealand bryozoans

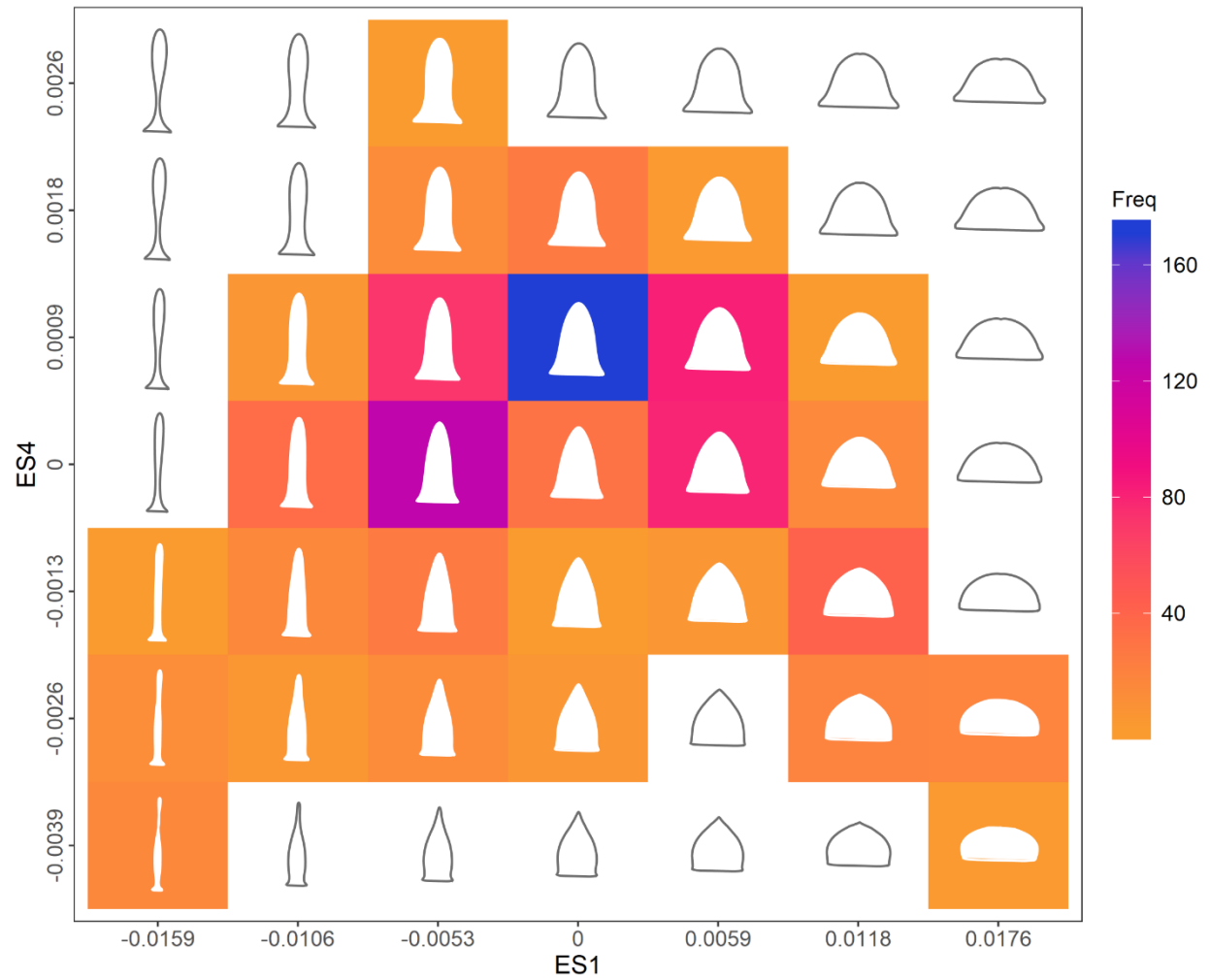


Figure S1: Heatmap of model shapes generated by the CP-EES along the eigenshape axes ES1 and ES4, which describe 78.59% and 2.31% of the shape variation, respectively. Color represents the frequency of that mandible shape (i.e. score combination) in the sample.

