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ORIGINAL ARTICLE

The evolution of colony-level development in the Siphonophora (Cnidaria:Hydrozoa)

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Abstract Evolutionary developmental biology has focused almost exclusively on multicellular organisms, but there are other relevant levels of biological organization that have remained largely neglected. Animal colonies are made up of multiple physiologically integrated and genetically identical units called zooids that are each homologous to solitary, free-living animals. Siphonophores, a group of pelagic hydrozoans (Cnidaria), have the most complex colony-level organization of all animals. Here the colony-level development of five siphonophore species, strategically sampled across the siphonophore phylogeny, is described from specimens collected using deep-sea submersibles and by self-contained underwater breathing apparatus diving. These species include three cystonects, *Bathyphysa sibogae*, *Rhizophysa filiformis*, and *Rhizophysa eysenhardti*, and two "physonects", *Agalma elegans* and *Nanomia bijuga*. These data, together with previous findings, are analyzed in a phylogenetic framework to reconstruct key features of the history of colony-level organization and development in the Siphonophora. It is shown that gonodendra and gastrozooids of the examined cystonects arise as independent buds directly on the stem, whereas probud subdivision (the origin of feeding, reproductive, and other zooids from a single bud) is a synapomorphy of the Codonophora. The origin of probud subdivision is associated with the origin of cormidia as integrated units of colony organization, and may have allowed for greater morphological and ecological diversification in the Codonophora relative to the Cystonectae. It is also found that symmetry is labile in siphonophores, with multiple gains and/or losses of directional asymmetry in the group. This descriptive work will enable future mechanistic and molecular studies of colony-level development in the siphonophores.

Keywords Major transition in evolution · Asexual reproduction · Animal colonies · Division of labor · Functional specialization

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Introduction

The siphonophores, a group of pelagic colonial hydrozoans (Cnidaria), include the longest animals in the world (Robison 1995) and are among the most abundant carnivores of the open ocean (Pugh 1984). Even so, they have largely escaped the public eye, and many biologists are not aware of their existence. Siphonophores have not always been so obscure. They were of central interest to zoologists of the nineteenth century because of another distinguishing feature—they are the most complex of all colonial animals. Colonial animals have a life cycle wherein multiple asexually produced zooids, each of which is homologous to a free-living solitary animal, remain attached and physiologically integrated throughout their lives. Siphonophores have both the highest degree of functional specialization between zooids and the greatest precision of colony-level organization of any group of

colonial animals (Beklemishev 1969; see Fig. 1 for a schematic of the organization of a siphonophore colony).

There has been much interest in the origin of developmental mechanisms and organization at new hierarchical levels of individuality (Buss 1987; Maynard Smith and Szathmáry 1995). Most of this work has been concerned with the transition from unicellularity to multicellularity (Bonner 2001) and includes important empirical studies on diverse multicellular organisms such as *Volvox* and *Dictyostelium*. It will not be possible, however, to know whether phenomena revealed by these studies are unique to the transition from unicellularity to multicellularity or are general properties of transitions in biological organization unless there is an empirical push to characterize development and morphology at other levels of biological organization. These necessarily include coloniality, and the extreme form of colonial organization found in siphonophores promises to be particularly informative.

There have been few studies of the colony-level development and organization of siphonophores largely due to the difficulty of collecting them. Although they are often large and relatively abundant, siphonophores are also extremely fragile, and most species are found only in the open ocean. There are published accounts of the early embryology of several siphonophores (Carré and Carré 1993; Gegenbaur 1853; Haeckel 1869), but these studies stopped short of describing the origins and structure of the growth zones that are responsible for the colony-level development that continues throughout the life of a siphonophore. The colony-level development of only three species has been described (Chun 1885; Dunn 2005; Schneider 1896). These three Codonophora taxa share a common mode of development called probud subdivision. Probuds arise sequentially within the growth zone, which is also the site of stem elongation. Each cormidium, the well-defined group of multiple zooids that is reiterated along the length of the siphosomal stem (Fig. 1), arises from a probud through a stereotypical series of subdivisions.

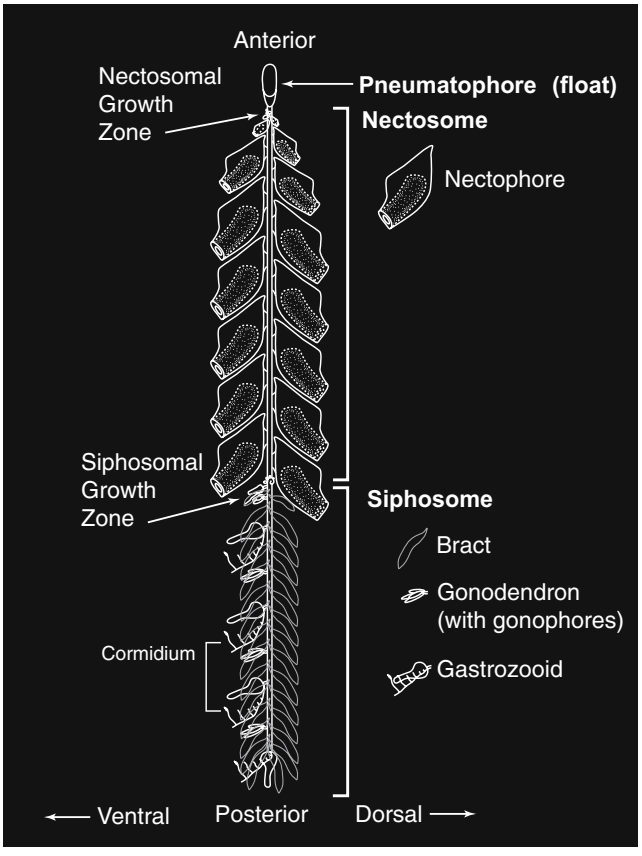

Fig. 1 Schematic overview of siphonophore structure modified from Dunn (2005). The depicted colony is a physonect and possesses a pneumatophore (gas-filled float), nectosome (a region with nectophores, the propulsive zooids), and siphosome (a region with zooids of mixed functional types). Cystonects lack a nectosome, whereas calycophorans lack a pneumatophore

The ability to safely dive using self-contained underwater breathing apparatus (SCUBA) from ships in the open ocean (Hamner 1975) and the refinement of midwater sampling devices that can be mounted on submersible vehicles (Youngbluth 1984) now make it possible to collect many species of siphonophores intact for the first time. These new tools have enabled us to make the first comparative study of siphonophore colony-level organization and development that draws on taxa sampled across the full diversity of the group. In conjunction with a recent molecular phylogeny (Dunn et al. 2005), this allows us to reconstruct the history of major changes in colony-level development and organization within siphonophores and to begin to understand how a diversity of form has been realized at this poorly understood level of biological organization. This descriptive work also provides a foundation for future molecular and mechanistic studies of siphonophore development that are relevant to questions of general interest regarding the origins and evolution of individuality.

