J. Mar. Biol. Ass. U.K. (2005), **85**, 695–707
Printed in the United Kingdom

A re-examination of siphonophore terminology and morphology, applied to the description of two new prayine species with remarkable bio-optical properties

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Siphonophores (Cnidaria: Hydrozoa) are dominant members of the carnivorous plankton, and they are known for their ability to produce bioluminescence. Here we describe two new calycophoran species (sub-family Prayinae) that are unique in their morphological and optical traits. One species, *Gymnopraia lapislazula* gen. nov., sp. nov., displays a dramatic form of blue structural coloration, and the other, *Lilyopsis fluoracantha*, sp. nov., bears an exceptional amount of fluorescence—enough to give a greenish cast during white-light illumination. We also introduce a consistent terminology for siphonophore axes and zooids, discuss characters important for distinguishing the known prayine genera, and suggest that the presence or absence of a disjunct pedicular canal could be of diagnostic value for the group.

INTRODUCTION

Siphonophores are large, abundant, and ecologically important oceanic hydrozoans (Totton, 1965; Kirkpatrick & Pugh, 1984). There are approximately 160 described species, but this is a biased sample of siphonophore diversity that is skewed in favour of robust species found at shallow depths. Recent innovations in collection technology, especially the improvement of research submersibles, have revealed the existence of fragile species that have never been seen in trawls (e.g. Pugh & Harbison, 1987; Pugh & Youngbluth, 1988; Dunn et al., in press a). Here we describe two such species, *Gymnopraia lapislazula* gen. nov., sp. nov. and *Lilyopsis fluoracantha* sp. nov., which were collected by remotely operated underwater vehicles (ROVs). They are so fragile that even when successfully collected, specimens quickly deteriorated during shipboard observations, and fixation was nearly impossible.

Both *Gymnopraia lapislazula* and *Lilyopsis fluoracantha* belong to the Calycophorae, a group that contains most of the described siphonophore diversity and that was found to be monophyletic in a recent molecular phylogenetic analysis (Dunn et al., in press b). Most siphonophores are bioluminescent (Haddock & Case, 1999), and many, especially calycophorans, have fluorescence and structurally based optical properties (S.H.D.H. personal observations; Mackie & Mackie, 1967). The species described herein present some of the most dramatic examples of both structural colour (*G. lapislazula*) and fluorescence (*L. fluoracantha*) yet found in the plankton.

TERMINOLOGY

As there is considerable confusion regarding the terminology used to specify the axes of siphonophores, we explicitly define our nomenclature below in an effort to ameliorate the ambiguities and contradictions often encountered when describing these organisms. There are multiple implicit terminologies currently in use, and it is often not clear which one is employed in any particular publication. Besides being inconsistent with each other, these nomenclature systems can be internally inconsistent and unintuitive because directions are often defined with reference to the traditional orientation of structures on the page, rather than their actual orientation within the colony. Throughout the current manuscript we restrict the use of terms that have multiple meanings to one usage only when possible, and refer to absolute axes rather than traditional orientations. The scheme is far from comprehensive and will need to be amended in order to formally define other features of siphonophore morphology not addressed here.

At the level of the colony as a whole we use the terms anterior and posterior as they have historically been employed to define a longitudinal axis that runs

through the main stem (Figure 1). The *anterior* end of the siphonophore is that with the nectophores (or the pneumatophore, when present), while the oldest cormidium is at the *posterior* end. We do not imply homology of this axis, or of the other axes described here, to any axes of the Bilateria. In fact, recent gene expression data suggest that the oral end of cnidarians may be homologous to the anterior end of other animals (Finnerty et al., 2004). In siphonophores, the oral end of the embryonic axis corresponds to the posterior end of the mature colony (Carré & Carré, 1993), so we are left with the strange situation where the anterior end of siphonophores, as historically defined and used here, may be homologous to the posterior end of other animals. No attempt is made to remedy this aspect of the nomenclature at the present time, as there are already far too many precedents in the literature.

A dorsal/ventral axis is arranged perpendicular to the anterior/posterior axis of the colony. We follow the well accepted convention that the *ventral* side of the stem is that which bears the siphosomal zooids, and the *dorsal* side is opposite this (e.g. Haeckel, 1888). It is important to keep in mind that nectophores can be attached to either the ventral or dorsal side of the stem. (See Dunn et al. (in press b) for a phylogenetic analysis of this character.)