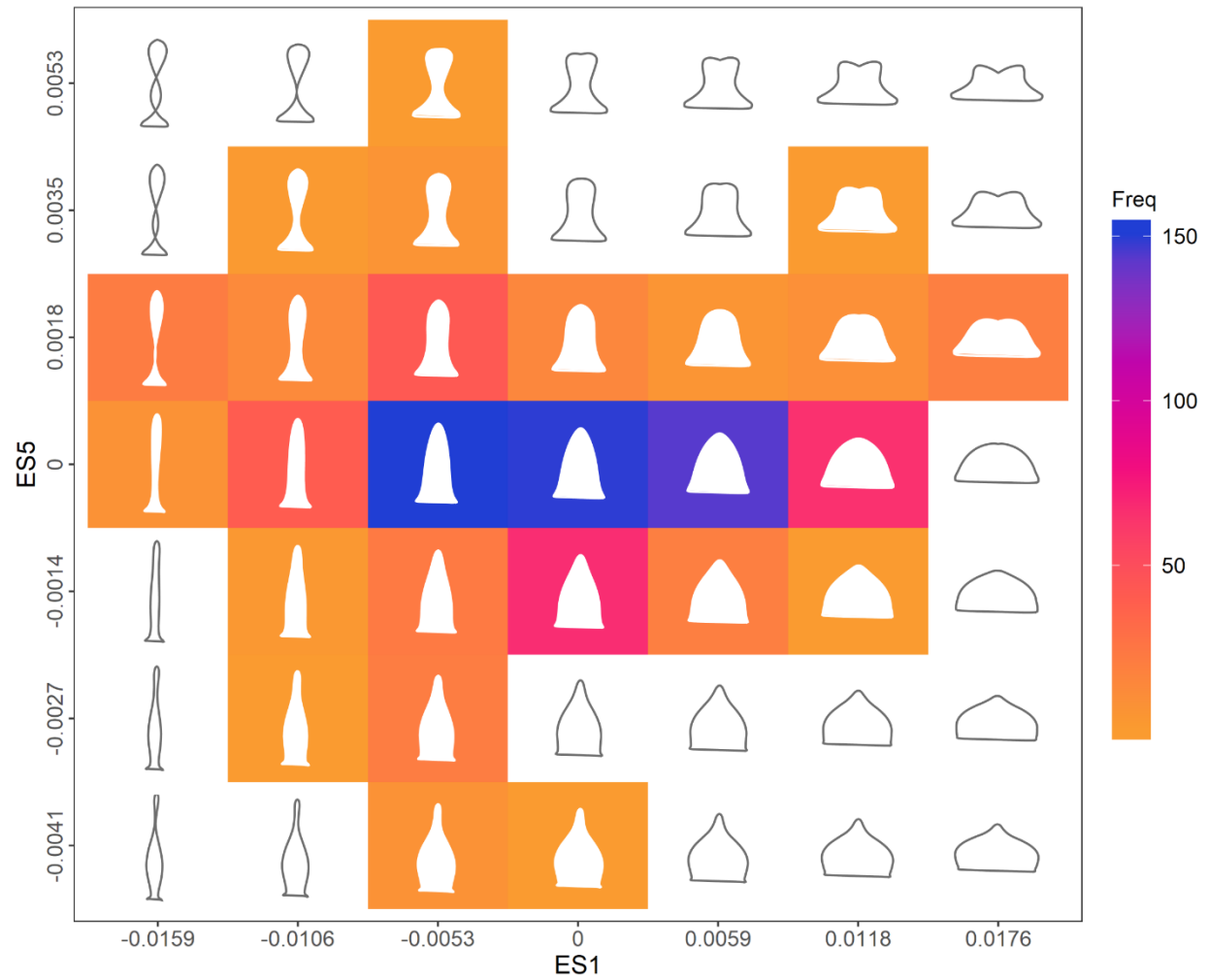


Figure S2: Heatmap of model shapes generated by the CP-EES along the eigenshape axes ES1 and ES5, which describe 78.59% and 1.61% of the shape variation, respectively. Color represents the frequency of that mandible shape (i.e. score combination) in the sample. Note the concentration of mandibles with a score of zero along on ES5.