Materials and methods

Fresh specimens were collected by manned submersibles, remotely operated vehicles, blue-water diving, and land-based diving. This material was prepared for scanning electron microscopy (SEM) as previously described by Dunn (2005). Preserved siphonophores from Dr. Philip R. Pugh's collection (most of which had been fixed with 4% formaldehyde in borax-buffered seawater) were also successfully prepared for SEM despite the fact that some specimens were decades old. The same protocol was used as for fresh material, beginning part way through with the 500-mM NaCl wash.

The budding sequence of each species was inferred through static observations of the ontogenetic sequence of developing zooids. The organization of zooids along a mature portion of the stem was first established. The regularity of siphonophore organization made it possible to then determine the identity of immature zooids further to the anterior based on their relative position.

Results

A note on terminology

Haddock et al. (2005) consolidated and standardized the usage of terms for axes and orientation in siphonophores, and the present article adheres to this clarified scheme. The nomenclature used to describe the functionally specialized zooids of siphonophores is similar to that used for the Hydrozoa in general, but also includes several terms that are specific to the group (Dunn 2005). The term gonodendron is used here to describe any compound reproductive structure consisting of multiple zooids that are attached to the stem via a common peduncle. The structure and zooid composition of gonodendra vary widely across taxa; it is not at all clear if they are homologous across siphonophores or have independently arisen multiple times.

Material examined

Specimen data for the examined material are listed in Table 1. Specimens of *Apolemia* sp. and the cystonects are from Philip R. Pugh's collection. Most *Agalma elegans* material was collected in the bay at Villefranche-Sur-Mer, France, in the months of March–May in 2003 and 2004; the remaining *A. elegans* specimens were collected by blue-water diving from the *RV Oceanus* in the summers of 2000–2002 along the east coast of the USA. *Nanomia bijuga* and *Forskalia formosa* were both collected in Monterey Bay, CA, and the bay at Villefranche-Sur-Mer, France. These newly acquired specimens have been deposited in the Yale Peabody Museum (catalog numbers can be found in Table 1). The *Lychnagalma utricularia* specimen quickly deteriorated after being caught and was not preserved.

Table 1 Specimens examined

Species	Specimen	Collection
Bathyphysa sibogae	BWP796-20-7	PRP
Rhizophysa filiformis	BWP1077-3	PRP
	BWP567-8	PRP
	BWP566-17	PRP
	BWP566-16	PRP
	BWP588-21	PRP
	BWP442	PRP
Rhizophysa eysenhardti	BWP539-13	PRP
	BWP1611-12	PRP
	BWP634-6	PRP
	BWP741-3	PRP
	BWP778-12	PRP
	BWP465	PRP
	BWP465	PRP
	BWP802-1	PRP
	BWP1086-6	PRP
	BWP814-12	PRP
Agalma elegans	YPM 36365-36390	YPM
Nanomia bijuga	YPM 36427-36444	YPM
Apolemia sp.	JSLII 1450-SS3	PRP
Forskalia formosa	YPM 36445-36448	YPM
Lychnagalma utricularia	Tiburon 676-D6	(specimen destroyed)

The numbers given in YPM rows are museum catalog numbers. The specimens in PRP's collection are identified by their mode of collection and a unique identifier.
PRP Personal collection of Philip R. Pugh (National Oceanographic Centre, UK); *YPM* the Yale Peabody Museum (New Haven, CT); *BWP* blue-water plankton series; *Tiburon* the remotely operated vehicle *Tiburon* (Monterey Bay Aquarium Research Institute); *JSL II* the manned submersible *Johnson Sealink II* (Harbor Branch Oceanographic Institute)

Bathyphysa sibogae

The gastrozooids and gonodendra of *Bathyphysa sibogae* were found to be in a uniserial sequence (Fig. 2b). The gastrozooids first appear as isolated buds at the anterior end of the siphosome and grow a tentacle on their anterior side as they mature and are carried to the posterior by the elongating stem. The first several gastrozooids at the anterior end of the sequence (i.e., the youngest gastrozooids) have no sign of gonodendra between them. Gonodendra arise further to the posterior as isolated buds between and in line with the maturing gastrozooids.

The young gastrozooids of *B. sibogae* have pronounced lateral ridges known as ptera (Leloup 1936), which give the gastrozooids a bract-like appearance. The striking similarity of these *Bathyphysa* gastrozooids to "physonect" bracts may indicate that physonect bracts are in fact modified polyps. Each young gastrozooid also has a lamella extending part way up its posterior side and further anchoring it to the stem, just as physonect bracts do. This holds the young gastrozooid so that its distal end faces to the posterior. Although conspicuous in young gastrozooids, the ptera and lamellae are absent by the 30th gastrozooid in the examined specimen.

Leloup (1936) figured and described the early stages of development of *Bathyphysa* gonodendra. He did not, however, trace the fate of the young buds or describe their