The anterior/posterior and dorsal/ventral axes are contained in a plane that divides the stem into two halves that are roughly bilaterally symmetric. A left/right axis can be drawn perpendicular to this plane, distinguishing the two halves. *Left* and *right* are defined in the same way as they would be for other animals, including humans, so that a dorsal view of a siphonophore with its anterior end at the top of the page would have its right side facing to the right of the page. Right and left have sometimes been used in the opposite sense at the level of the colony (e.g. Mapstone, 2003).

Regarding structures attached to the stem, we restrict the usage of proximal and distal to refer to positions within any such structure, with *proximal* being closer to the stem, and *distal* being further from the stem. These terms are often used in a very different way to describe the relative attachment positions of structures to the stem, with proximal indicating the direction towards the anterior end of the stem and distal indicating the direction towards the posterior end. We have avoided this usage because for any structure attached perpendicularly to the stem, these two connotations, if not qualified, would indicate orthogonal directions.

With respect to nectophores, we use *distal* and *proximal* to describe the axis that runs from the centre of the ostium to the point where the pedicular canal attaches to the stem (Figure 1). The historical definitions for the other nectophore axes—dorsal/ventral and left/right—are problematic because these terms have already been used to describe the colony itself. Because the nectophores can attach to the dorsal or ventral side of the stem, and join the stem at different angles, there is no way to define dorsal and ventral at the level of the nectophores so that they are always consistent with the axes of the colony as a whole. We therefore use the terms *upper* and *lower* in their place. The upper surface is to the anterior of the proximal/distal axis, and the lower surface is to its posterior. The upper radial canal is anterior to the point where the pedicular canal reaches the nectosac, and the lower radial canal is posterior to this junction.

Left and right are more difficult to replace, so we retain them, while stressing that it is important to specify whether one is discussing a nectophore or the entire colony when using these terms. Various authors have oriented the right/left axis of nectophores in different directions, a practice which Totton (1932) noted but which continues to the present time, so we again define our usage here. When the upper surface of a nectophore is drawn with the proximal end facing the top of the page, the *right* side of the nectophore faces the right of the page. This is consistent with the bulk of the contemporary literature (e.g. Pagès & Gili, 1992).

There are several other terms that are sometimes used to describe directions within nectophores. These include ‘up’ and ‘down’ to indicate proximal and distal directions (e.g. Pugh, 1998), as follows from the traditional orientation of nectophore figures. Because they can be confused with the upper/lower axis of the nectophore, we do not use these terms. We do, however, use *ascend* and *descend* to describe the course of canals relative to the main stem in the anterior and posterior directions, respectively, because they have been used uniformly in this sense throughout the literature.

Any descriptions of bracts face similar challenges to descriptions of nectophores, so most of the same terms can be used. The *upper* surface of a bract faces away from the stem, and the *lower* surface is adjacent to it. Variability in the attachment point of bracts can complicate the identification of a proximal and distal end. For bracts that have a lobe extending to the anterior of the attachment, it is more convenient to use anterior/posterior, as defined for the colony, to help describe the bract. *Left* and *right* are defined for bracts such that the upper surface of a bract, when figured with the anterior-facing end at the top of the page, will have its right side facing the right of the page. Note that for a bract borne on the ventral midline, the right side of the bract will be on the left of the colony.