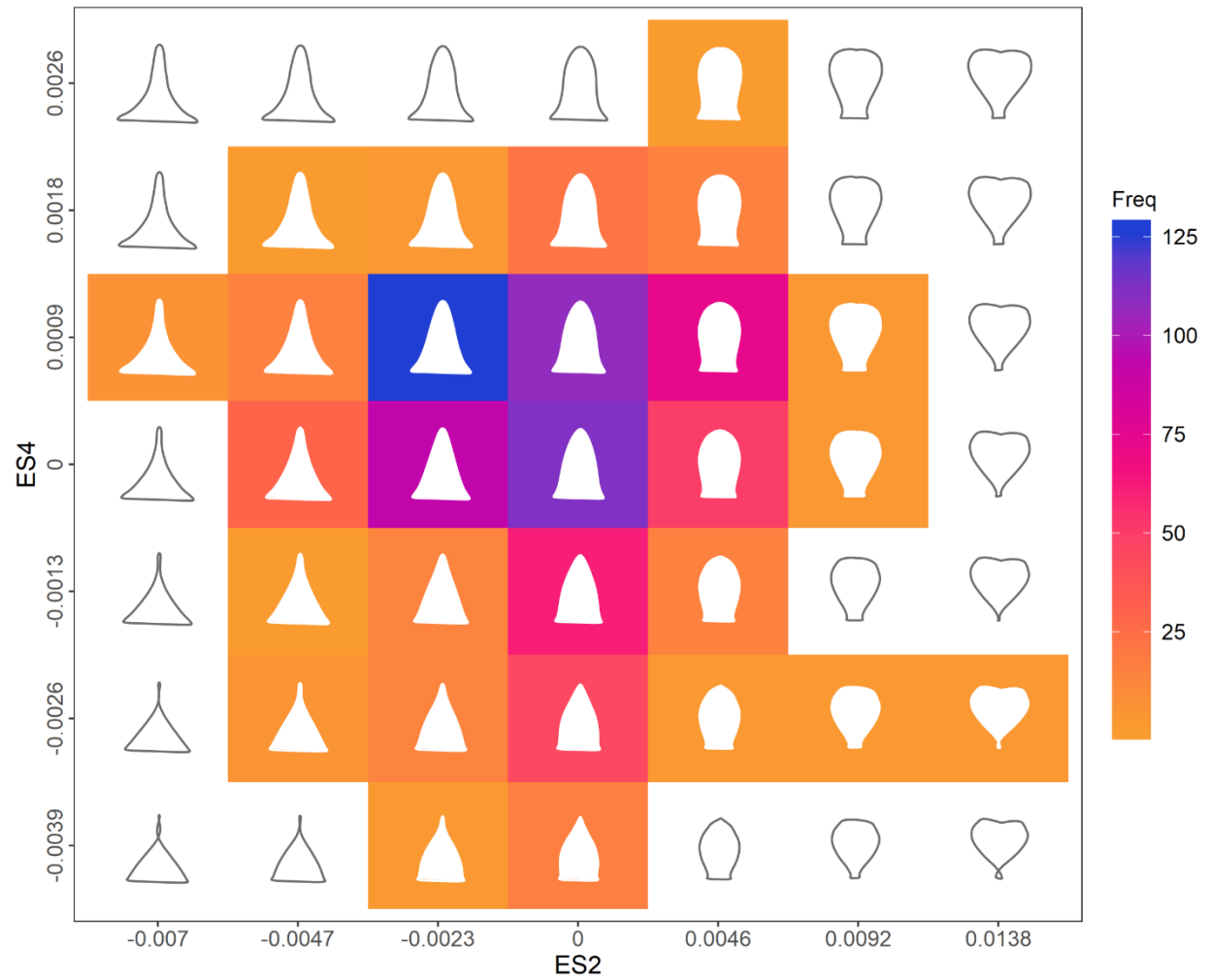


Figure S3: Heatmap of model shapes generated by the CP-EES along the eigenshape axes ES2 and ES4, which describe 12.06% and 2.31% of the shape variation, respectively. Color represents the frequency of that mandible shape (i.e. score combination) in the sample.