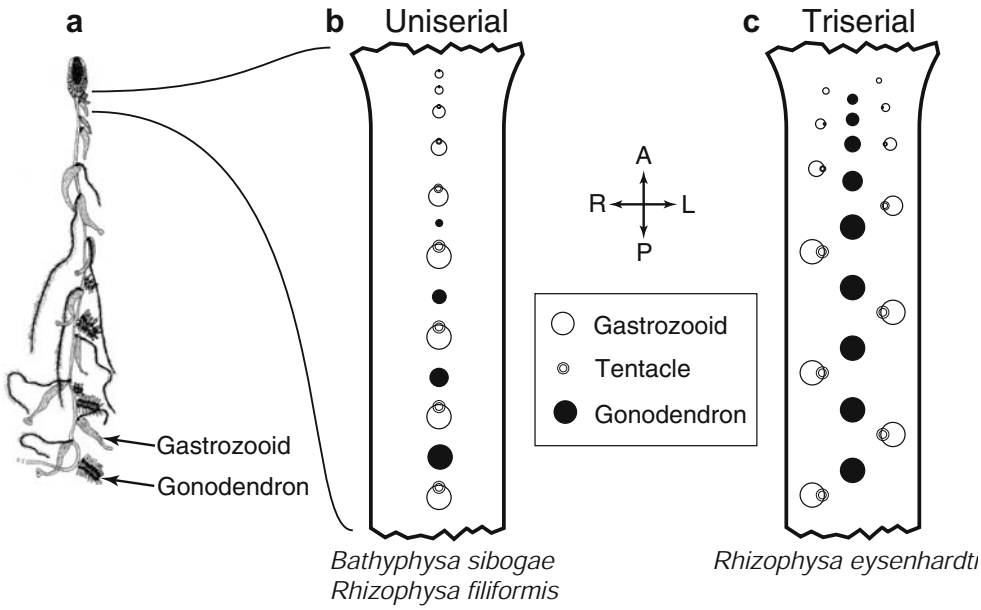

Fig. 2 Schematic illustrations of the budding sequences of long-stemmed cystonects (the Rhizophysidae). (**b**, **c** ventral view) **a** *Rhizophysa eysenhardti* (adapted from Kawamura 1910). **b** Uniserial budding, in which the gastrozooids arise in a single line, and the gonodendra are later intercalated between them. The tentacles are borne on the anterior side of the gastrozooids. **c** Triserial budding, in which the gastrozooids arise in two outer rows, and the gonodendra are found in a row between them. The tentacles are borne on the side of the gastrozooid facing the ventral midline

later stages of development. The material examined here was in good condition, and the full ontogenetic sequence of gonodendron development was described (Fig. 3). The youngest gonodendron buds are at first simple, smooth evaginations that protrude from the stem (Fig. 3a). These then elongate and become warty in appearance (Fig. 3b,c). Each wart-like protuberance then elongates and develops into a gonodendron branch (Fig. 3d–g). The branches do not ramify further, and are all attached directly to the central style (i.e., the main axis of the gonodendron). A single bud first arises on the side of the branch (Fig. 3d). This bud takes on a distinctive shape and matures into a nectophore. The portion of the branch distal to the nectophore matures into the palpon that terminates the branch. Gonophores then form from evaginations that arise along the branch (Fig. 3e), some of which arise distal to the nectophore. Each branch bears on the order of seven to nine gonophores at maturity. The gonodendra of this species,

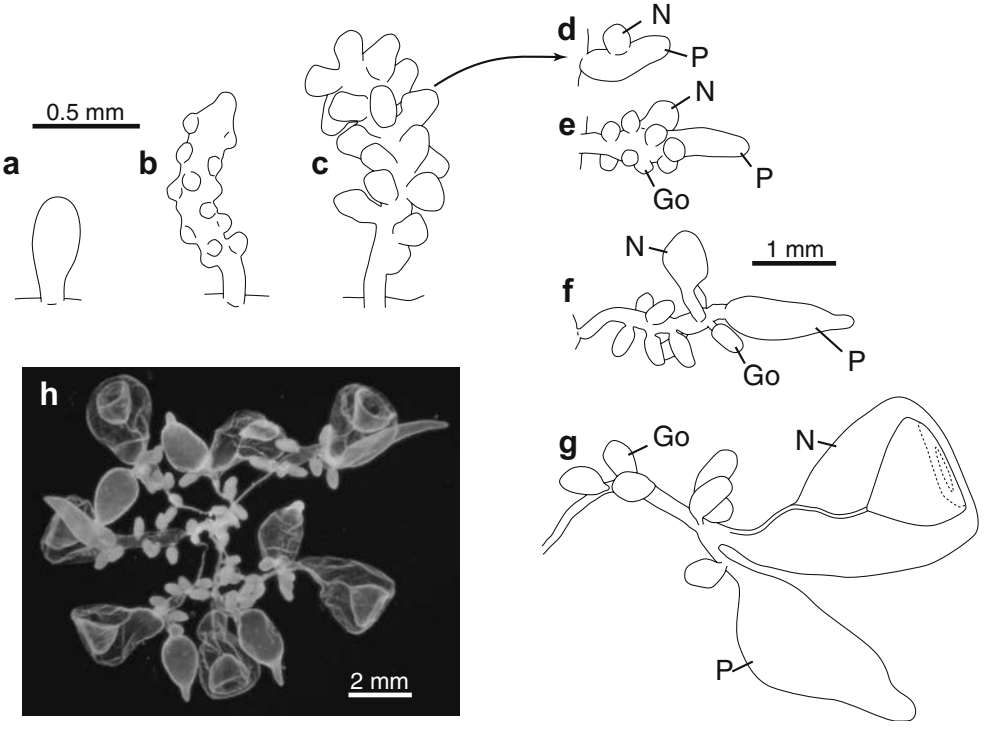

Fig. 3 Developmental sequence of *Bathyphysa sibogae* gonodendron. **a**–**c** Young gonodendra showing the origin of the side branches as evaginations of the main gonodendron axis. **d**–**g** Close-ups of isolated side branches following the developmental stage shown in (**c**). **h** Photograph of preserved gonodendron. *Scale bar* (0.5 mm) applies to **a**–**e**, *Scale bar* (1 mm) applies to **f**, **g**; *scale bar* (2 mm) applies to **h**. *Go*, gonophore; *N* nectophore; *P* gonopalpon

like those of the other Cystonectae, are thought to break away from the colony at maturity before releasing their gametes (Totton 1965, P.R. Pugh, personal communication).

Rhizophysa filiformis

All examined specimens of *Rhizophysa filiformis* had the same uniserial development of gastrozooids and gonodendra as *B. sibogae* (Fig. 2b). There were from one to six gonodendra found between the mature gastrozooids. The gonodendra develop in the same manner as those of *B. sibogae* (Fig. 3) and have the same general structure at maturity. The gastrozooids of *R. filiformis* do not have pronounced ptera at any stage.

Rhizophysa eysenhardti

The origin of the siphosomal elements of *Rhizophysa eysenhardti* is triserial (Fig. 2c). The buds of the two outer rows give rise to gastrozooids. The gastrozooids are staggered with respect to each other. The single tentacle of each gastrozooid forms on the side facing the midline. When the gastrozooids are mature, it is not entirely obvious that they arose in two separate rows, but the alternate attachment of the tentacle to the left and right sides of the gastrozooids is clearly discernable.

The median row of siphosomal elements, found between the left and right rows of gastrozooids, consists solely of gonodendra. Gonodendron buds are found just as far to the anterior as the gastrozooid buds are. The gonodendron buds are not exactly in phase with the gastrozooid buds, and there are a variable number of gonodendra between gastrozooids at maturity (but always at least one). The gonodendra of *R. eysenhardti* develop in the same manner as those of *B. sibogae* (Fig. 3).