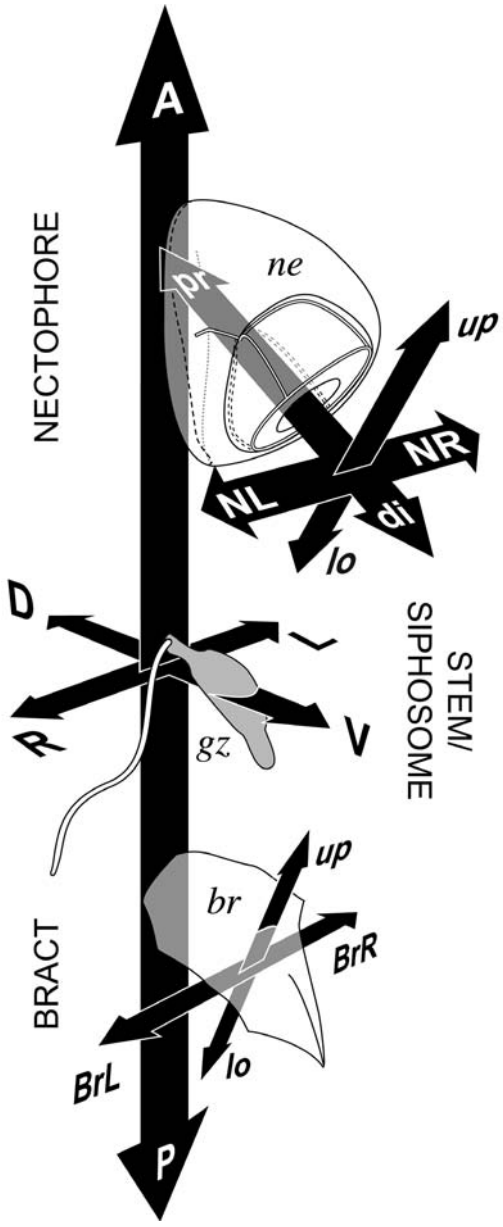


Figure 1. Siphonophore axes defined. With reference only to the stem itself, the primary siphosomal axis is defined as running anterior (A) to posterior (P). The dorsal (D) to ventral (V) axis is defined in relation to where the siphosomal zooids are attached to the stem. (A gastrozooid (*gz*) is illustrated as arising, by convention, from the ventral side of the stem.) The left (L) to right (R) axis is also defined for the stem as a whole. The axes that we apply to the zooids themselves, as defined in the text, are abbreviated as follows: proximal (pr); distal (di); upper (up); lower (lo); nectophore left (NL); nectophore right (NR); bract left (BrL); bract right (BrR); nectophore (*ne*); bract (*br*).

The names of the bracteal canals are particularly problematic. Here we only address those of *Lilyopsis*. We follow Carré (1969) in his use of *longitudinal bracteal canal*, and we employ his alternative names *anterior* and *posterior* for the left and right hydroecial canals, respectively (note that in his figure 2, labels for left and right hydroecial canals (*gauche/droit*) have been inadvertently transposed, though they are defined correctly in the text). This schema is preferred because the canals are on the left side of the bract, and derived their previous names from the traditional right-side-up orientation of bract figures rather than the actual axes of the bract. However, his usage of the names dorsal and ventral for the other two canals are not consistent with absolute axes, and we will use the names *lateral* for his ventral, and *upper* for his dorsal.

Our interpretation of what constitutes the somatocyst and the pedicular canal of calycophoran siphonophores is considered in the Discussion section.

SYSTEMATICS

Sub-order CALYCOPHORAE Leuckart, 1854
Family PRAYIDAE Kölliker, 1853
Sub-family PRAYINAE Chun, 1897
Gymnopraia gen. nov.

Monotypic genus for *Gymnopraia lapislazula* sp. nov. whose diagnosis is given below.

Etymology

The generic name is derived from the Greek γυμνοσ, meaning 'naked' and referring to the lack of bracts on the siphosome, combined with *praia*, referring to the generic name *Praya*, which was in turn derived from the port of Praia on the Cape Verde Islands (Quoy & Gaimard, 1833).

Gymnopraia lapislazula sp. nov.
(Figures 2 & 3)

Type Material

Holotype: specimen collected during ROV 'Ventana' dive 2623 from a depth of 462 m (7 February 2005; 36°42'N 122°04'W). Specimen photographed, preserved in 2% glutaraldehyde, and deposited at the National Museum of Natural History, Washington, DC. Nectophores preserve very poorly.

Paratype: specimen from ROV 'Tiburon' dive 105 on 13 January 2000. Sample was collected at 36°42'N 122°02.4'W at a depth of 420 m. Material exists only in photographs, drawings, and molecular sequences.

Other material examined: Nine specimens collected and *in situ* video of 21 specimens from the seas around Monterey Bay, California, between 1999 and 2005. (Table 1; See Distribution below.)