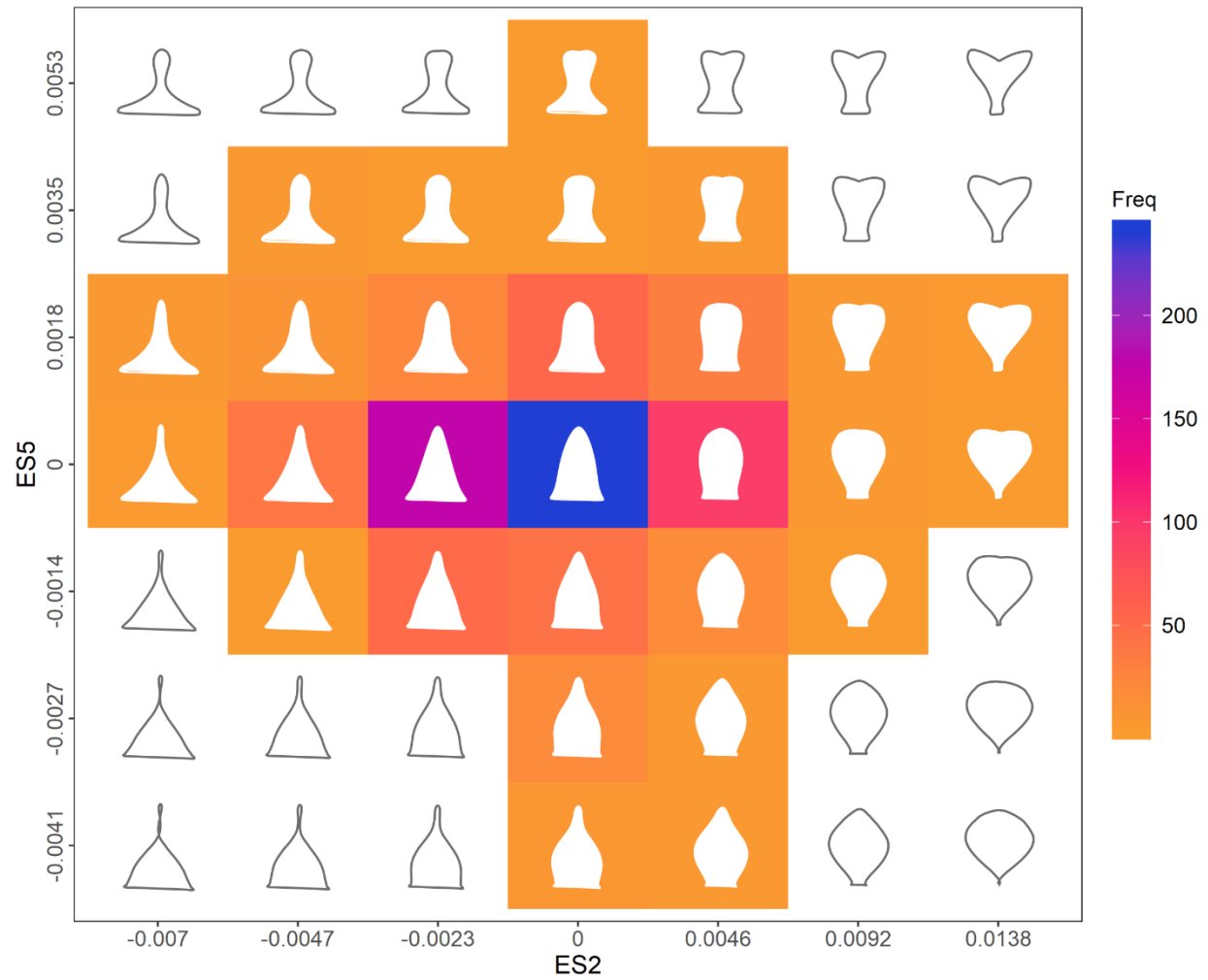


Figure S4: Heatmap of model shapes generated by the CP-EES along the eigenshape axes ES2 and ES5, which describe 12.06% and 1.61% of the shape variation, respectively. Color represents the frequency of that mandible shape (i.e. score combination) in the sample.

Data S1: An excel file containing 1) CP-EES scores for all avicularia; 2) measurement error; 3) data used to estimate mandible thickness; 4) integral approximations for mandibles with tendons; 5) calculated inertia, water displacement, force, drag, torque, and work for mandibles with tendons; 6) calculated inertia, water displacement, and domain surface area for mandible shapes generated by the CP-EES.

File S1: “shape_extraction”. R-code to extract a shapefile from a png image.

File S2: “Shape_integration”. R-code to calculate moment of inertia, water displacement, force, drag, torque, and work from shapefiles.

File S3: “avi.df” Sample data to be run with r-code in File S1 and S2

File S4-S7: Sample pngs to be run with R-code in File S1

File S8-S10: Sample shapefiles to be run with R-code in File S2

Chapter 6: Discussion

1. Overview

The goal of this thesis was to investigate the morphology, ecology, and energetics of cheilostome modular polymorphism in relation to environmental parameters and evolvability. The degree of modularity (i.e. degree of dissociation between zooids) appears to be an important factor in the evolution of polymorphism, at least in bryozoans (Chapter 2). Modularity enhances adaptive potential by allowing selection to act separately on dissociated modules, while redundant modules reduce the impact of deleterious mutations [1]. Resource translocation between modules (zooids) allows the existence of non-feeding zooids like kenozooids and avicularia. Dissociation between module components (e.g. operculum, polypide, cystid) can also enhance adaptive potential by allowing zooids to generate “appendages” (e.g. cystid projections that do not influence polypide structure).

Rootlets can be either zooidal (modified kenozooids) or appendages (evaginations of the body wall). Regardless of type, these structures were likely a “key innovation” in bryozoan evolution (Chapter 4). In particular, they freed cementing cheilostomes from hard substrata, allowing them to colonize muddy and sandy environments. This may have triggered a species radiation in the presence of a new habitat, though the degree to which rootlets may have contributed to the cheilostome evolutionary radiation will have depended on the availability of new niches in the soft-substratum environment (which may be small).

Interestingly, rootlets do not appear to be important in the radiation of cyclostomes. In cyclostomes, rootlets are only present in the articulated-erect family Crisiidae [2]. The earliest potential members of this family appeared in the Late Valanginian (135-140 mya), in the middle of the Mid-Mesozoic cyclostome radiation and 50 million years after the development of erect growth [3–5]. It is unclear whether these Early Cretaceous cyclostomes are true “crisiids”, and definite crisiids do not appear until the Maastrichtian (72.1-63 mya)[5,6]. The late timing and restriction to single family suggests that rootlets did not contribute to the radiation of cyclostomes. This also supports the idea that decreased modularity and stronger developmental constraints made kenozooids harder to evolve in cyclostomes (Chapter 2).

Dissociation of components within zooids and a resulting lack of strong developmental constraints may be responsible for the high evolvability of mandible shapes in avicularia (Chapter 5). Mandible shape is likely driven by biomechanical trade-offs and selection pressures. However, there may be developmental constraints within lineages (potentially linked to pivot structure). Mandible morphology strongly

influences both energetic cost and biomechanical optimization for different functions (Chapter 5). However, the morphology of avicularia does not appear to change along environmental gradients (Chapter 4). This suggests that the degree of energetic investment in each type of avicularium, rather than simple presence/absence of certain morphologies, may respond to changes in the environment (discussed below).

In contrast to avicularia, colony form, degree of brood-chamber immersion, and presence of costae (rib-like spines) all changed along environmental gradients (Chapter 4). Brood-chamber immersion decreased in low oxygen conditions, and cribrimorphs were predominate in areas with low CaCO_3 concentrations. Better defenses (internal brooding, calcified frontal walls) were more common in shallow environments that likely experienced high predation. These results confirm previous studies on colony form [7], but conflict with studies on polymorphism that found no relationship the environment [8,9]. However, previous studies examined presence/absence of polymorph types (rather than morphology) of < 100 species, over a small depth range (0–25m). These results hint at the potential importance of environmental conditions in the ecology and evolution of bryozoan polymorphism, instead of being driven solely by small epibionts [8,10]. Of course, these trait-environment relationships need to be confirmed with data on predation intensity and phylogeny (to rule out potential phylogenetic signals).

While geometric morphometrics is a highly useful tool for describing shape, it is extremely time consuming and requires direct access to specimens. The classification system presented in Chapter 3 will be highly useful for those that only have access to the literature or have limited time. Polymorphs described using this system will be easy to compare, even if classified by different workers.