Agalma elegans

Our observations of the zooid organization of *A. elegans* (Fig. 4b,c) are consistent with Totton's (1954, frontispiece—reproduced here as Fig. 4a). Each cormidium consists, from posterior to anterior, of a large palpon (marked as "B" in Totton's (1954) frontispiece so it will be referred here as the B-palpon), a female gonodendron with an associated palpon, a gastrozooid with multiple gastric palpons attached to its peduncle, and several clusters of male elements. Each cluster of male elements has a palpon, several male gonophores, and bracts in various stages of development. Bracts also are spread along the full length of the stem. Each cormidium can span up to 4 cm or more of the stem, and there are few developing cormidia within the growth zone. This makes the inference of the budding sequence quite difficult in this species because there are few intermediate stages of cormidial development in each colony. It also implies that zooids may be added to the colony at a very slow rate.

There is not a pronounced horn (sensu, Dunn 2005) in the siphosomal growth zone of mature specimens of *A. elegans*, although the tip of the siphosomal growth zone does form a slight overhang (Fig. 4d–j, marked "T" in each pane). Totton (1956) noted a well-developed horn (which he called a nectostyle) in the larvae of this species, so it seems to recede as the colony matures. The anteriormost-differentiated cormidium consists of six buds on a common peduncle (as seen in cormidium 1 of the specimens figured in Fig. 4d–g and h–j). The largest of these is a bud that was inferred to be the gastrozooid based on its rounded shape and consistent position relative to the other buds. There are two buds on the anterior side of the gastrozooid base. The proximal bud, which was identified at various stages of development by its anteriormost position within the cormidia, differentiates into the primary male reproductive elements. The distal bud on the anterior side of the gastrozooid peduncle differentiates into one, or perhaps several, gastric palpons. There is also a bud on the left side of the gastrozooid base at the same level as the bud of the male reproductive elements. This lateral bud was inferred to be a bract based on the differentiation of buds in the same position in more posterior cormidia. Finally, there are two buds to the posterior of each young gastrozooid. The one furthest to the posterior had a characteristic shape even early in development that indicated that it was a palpon, and its precocious development and location indicate that it is the B-palpon. Between the B-palpon and the gastrozooid is a bud that in posterior cormidia differentiates into the female reproductive elements. The B-palpon grows larger than all the other zooids of a cormidium by the point where the cormidium is second in the sequence. Totton (1954) considered the gastrozooid to be the posterior element of each cormidium. In light of the developmental sequence, we differ with him on this point and consider the B-palpon to be the posteriormost structure.

In addition to the bract that arises on the left of the base of each gastrozooid, there is a lateral row of bracts on both the left and right sides of the stem. These bracts are further from the ventral midline than any other zooids and are more developed than adjacent bracts arising from the base of the gastrozooids. No buds that could give rise to these lateral bracts were ever observed within the derivatives of the probud, and the lamellae of these bracts sometimes extended along the stem slightly to the anterior of the tip of the siphosomal growth zone. These findings suggest that they arise directly on the side of the stem.

The male bud comes to be attached to the stem independently from the gastrozooid, gastric palpon, and gastrozooid-associated bract, and the female bud comes to