Diagnosis

Prayine siphonophore with a pair of rounded, apposed nectophores that appear blue in life when acutely illuminated with white light. Nectosac occupy-

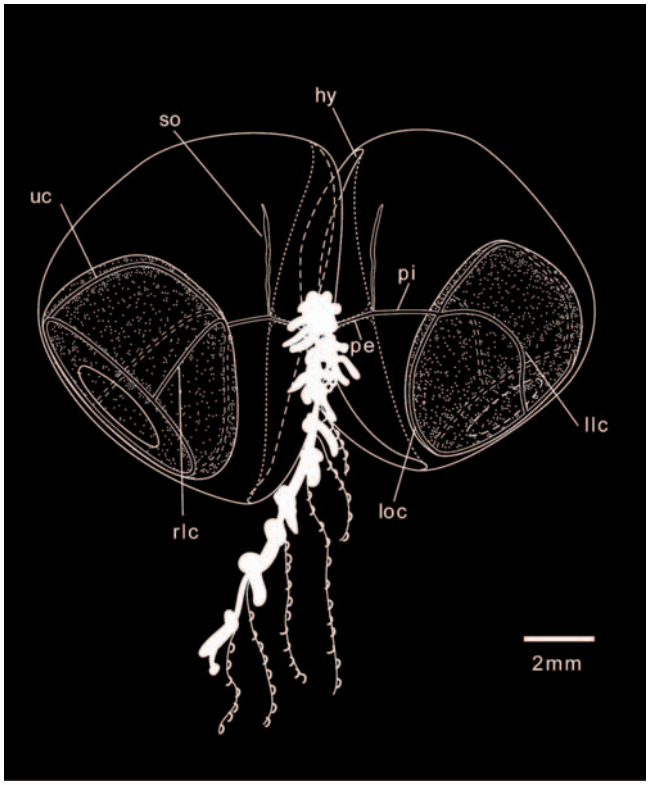


Figure 2. *Gymnopraia lapislazula* gen nov., sp. nov. Approximately lateral view of the whole colony. Abbreviations: somatocyst (so); hydroecium (hy, fine dashed line); internal pedicular canal (pi); external pedicular canal (pe); right lateral canal (rlc); left lateral canal (llc); upper radial canal (uc); lower radial canal (loc).

ing distal half of nectophore, with straight or very slightly curved radial canals. Somatocyst simple, penetrating into the mesogloea as an ascending branch at its point of origin. Pedicular canal running directly from the stem to the nectosac. Siphosome unique among prayine species in being devoid of bracts. Gastrozooids coloured bright carmine.

Etymology

Together, the specific name is a variation of *lapis lazuli*, a stone that contains blue flecks, much like the nectophores. *Lapis*, Latin for 'stone' or 'milestone', also commemorates, much to his embarrassment, the 25th siphonophore description by P.R.P. *Lazula* derives from the Farsi word لاجورد (lajevard) meaning 'cobalt-blue', and here refers to the blue colour of the mesogloea.

Description

Nectophores. The paired nectophores were roughly spherical, 11 mm long and 10 mm wide (Figures 2 & 3A). They were almost identical, but one was slightly larger and had a wide and shallow hydroecial furrow, extending the full length of the nectophore, while the other had a slightly deeper hydroecium, with its short lateral flaps tucked between the broader wings of the apposing nectophore. Both nectophores were fragile and soft, and entirely without exterior ridges.

The pedicular canal ran at a right angle from the stem and connected to the hydroecial wall. At this point it could appear, in detached nectophores, to give rise to a descending branch (Figure 3B). However, we interpret this tissue as the scar of the attachment lamella, since on intact animals it was clear that there was no separate descending portion of the pedicular canal. Because of the small size of the attachment lamellae and the flaccidity of the mesogloea, the loosely connected nectophores could rotate rather freely. Directly upon passing through the hydroecial wall, the pedicular canal bent towards the lower side of the nectophore and ran straight to the wall of the nectosac, which itself extended to just under one half the length of the nectophore. The upper and lower radial canals (historically, dorsal and ventral) and the left radial canal originated at the point where the pedicular canal reached the nectosac. The right radial canal branched from the upper canal a short distance from the intersection of the pedicular canal, and all radial canals proceeded straight, or with a very slight bend, to the circumostial canal.

At the point where the pedicular canal met the hydroecial wall, the somatocyst originated and immediately penetrated into the mesogloea, essentially becom-

Figure 3. *Gymnopraia lapislazula.* Photographs of live specimens. (A) Holotype specimen showing internal (pi) and external (pe) portions of the pedicular canal, with the external canal surrounded by the attachment lamella; (B) close-up view of isolated nectophore showing: scar (sc) where the lamella was attached, internal pedicular canal (pi) running to the nectosac (ne), somatocyst (so) and blue mesogloeal specks (sp; inset); (C) *in situ* photograph showing bluish tint and the meekly deployed gastrozooids; (D) details of the siphosome, with somatocyst (so) in the background, showing gonophores (go) and gastrozooids (gz); (E) view of tentacles and tentilla. Scale bars: A, 2 mm; B, 1 mm; C, 0.5 mm.