Overall, this thesis highlights the complexity of bryozoan polymorphism and addresses several gaps in the literature. For the first time, the relative energetic cost of different mandible shapes has been calculated and detailed polymorph morphology has been included in ecological analysis (facilitated by a new classification system). These results lay important groundwork for further energetic and ecological studies. In addition, the results of this thesis suggest cheilostome avicularia could provide a unique evolutionary system to study. Heterogeneous filling of morphospaces is the norm, highlighting the ubiquity of developmental constraints [11–13]. Therefore, the high evolvability of the avicularian mandible may provide a rare glimpse into the evolution of traits that are (relatively) developmentally unconstrained —particularly since avicularia evolved multiple times [14].

2. Avicularia

2.1 Environmental conditions

Avicularia represent a persistent cost (passive upkeep, operation of the mandible, repair) and are not shed as the colony grows (as with some spines). Indeed, avicularia cannot be modified once budded (except through regeneration after breakage). In colonies with multiple types of avicularia, how heavily should colonies invest in each type? If colonies produce certain avicularia in response to environmental conditions, then older zooids may be stuck with relatively useless avicularia when conditions shift. Therefore, long-lived colonies in rapidly fluctuating environments should have consistent investment into all types of avicularia to benefit older zooids. Constant investment should also occur when the lag time between an environmental cue and avicularium production is longer than the rate of environmental change. For inducible spines, the lag time between the cue and spine production is only two days (and only one day to cease production) [15]. However, it is unlikely that avicularia can be produced in such a short time. Short-lived colonies or colonies in rapidly changing environments (where conditions change during the colony lifespan but are constant during the zooid lifespan) should have plastic investment that strongly reflects external conditions, since zooids will die before being hindered by “obsolete” avicularia.

2.2 Spatial constraints

Investment in avicularia is complicated by spatial constraints. Vicarious and interzooidal avicularia are less hindered by spatial constraints than adventitious avicularia. Occasionally, vicarious avicularia provide a similar space-filling function to kenozooids, budding in areas that are too small for an autozooid. This could explain why clusters of vicarious avicularia sometimes occur (Fig. 1). Such clusters represent a significant resource sink into a small area of the colony but may provide an effective defensive line against predators (but not against other bryozoans [16]).

Adventitious avicularia are constrained by the amount of space available on the calcified frontal shield or the gymnocyst (in taxa with a membranous frontal wall). Additional or larger avicularia can be accommodated by elevating them on rigid columns (columnar avicularia) or flexible stalks (pedunculate avicularia) that have a small locus of attachment. The space available on the gymnocyst/frontal wall is not constant across zooids (Fig. 2), and this internal factor may have a greater influence on avicularium type/size than any external condition (though it may be influenced by substratum irregularities).

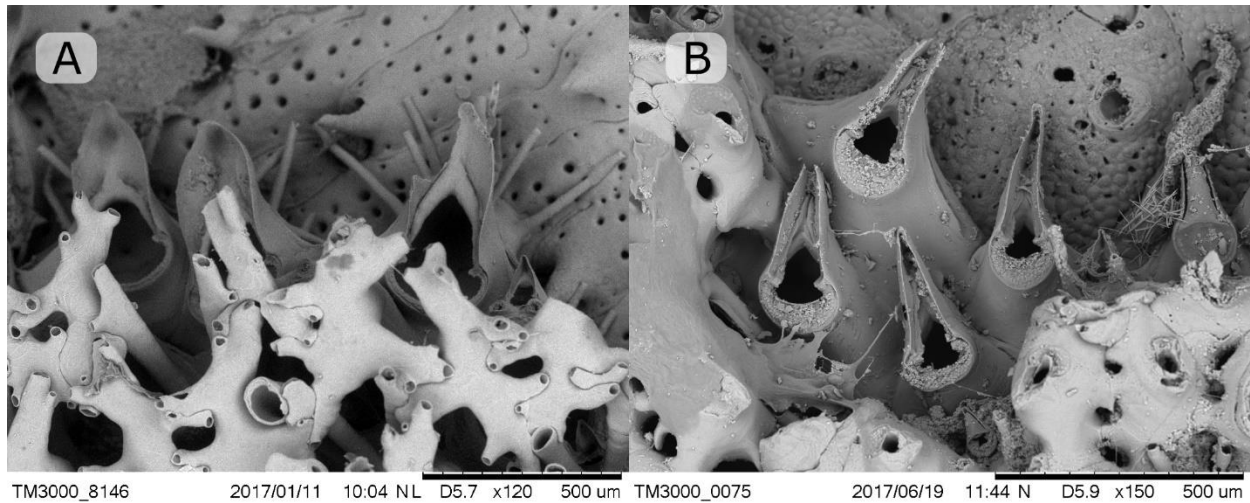


Figure. 1: Clusters of vicarious avicularia at colony edges in *Chaperiopsis* sp. (A) and *C. funda* (B).