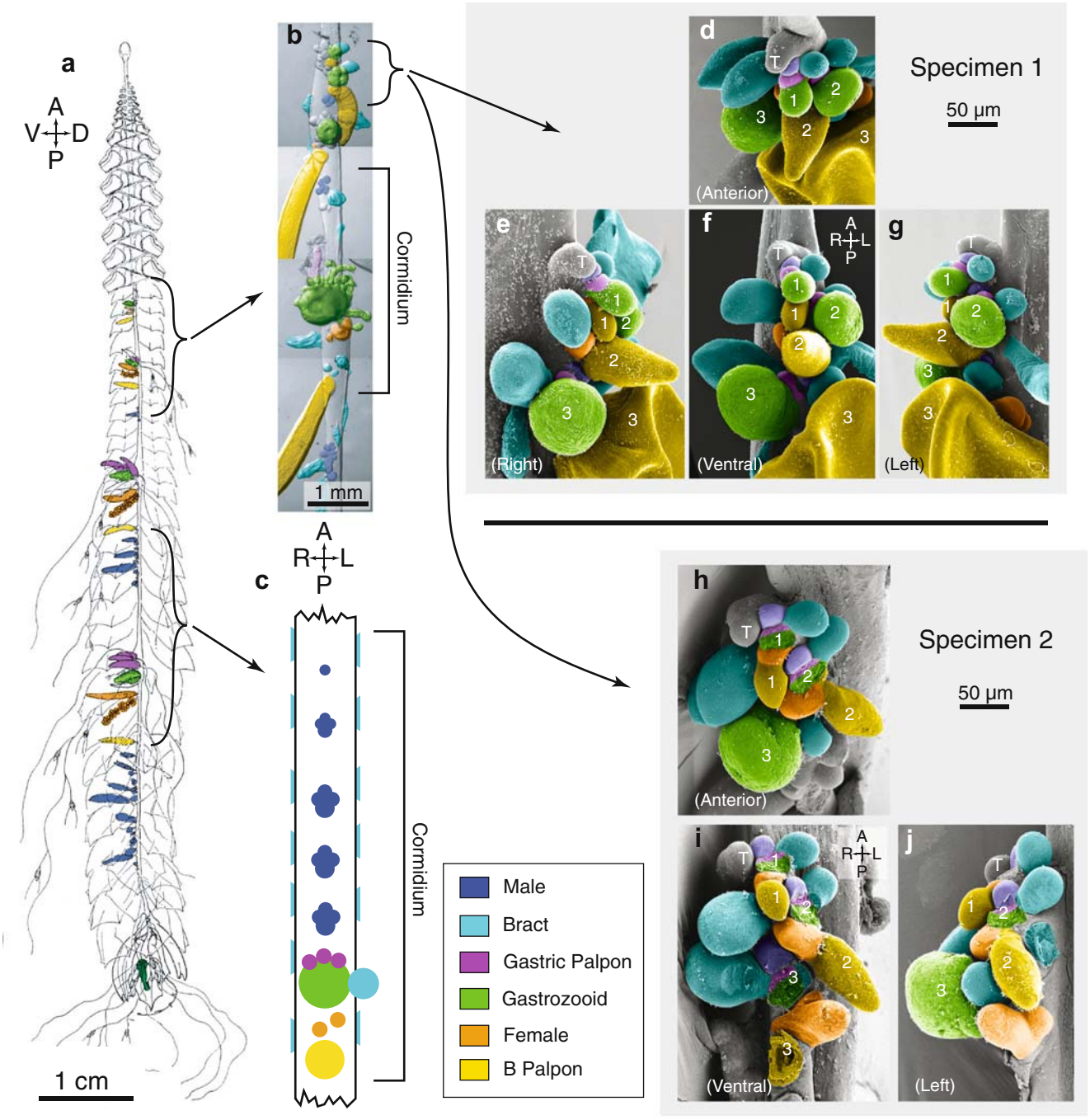

Fig. 4 *Agalma elegans*—false coloring indicates zooid identity. Anterior faces the *top* of the page, unless noted otherwise. **a** Left lateral view of the mature colony (adapted from Totton 1954). The bracts, which sheathe the siphosome, have not been colored. **b** Photographic collage showing the growth zone and young siphosomal stem of an anesthetized living specimen. *Ventral view*. Note that both the female gonodendron and the female-associated palpon have been colored *orange*, and that the male gonophore and male associated bract and palpon buds are colored *blue*. Buds whose fate could not be assigned with confidence have been left *uncolored*. **c** Schematic of a mature corniculum. *Ventral view*. The lateral bracts are not shown in their actual position along the stem relative to the other zooids. **d**–**g** SEM of the growth zone of specimen 1, shown from four different views. **h**–**j** SEM of the growth zone of specimen 2, shown from three different views. In **d**–**j**, the tip of the growth zone is labeled with a T, and the gastrozooids and B-palpons are numbered according to the cormidium they belong to, beginning with the first differentiated cormidium. Gastrozooids 1–2 and B-palpon 3 have been broken away (**h**–**j**). Additionally, gastrozooid 3 has been broken away (**i**). Where young gastrozooids were broken away, the buds of the gastric palpons, and sometimes the left bracts, also broke off with them. *A* Anterior; *D* dorsal; *L* left; *P* posterior; *R* right; *V* ventral

be attached to the stem independently from the B-palpon (this is most easily seen in Fig. 4i). Bracts later bud at the foot of the B-palpon. After the female bud separates from the B-palpon, it takes on a bilobed shape (as in cormidium 3 of Fig. 4i,j), and further to the posterior, one lobe was seen to be the female-associated palpon and the other the female gonodendron (not shown). These are attached to the stem independent from each other at maturity. Close to the growth zone, there is only one male cluster per cormidium. In cormidia slightly to the posterior, it can be seen that a new male cluster arises directly on the stem, separate from and to the anterior of the original one. This process repeats several times, with new male clusters being added anterior to the existing ones.

There are multiple gastric palpons attached to the peduncle of gastrozooids in mature cormidia. It was not determined if these arise by subdivision of the single gastric palpon bud that arise within the growth zone or if each gastric palpon arises as an independent bud on the peduncle of the gastrozooid. There are more gastric palpons in cormidia further to the posterior.

Large specimens of *A. elegans* have an irregular organization of zooids in their posterior cormidia, but in all examined cases, the irregularity was consistent with zooid loss. In these specimens, cormidia close to the growth zone still have the regular organization originally described by Totton (1954) and reconfirmed here.

The nectosomal growth zone of a single specimen was examined using SEM. It was found that each young nectophore had a small bud on the posterior side of its peduncle. A similar bud has previously been found in the nectosomal growth zone of *Bargmannia elongata* (Dunn 2005).

Nanomia bijuga

Totton (1965, Fig. 35) figured the organization of zooids within a cormidium of *N. bijuga*. He found that each cormidium consisted of a gastrozooid and a series of palpons, all attached independently to the stem. A female gonodendron and a cluster of male gonophores flanked each palpon and alternated sides from palpon to palpon. Totton noted that secondary palpons, also with male and female structures at their base, were sometimes intercalated between the primary palpons in mature cormidia.

Our own observations of *N. bijuga* confirm Totton's earlier findings. In addition, we are also able to describe the budding sequence by which the zooids of this species arise. There is a pronounced horn within the siphosomal growth zone, and each cormidium arises as a simple probud close to its tip (Fig. 5d). The bulk of the probud gives rise to the gastrozooid, with most of the other zooids arising on the anterior side of its peduncle. A palpon and two flanking bracts are the first such zooids (Fig. 5e). Additional bracts are added on both sides of the gastrozooid peduncle, with the youngest (i.e., the smallest) being the most distal (Fig. 5f). Additional palpons bud on the anterior side of the primary palpon, and all palpons initially share a common base and form a fanlike structure (Fig. 5g). The original palpon is the most distal in this structure, and the smallest (youngest) is the most proximal. A lateral bract forms on each side of each palpon as it matures.

The zooids of successively more mature cormidia come to be spread out along the stem such that each zooid is eventually attached to the stem independently (Fig. 5h,i). The bracts that arose on the gastrozooid peduncle come to be arranged in two lateral rows that flank all the other zooids (the lamellae of the left row of bracts can be seen in Fig. 5i). The oldest (i.e., largest and most proximal) bract to arise on the gastrozooid peduncle comes to be located furthest to the anterior, and the youngest (i.e., the smallest and most distal) remains the closest to the gastrozooid. As the palpons spread out, the youngest (i.e., the smallest and most proximal) comes to be located the furthest to the anterior of the cormidium, and the first palpon to arise (i.e., the largest and most distal) remains closest to the gastrozooid. The lateral bracts associated with each palpon move away from the ventral midline and come to be arranged in rows just inside of the rows of bracts that arose on the gastrozooid peduncle. Kawamura (1911) had already noted that the bracts of this species are arranged in two rows along each side of the stem.

The male and female gonodendra arise at the base of the palpons just inside of each palpon-associated bract (Fig. 5i). Additional clusters, each consisting of a palpon, a male gonodendron, a female gonodendron, and bracts, are added directly on the stem at the anterior end of each cormidium after all zooids have spread out. Clusters may also sometimes be added between existing palpons.

There is a single tentacle on the anterior side of each gastrozooid. Likewise, each palpon has a palpacle (as the tentacle of a palpon is called) on its anterior side. Tentacles and palpacles form as simple evaginations. The bud of the gastrozooid tentacle can be seen in Fig. 5f–h, and the base of the tentacle, which has been broken away, can be seen in Fig. 5i. The palpacle rudiments can be seen in Fig. 5i. Some palpons also have a large basal swelling on their anterior side just distal to the palpacle.