Figure 6. *Lilyopsis fluoracantha* sp. nov. Photographs of live specimens. In all images except (B), the green colour is from fluorescence visible under white-light illumination with no barrier filters. *(*A) Whole animal image of holotype; (B) fluorescence image of whole animal, excited with 440 nm strobe, using long-pass barrier filters; (C) *in situ* video image of a detached siphosome, showing the yellow-coloured tentilla; (D) detailed view of interconnected bracts, showing cormidial bell (cb), gastrozooid (gz) and characteristic spurs (sp); (E) lateral view of nectophores, in a similar orientation to the illustrated nectosome (Figure 5). Scale bar: A, 5 mm.

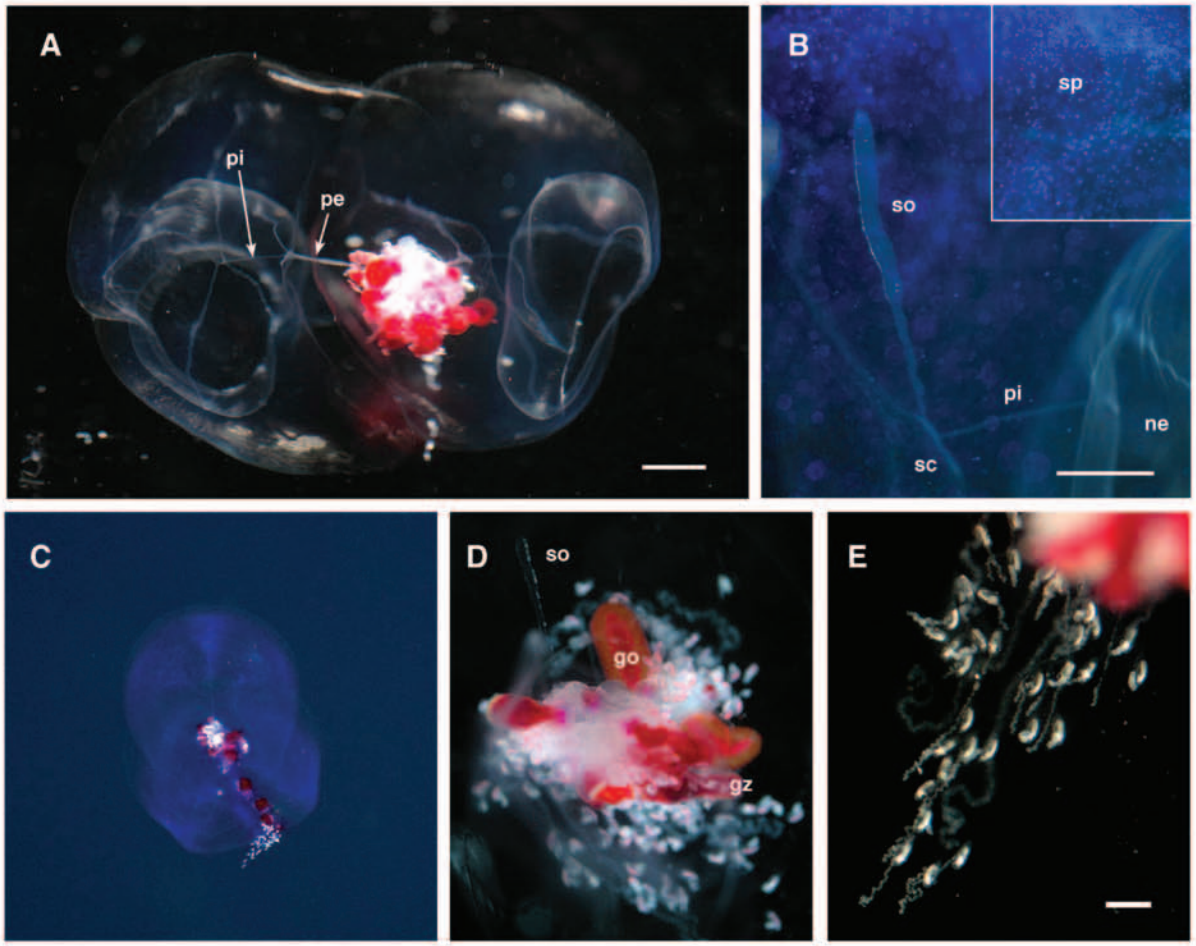


Figure 3. ▲

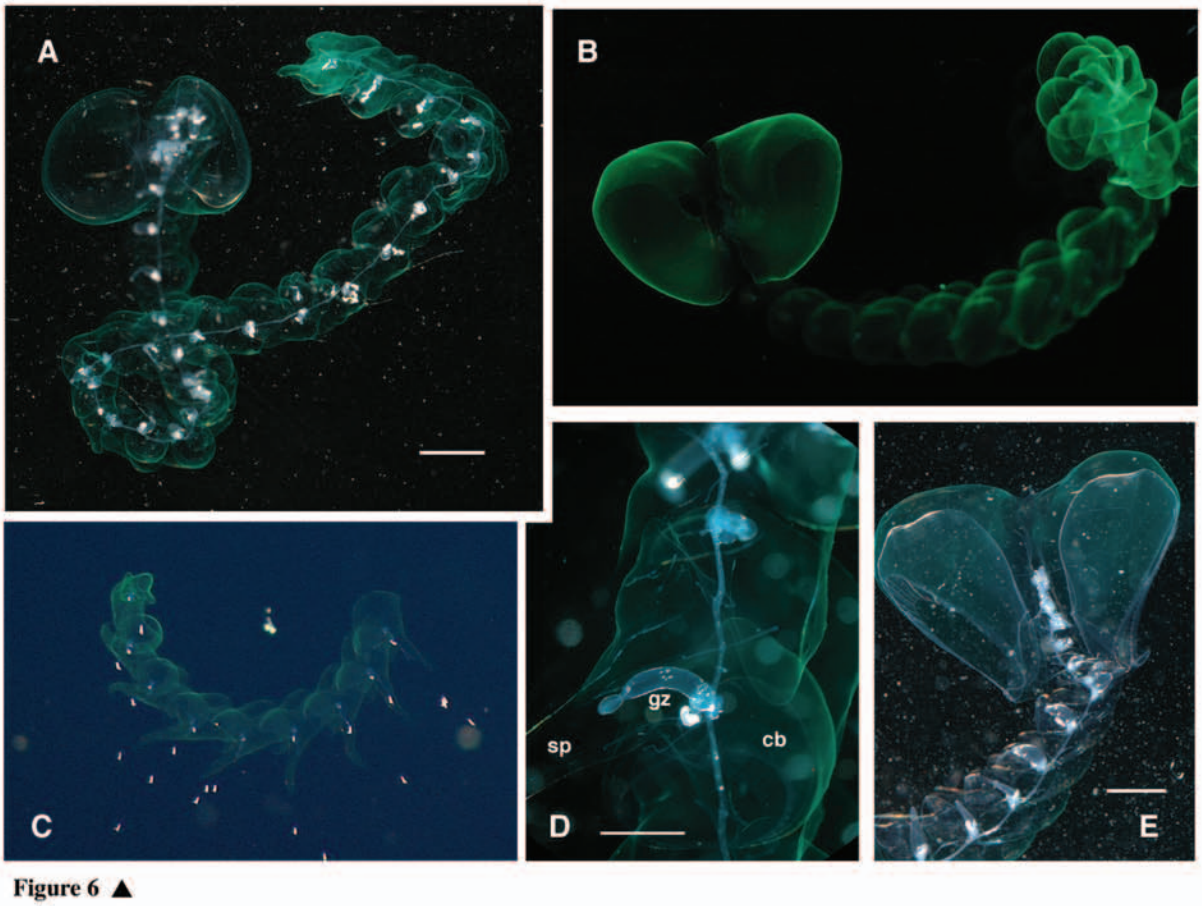


Figure 6 ▲

Table 1. *Observations of* Gymnopraia lapislazula *from ROVs, including specimen collection data where applicable.*