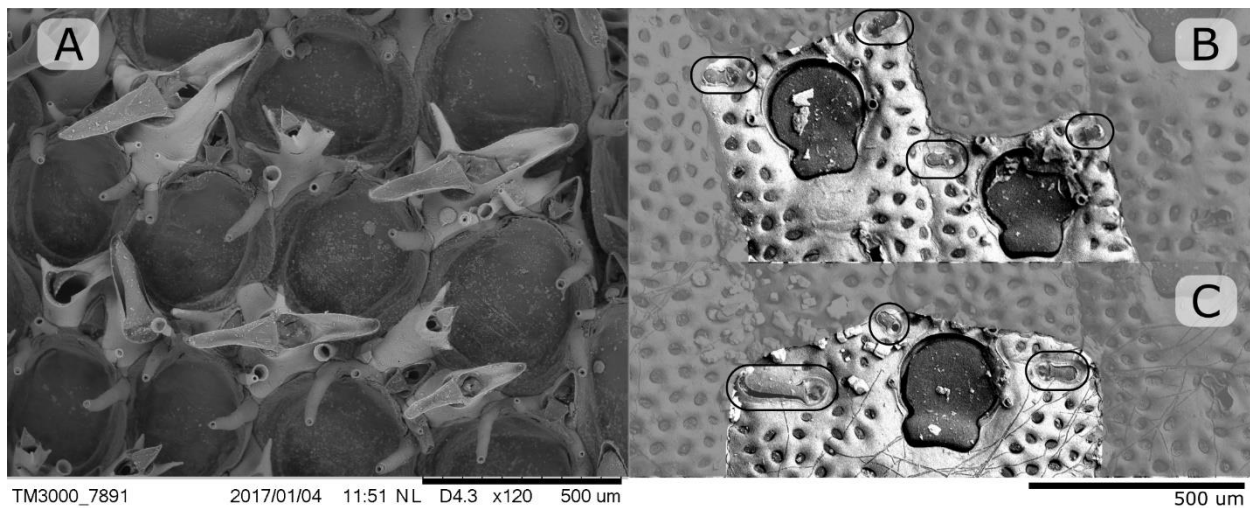


Figure 2: Spatial limitations and avicularia. Gymnocyst size appears to influence type/size of avicularia in *Chaperiopsis rubida* (A). In *Hippomenella vellicata* narrow zooids (B) appear to host fewer/smaller avicularia than wide zooids (C). Avicularia are circled in B-C and contrast is enhanced for clarity. Images B-C were photographed by Charlotte Bridger.

Naturally, avicularia should be located where they will be most effective. Potentially defensive avicularia are often found adjacent to the orifice, inside peristomes, or crowning ovicells. Avicularia on the proximal gymnocyst likely protect the orifice of the proximal zooid(s) in addition to the frontal surface of its host zooid. This serves to further limit the number of avicularia per zooid, since there are only a few locations where avicularia will be beneficial. Avicularia with chemical defenses or those that direct wastewater should have different optimal locations than mechanically defensive avicularia. The positions of these avicularia should reflect water flow over the surface of the colony and thus will be influenced by colony size and orientation.

If two types of avicularia can only appear in the same location (e.g. distal to the orifice), then the colony should allocate resources (space and energy) to the most appropriate avicularium, determined by external/internal cues. I can construct a potential decision-tree for avicularium production that takes these factors into account (Fig. 3). Expensive avicularia should be produced in response to a cue only if there is sufficient space and energy. Cues could be external (predation, sedimentation, fouling), or internal (colony size, neighboring avicularium types, presence of ovicells). The number and type of nearby avicularia may be particularly important for some avicularian functions. For example, a high-density of cheap avicularia may be necessary to provide effective defense [17], while there be a optimal distance between avicularia that direct waste-water. Internal cues may drive investment in long-lived colonies, while external cues may be more important in short-lived colonies. If the cue is absent, but there is abundant space and energy, then multiple cheap avicularia or a larger size class (isometric scaling) may occur. Gradients in size likely relate to spatial constraints more than external cues. If isometric scaling is prohibited due to high energetic cost or developmental mechanisms, then allometric scaling (contingent on available space but invariant of external cues) may produce mandibles capable of different functions. Otherwise, cheap avicularia should be produced unless the colony has a severe energy shortage (i.e. starvation or extreme stress).

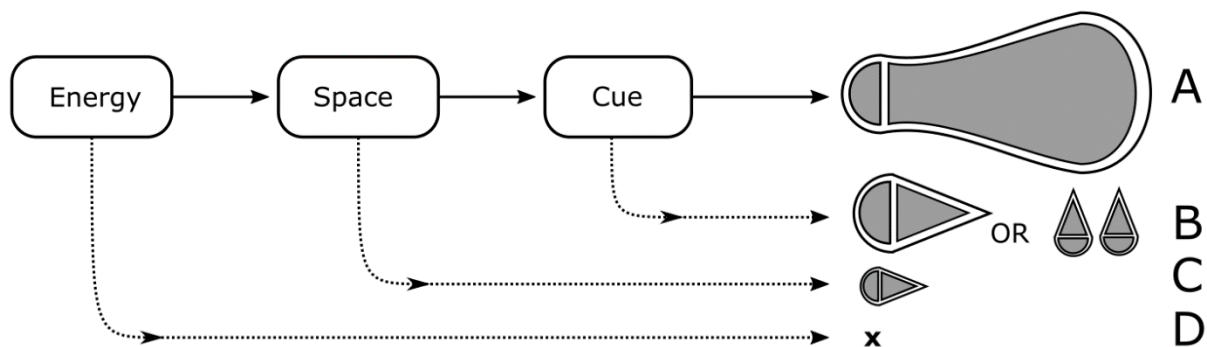


Figure 3: Hypothetical factors influencing investment in avicularia. **A** large, expensive avicularium should only be produced if there is sufficient energy, space, and an external cue. **B** when there is only sufficient space and energy, then multiple inexpensive avicularia or a larger size class (isometric scaling) may be produced. **C** when space is limiting, only small inexpensive avicularia can be produced. **D** under extreme energy limitations (starvation) no avicularia should be produced.