Apolemia sp.

One specimen of *Apolemia*, belonging to the same undescribed species as "*Apolemia 3*" in the molecular

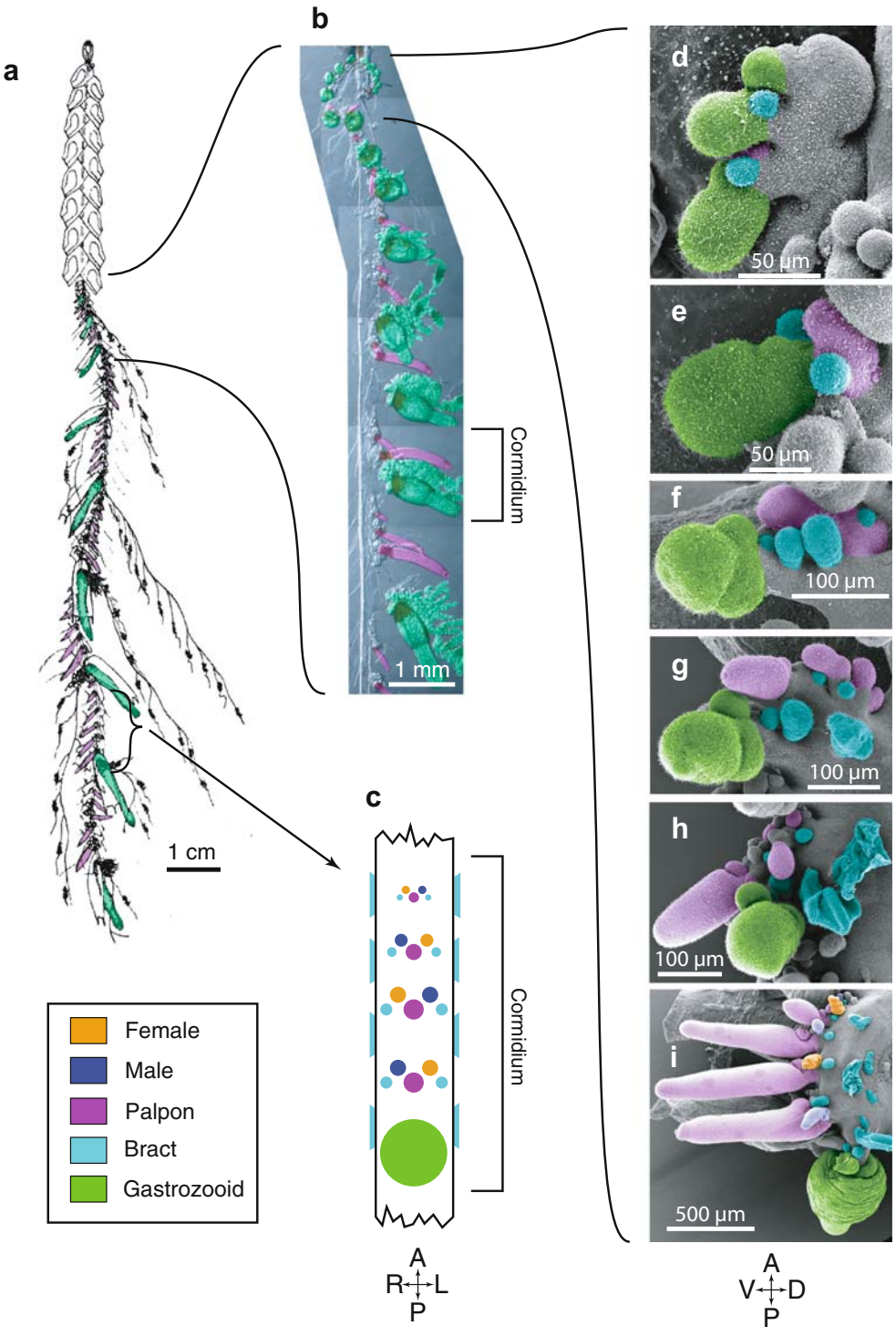

Fig. 5 *Nanomia bijuga*. False coloring indicates zooid identity. Anterior faces the *top* of the page in all panes. **a** Mature colony (adapted from Kawamura 1911). The stem is twisted, but the view is generally lateral. **b** Photographic collage showing the growth zone and young cormidia of an anesthetized living specimen. The stem is twisted such that the youngest cormidia are shown from their left side, and the older (posterior) cormidia are shown from their right side. **c** Schematic of a single mature cormidium. *Ventral view*. The lateral bracts are not shown in their actual position along the stem relative to the other zooids. **d**–**i** SEMs of the youngest cormidia, shown from their left side in an ontogenetic sequence (from *anterior* to *posterior*). The largest bracts have been removed, leaving only their lamellae. **d** The three youngest cormidia at the tip of the horn; all other *panes* show only one cormidium. *D* Dorsal; *L* left; *R* right; *V* ventral

phylogeny of Dunn et al. (2005), was prepared for SEM. The siphosomal growth zone was extremely dense, with many tightly packed cormidia. There was a pronounced horn, and the young cormidia close to the tip were regular in organization and appeared to arise by probud subdivision (data not shown). The budding sequence and mature organization of cormidia were not determined.

Forskalia formosa

SEM of the siphosomal growth zone of *F. formosa* indicates that the zooids arise by probud subdivision (not shown), although we did not describe the exact budding sequence. The mature cormidia consist of a posterior gastrozooid, a palpon, and a gonodendron bearing several gonopalpons and gonophores (Fig. 6). This is consistent with previous