However, if two types of avicularia appear in different locations, then the colony can always invest in both types if it has sufficient energy. For example, an erect colony may possess defensive avicularia near the zooid orifice and a cleaning vibraculum on the underside of a branch (as in *Caberea*). Spatial partitioning of avicularia by type may be easier in erect colonies (multiple surfaces) than encrusting ones (single

surface). This could explain why cormidia in erect colonies appear to be fixed, while cormidia in encrusting colonies appear more plastic.

3. The cost of defense

The energetic cost required to operate the mandible of an avicularium appears to be quite small (1.24×10^{-16} to 8.82×10^{-11} cal). However, the energetic cost can stack up for a colony with many avicularia that close multiple times per day. There are also additional costs for growth, maintenance, production of chemical secretions (in avicularia that possess them), and regeneration. This is likely why taxa with avicularia tend to lose competitive interactions – particularly if they possess large vicarious avicularia. While ascophorans (which possess a calcified frontal shield) typically lose to anascans, ascophorans win if the anascans possess a vicarious avicularium [16]. Similarly, colonies with oral spines (or spines in general [18,19]) lose to unarmored colonies, and colonies with adventitious avicularia lose to those with spines [16]. It is clear that there is an energetic trade-off between defending against predators and investing in competitive ability.

However, competitive exclusion appears to be rare between bryozoans. Good competitors are usually rare, and poor competitors with fast growth rates can be the most abundant under disturbed conditions (e.g. ice scour [18]) and when space is not limiting [20]. While heavily armored taxa likely have reduced growth rates [15], it is unlikely they would be excluded from communities. Indeed, high predation should allow heavily armored taxa to flourish.

This reveals the importance of including some measure of abundance (be it biomass, number of zooids, or percent cover) when examining trait-environment relationships of bryozoan communities. Most environmental filters are weak and combine pressure from competition to generate community-level trends in functional traits [21]. If competitive exclusion is non-existent, then mere presence/absence data (like that used in chapter 4) makes it difficult to distinguish which environments are favorable for certain traits.

It is also worth noting that other factors (zooid size, colony angle, ability to produce stolons) also strongly affect competitive ability [16,22–24]. Since zooid size is a key predictor of competitive ability, defensive polymorphs may be more favorable in colonies with larger zooids. Size may also offset the energetic cost of defensive polymorphs: larger zooids with larger tentacle crowns could capture more food (as long as metabolic requirements scale sub-isometrically with zooid size). If true, then species with larger zooids could exhibit increased polymorphism. The above trends in competition only refer to encrusting taxa.

Erect colonies are able to escape competitive interactions by growing away from the substratum [7]. Erect growth may also allow colonies to invest more in defensive polymorphism – indeed, a significantly greater proportion of erect taxa have pointed and setiform avicularia compared to encrusting species (two tailed z-test of proportions, $p < 0.001$ in both cases, $n_{\text{erect}} = 211$, $n_{\text{encrusting}} = 385$). This may be particularly important for erect bryozoans, since their elevated profile and high surface area may attract more epibionts and fouling organisms.

4. Future directions

4.1 Avicularia

It is clear that future investigations into avicularian morphology and environmental conditions (both abiotic and biotic) need to consider 1) colony and zooid life span, 2) frequency of environmental change, and 3) degree of investment. Degree of investment could either be measured as the number of avicularia of each type per zooid (counting all zooids in the colony), or the number of avicularia of each type per zooid size class (which accounts for spatial requirements of different adventitious avicularia). Controlling each factor experimentally would be ideal. While much of the variation in avicularia shape is explained by changes in aspect-ratio and a general descriptor of shape (Chapters 2 and 4), geometric morphometrics should be employed to determine if differences between avicularium types are continuous or discrete.

As mentioned in Chapter 5, the closing speed of different avicularia needs to be measured in order to truly compare different mandible shapes. Mandibles with a high moment of inertia can reduce the energy required to close them by reducing their angular acceleration. Knowing closing speed will allow for more accurate energetic comparisons of different mandible types or avicularian functions. In addition, behavioral studies (similar to those of Winston [25]) should be performed to quantify the effectiveness of different avicularia at performing different functions / defending against different epibionts.

The multiple independent evolutions of avicularia [14] serve as natural replication studies. Comparing evolutionary trajectories (i.e. rate morphospace exploration over time) between different lineages could determine the intensity and direction of potential selection pressures and developmental constraints.