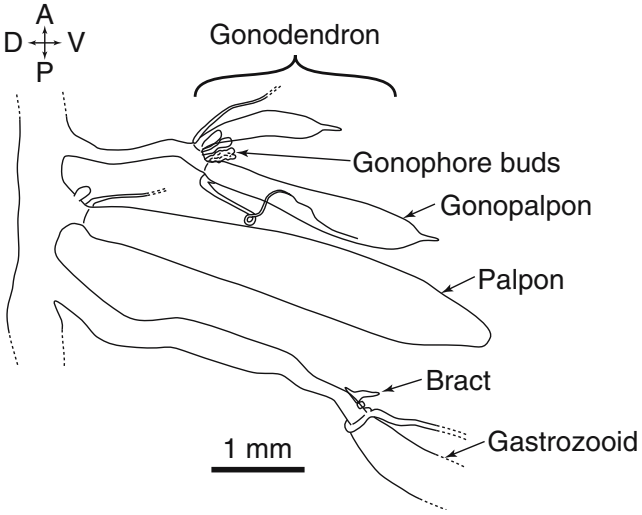

Fig. 6 Schematic of a single cormidium of *Forskalia formosa* shown from the right side. Multiple bracts are attached along the gastrozooid peduncle (not shown). The gonodendron is immature. *A* Anterior; *D* dorsal; *L* left; *P* posterior; *R* right; *V* ventral

descriptions of the cormidia of other species of *Forskalia* (Pugh 2003). The gastrozooids bear multiple bracts along their peduncles, and there are several small buds and juvenile bracts adjacent to the gastrozooid. The peduncle of the palpon does not significantly elongate. The gonodendra we observed were immature, so it was not possible to describe their organization in detail. It was clear that the gonodendron arises from the base of the palpon. The peduncle of the gonodendron appears to be an evagination of the stem, which at first bears only a single gonopalpon. Gonophores and further gonopalpons are added later in development.

SEM of the nectosomal growth zone revealed a simple bud on the posterior base of the peduncle of each young nectophore, as was the case for *A. elegans*.

Lychnagalma utricularia

We were able to make preliminary notes on the organization of the cormidia of *L. utricularia*, although we have not yet looked at the siphosomal growth zone. We found that the gastrozooids are borne on long peduncles, as had been noted previously (Pugh and Harbison 1986). We also found that there is a palpon at the distal end of this peduncle just proximal to the gastrozooid. This gastric palpon is associated with at least one bract, and several other bracts are scattered along the length of the peduncle. Palpons, male and female gonodendra, and bracts are attached to the stem between the gastrozooid peduncles. There were no palpons within gonodendra of either sex. Gastrozooids alternate with large palpons close to the growth zone.

Discussion

Siphosomal elements arise independently in the Cystonectae

Gastrozooids and gonodendra arise independently on the stem in the three long-stemmed cystonects examined here. The arrangement of these elements can be either uniserial or triserial (Fig. 2). The independent origin of the gonodendra and gastrozooids, as well as the variable number of gonodendra between gastrozooids within a specimen, bring into question the existence of cormidia in a biologically relevant organizational sense in these taxa.

There are five valid species of cystonects in total, two of which were not examined. Of these, preliminary observations indicate that the siphosomal elements of *Bathyphysa conifera* may arise in a uniserial pattern (P.R. Pugh, personal communication). Totton (1960) made an excellent description of the gross organization of *Physalia physalis* (the Portuguese Man o' War), the only cystonect that lacks a long stem. Although his description of colony-level development is incomplete, it is sufficient to determine that budding in this species is substantially different from that in the long-stemmed cystonects examined here.

Probud subdivision is a derived, shared mode of development for the Codonophora

The present study has described the origin of cormidia by probud subdivision in *A. elegans* and *N. bijuga*, and established that it is the mode of cormidial development in *F. formosa* and an undescribed species of *Apolemia*. The phylogenetic distribution of these taxa, and of those for which previous descriptions of colony-level development are available, indicates that probud subdivision is a synapomorphy of the Codonophora (Fig. 7).

Small buds were found to the posterior of young nectophores in both of the Codonophora taxa whose nectosomal growth zones were examined (*A. elegans* and *F. formosa*). Such a bud was previously found in the nectosome of *B. elongata* (Dunn 2005), and the nectophores of *Apolemia* are known to alternate with nectosomal polyps (Totton 1965). These findings indicate that the organization of the nectosome is more complex than had previously been appreciated. It may be that the nectosome arose by tandem duplication of the siphosome and subsequent simplification.

The developmental underpinnings of organizational diversity in the Codonophora

The molecular phylogeny (Dunn et al. 2005) suggests a single transition from dioecy to monoecy within the

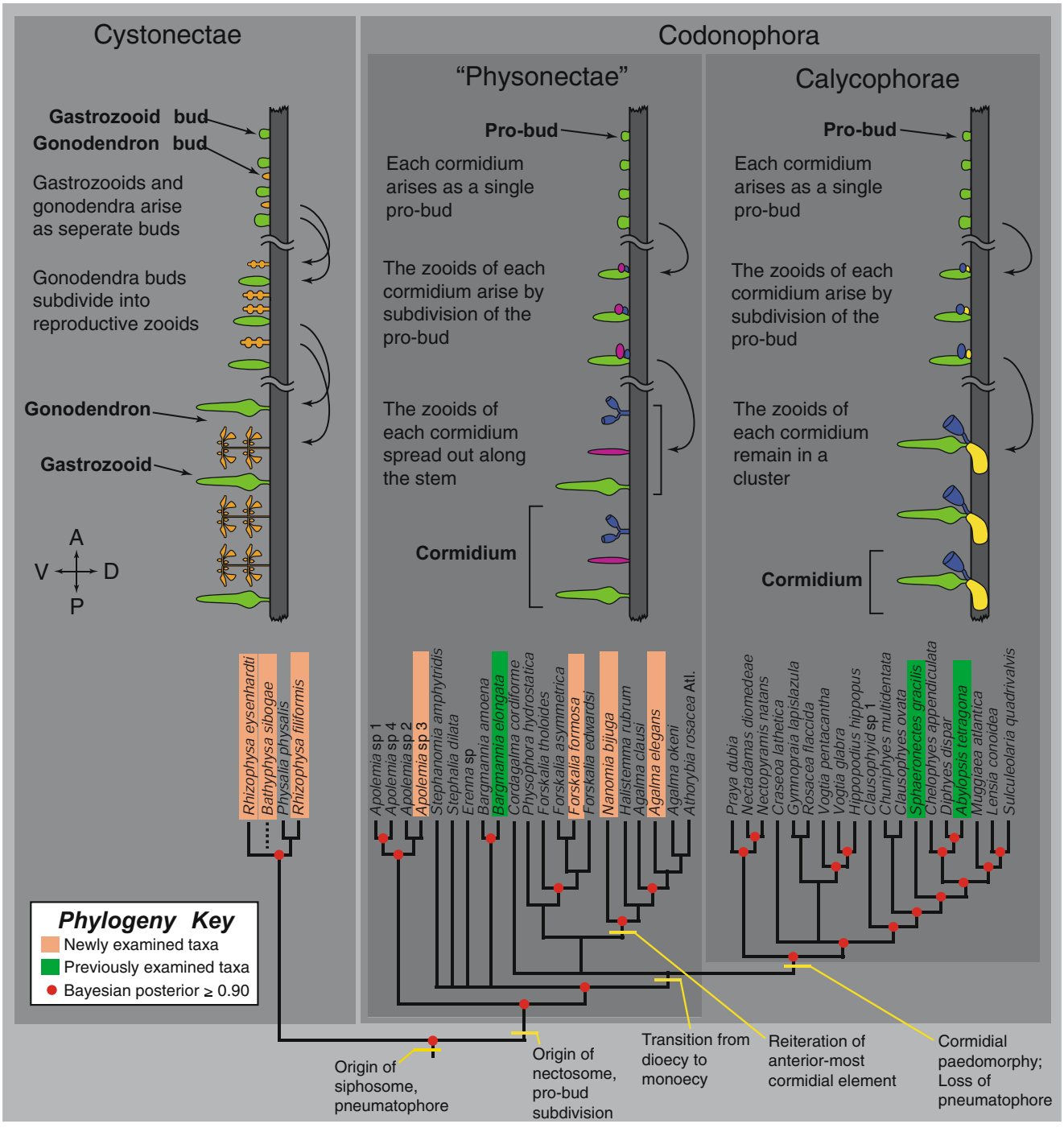

Fig. 7 Hypothesized phylogenetic reconstruction of the history of several important transitions in siphonophore colony-level organization. Those taxa that have been examined in sufficient detail to determine the presence or absence of probud subdivision are indicated with *boxes*. The rooted molecular phylogeny (using data from 16S and 18S) is from Dunn et al. (2005). The topology of the tree presented here differs from that in Dunn et al. (2005) in that the basal polytomy in the sister group to *Apolemia* has been resolved to show the monoecious species as being monophyletic. The previous molecular data were consistent with this topological hypothesis, but did not favor it more than other hypotheses where monoecy arose more than once. *Bathyphysa sibogae* was not included in the molecular phylogenetic analysis; in this figure, it is shown with the other cystonects

Codonophora (Fig. 7), although the relevant nodes are not well supported, and this hypothesis should be treated provisionally until more data are available. Within a dioecious species, colonies of different sexes are organizationally the same and differ only in the sex of the gonophores (Dunn 2005). Monoecious colonies are more complex in that both male and female gonophores must be organized along the stem. In the Calycophorae, each

cormidium bears only male or female gonophores (Totton 1965). Within the monoecious "physonects", each cormidium bears male and female gonophores. The organization and developmental origins of the male and female elements are very different in the two monoecious "physonects" examined in detail here, so better descriptions of colony-level organization in other monoecious "physonects" will need to be made before it is possible to make specific hypotheses regarding the origin (or origins) of monoecy.

The events following probud subdivision differ somewhat between the "Physonectae" and the Calycophorae. In the "Physonectae", the zooids within a cormidium spread out along the stem, whereas in the Calycophorae, the zooids of a cormidium remain attached to the stem by a common peduncle. The molecular phylogeny indicates that the calycophoran state is derived (Fig. 7). This derived calycophoran arrangement could have arisen by cormidial paedomorphosis, whereby cormidial development is truncated before the expansion of zooids along the stem, although the zooids within the cormidia mature fully.

Siphosomal budding has now been described in three physonect species, *B. elongata*, *A. elegans*, and *N. bijuga*. *Agalma elegans* and *N. bijuga* both belong to the Agalmatidae sensu stricto and are more closely related to each other than to *B. elongata* (Dunn et al. 2005). The cormidial organizations of *A. elegans* (Fig. 4c) and *N. bijuga* (Fig. 5c) are quite different. They are, however, developmentally similar in that some zooids are intercalated at well-defined locations within the cormidium and arise directly on the stem. In both of these species, the intercalated structures are reiterations of the anteriormost derivative of the probud (this is the cluster consisting of a palpon, bracts, and male gonophores in *A. elegans* and the cluster consisting of a palpon, male and female gonophores, and bracts in *N. bijuga*). Even when zooids arise directly on the stem outside of the growth zone, they do so at well-defined locations within the cormidia.

Directional asymmetry of siphonophore colonies

Dunn (2005) described the directional asymmetry of the cormidia of *B. elongata*, noting that many other descriptions of directional asymmetry are scattered throughout the siphonophore systematics literature. No directional asymmetries were identified in any of the Cystonectae examined here. Within the Codonophora, no directional asymmetries were found in *N. bijuga*, but there was a bract on the left side of each gastrozooid in *A. elegans*. Although data on directional asymmetries are still sparse, these findings suggest that directional asymmetry has been gained and/or lost multiple times within the siphonophores.

Conclusions

Comparisons between the Codonophora and Cystonectae, the broadest contrast that can be made among extant siphonophores, reveal some key differences in colony-level organization and development. The same two reiterated elements, the gastrozooids and gonodendra, are present in all long-stemmed cystonects. These elements arise independently, and there are a variable number of gonodendra between gastrozooids. The gonodendra, which consist of three types of zooids, are the most complex structures in the colony. The gonodendra arise by subdivision of the gonodendron bud, but this subdivision has nothing to do with the organizational context in which the gastrozooids arise. In the Codonophora, the organizational context of all siphosomal zooids is established by the process of probud subdivision that gives rise to cormidia. The cormidial organization of the Codonophora is extremely diverse across species (with respect to both the types of zooids present and the position of the zooids relative to each other), although it is highly consistent within species.

There is little known ecological diversity within the long-stemmed Cystonectae (all species are piscivorous), and there are only five extant cystonect species in total (*P. physalis*, the fifth species, being clearly morphologically distinct but still poorly known). The great morphological diversity of the Codonophora is accompanied by large ecological diversity (with respect to prey and habitat), and the Codonophora are much more speciose than their sister group, the Cystonectae. These patterns in diversity make it tempting to speculate that probud subdivision may have been a key colony-level developmental innovation that opened up a complex morphospace that is inaccessible to other colonial animals. Among other things, this morphospace includes an increased number of functionally specialized zooids and greater flexibility in the way these zooids are organized relative to each other.

It is possible that the organizational mechanisms at play in probud subdivision did not arise de novo but were co-opted from zooid development. The development of a zooid from a bud (whether that bud arises independently or by probud subdivision) requires the specification of fields that specify the location of various zooid structures. It may be that these already-existing fields are used in the probud to specify where the different zooids will arise during subdivision. Future mechanistic and molecular studies of colony-level and zooid-level development in the siphonophores will provide an opportunity to test this hypothesis.

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