Thorough study of avicularian morphology and biomechanics is not just useful for bryozoology. Bryozoans are capable of synthesizing chemical defenses [26,27], some of which may be secreted by avicularia [17]. Linking certain mandible shapes to the production or distribution of chemical defenses can narrow the search for marine natural products. In addition, understanding rotational motion at low Reynolds numbers can inform the construction of micro-robots for health and pest-control applications.

4.2 Ecology

The ecological analysis in Chapter 4 is one of the most thorough explorations of the ecology of bryozoan polymorphism so far. However, this analysis needs to be followed up with additional information. Once a phylogeny of New Zealand cheilostomes is complete, it can be plugged into the existing trait/community database to determine whether phylogenetic signals are truly present in the data. The analysis in Chapter 4 is also mostly limited to New Zealand Cheilostomes, but could easily be expanded to include more taxa from Australia [28] or compared with well-sampled fauna from other oceans (e.g. the North Atlantic or Mediterranean).

Ideally, a replication study would also be carried out to confirm the results of Chapter 4. Such a study would collect new samples of both the bryozoan and predator communities around New Zealand. While bryozoans would need to be sampled directly, towed underwater cameras or ROVs could be used to determine predator composition and abundance. Sediment type, benthic chlorophyll, the concentration of oxygen and calcium carbonate, and CTD measurements should also be taken to verify the modeled environmental data. Each bryozoan specimen would need to be identified, and then classified under the SEM. Classifying bryozoans from samples, rather than literature, allows plasticity to be accounted for, and would allow the classification system presented in Chapter 3 to be used to its full extent. For example, rootlet morphology and spine length could be directly measured. Abundance of each species in each sample, as well as degree of investment in each polymorphism in each specimen, should also be calculated. The addition of genetic analysis on voucher specimens would strengthen the analysis further.

4.3 Universal assembly rules

Analyses in Chapter 4 demonstrated shifts in colony form with depth (sedimentation rate) and substratum type. Similar trait-environment relationships may exist for other sessile marine taxa, such as sponges and ascidians.

Deep-sea sponges often have a stalked or elongate colony morphology [29], analogous to erect bryozoans. An RLQ analysis of sponge growth forms in Indonesian reefs found that massive (encrusting and creeping) forms were associated with shallow, inshore environments with clear water. In contrast, erect forms (fistulose and fan-shaped) were found in deeper, offshore environments with fluctuating sediment loads [30]. Similarly, the sponge *Biemna fortis* adopts an erect growth form under high sedimentation rates, and a massive growth form in clearer water [31]. The massive growth forms of *Biemna fortis* are unable to survive if transplanted to high-sedimentation rate areas [31], suggesting that sedimentation rate represents a strong environmental filter on colony form across sessile organisms.

In addition to possessing erect growth, sponges subject to high sedimentation loads are often hairy or rough-textured, which entrains sediment and keeps their oscula unclogged [30]. Surface texture of bryozoans (modified by spines, protrusions, rugosity of the frontal shield, and vibracula) and other taxa may be an overlooked but important aspect of deep-sea survival.

Contrary to sponges and bryozoans, erect ascidians appear to be more adversely affected by increased sedimentation rates than flat-form ones [32]. Flat-form species have lower respiration rates (due to their unfolded branchial sacs), which allow them to tolerate low oxygen and wait-out high-sedimentation conditions [32]. Erect taxa have higher respiration rates (folded branchial sacs) and under high-sedimentation rates continue filtering water (resulting in hypoxia from sediment-clogged branchial sacs) or engage in squirting behavior until exhausted [32]. Therefore, ascidian colony form seems less important for surviving sedimentation than branchial sac morphology and behavior. Indeed, deep sea ascidians possess simplified branchial sacs to combat sedimentation [33]. Erect growth may still be necessary to prevent burial and increase feeding performance, since these deep-sea taxa often adopt a stalked growth form [33].

Like bryozoans, sponges and ascidians require specialized structures to anchor themselves to soft, deep-sea sediments. In sponges, these take the form of rootlets, spicule tufts, or the ability to agglutinate sediment [29], while ascidians generate rhizoid-like protrusions from their tunic [33].

The shift from encrusting-cemented to erect-anchored in bryozoans, sponges, and ascidians suggests that substratum type and sedimentation rate are universal environmental filters for communities of sessile, marine organisms. Confirming this “universal assembly rule” would require further investigation, but it could prove useful for studying deep-sea colonization events and “key innovations”, in addition to improving conservation efforts in an era of deep-sea mining.

4.5 Brooding shifts

One of the unique findings in Chapter 4 was the shift from internal to external brooding with increasing depth, wither due to predation or reduced oxygen. These factors may result in shifts in brooding mode in other taxa, like crinoids and sea anemones [34,35]. However, comparisons across taxa must be made with caution due to differences in energetic cost and evolutionary history. Both crinoids and sea anemones lack specialized structures for external brooding: released eggs/embryos simply adhere to the maternal surface [34,35]. This suggests that external brooding is less expensive in crinoids and sea anemones than in bryozoans, which can possess external ovisacs or calcified ovicells and gonozooids. And while bryozoans

exhibited an evolutionary shift from external to internal brooding, external brooding in sea anemones is thought to have evolved from internal brooding [35]. Despite these differences, comparing shifts in brooding mode across taxa can reveal which environmental pressures were important to the evolution of internal and external brooding in marine invertebrates.

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