ROV Dive-Specimen	Date	Depth (m)	Lat./Lon.
Ventana 1606-SS6	12 May 1999	434	36°48'N 122°00'W
Ventana 1680-D3	27 Sep 1999	479	36°48'N 122°00'W
Ventana 1342	20 Nov 1999	489	36°43'N 122°05'W
Ventana 1342	20 Nov 1999	500	36°43'N 122°05'W
Tiburon 105-SS8	13 Jan 2000	420	36°42'N 122°02'W
Ventana 1797	28 Jul 2000	520	36°42'N 122°02'W
Ventana 1886-D1	7 Dec 2000	455	36°42'N 122°04'W
Ventana 2070	24 Sep 2001	421	36°42'N 122°04'W
Tiburon 410	22 Mar 2002	377	36°19'N 122°55'W
Tiburon 440	14 Jun 2002	486	36°42'N 122°04'W
Ventana 2210	25 Jul 2002	482	36°45'N 122°12'W
Tiburon 680-D1	26 May 2004	357	35°29'N 123°53'W
Ventana 2547	16 Jul 2004	474	36°42'N 122°03'W
Ventana 2558-SS4	13 Aug 2004	400	36°42'N 122°04'W
Ventana 2570	13 Sep 2004	419	36°42'N 122°04'W
Ventana 2570	13 Sep 2004	419	36°42'N 122°04'W
Ventana 2609	17 Dec 2004	476	36°42'N 122°04'W
Ventana 2623-D1	7 Feb 2005	388	36°42'N 122°04'W
Ventana 2623-D3	7 Feb 2005	358	36°42'N 122°04'W
Ventana 2623-D6	7 Feb 2005	462	36°42'N 122°04'W
Ventana 2636	7 Feb 2005	400	36°42'N 122°04'W

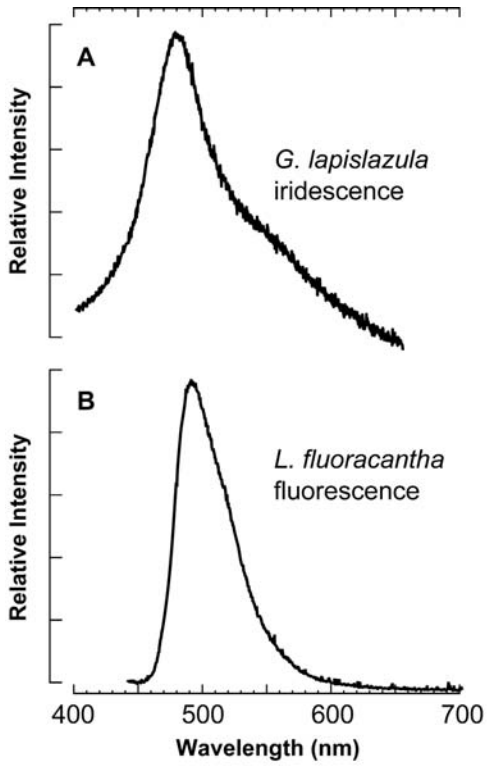


Figure 4. Optical spectra from new prayine species. (A) *Gymnopraia lapislazula* iridescence spectrum. Maximum emission at 485 nm; (B) *Lilyopsis fluoracantha* fluorescence emission spectra. Fluorescence could be excited by light between 410 nm (emission shown) and 470 nm.

ing a long ascending branch (Figure 3B). It was narrow and elongate, with only a slight thickening along its length. There was some variability between specimens: the somatocyst could be shorter and slightly swollen at the tip, wrinkle slightly along its length, or have a few extremely fine lateral offshoots.

A unique feature of the nectophores was that the mesogloea contained spherical inclusions 12 μm in diameter. They appeared as intense blue speckles when illuminated under white light (laboratory source with large red component) at an angle of up to 60° from the observer (Figure 3B,C). The emission spectrum was unimodal with a maximum wavelength of 485 nm (Figure 4A). The specks were not coloured under transmitted or perpendicular illumination, and they were not fluorescent or bioluminescent, although the surface epithelium of nectophores was bioluminescent. Thus the coloration seemed to be caused by a unique form of monochromatic light scattering, which merits further investigation. This may be difficult, however, as the blue iridescence was not maintained upon fixation.

Siphosome. The siphosome was frail, and the uncontracted portion of the stem was readily severed during collection and observation.

Bracts. In a feature unique to this genus of prayine siphonophore, no bracts were found in the examined

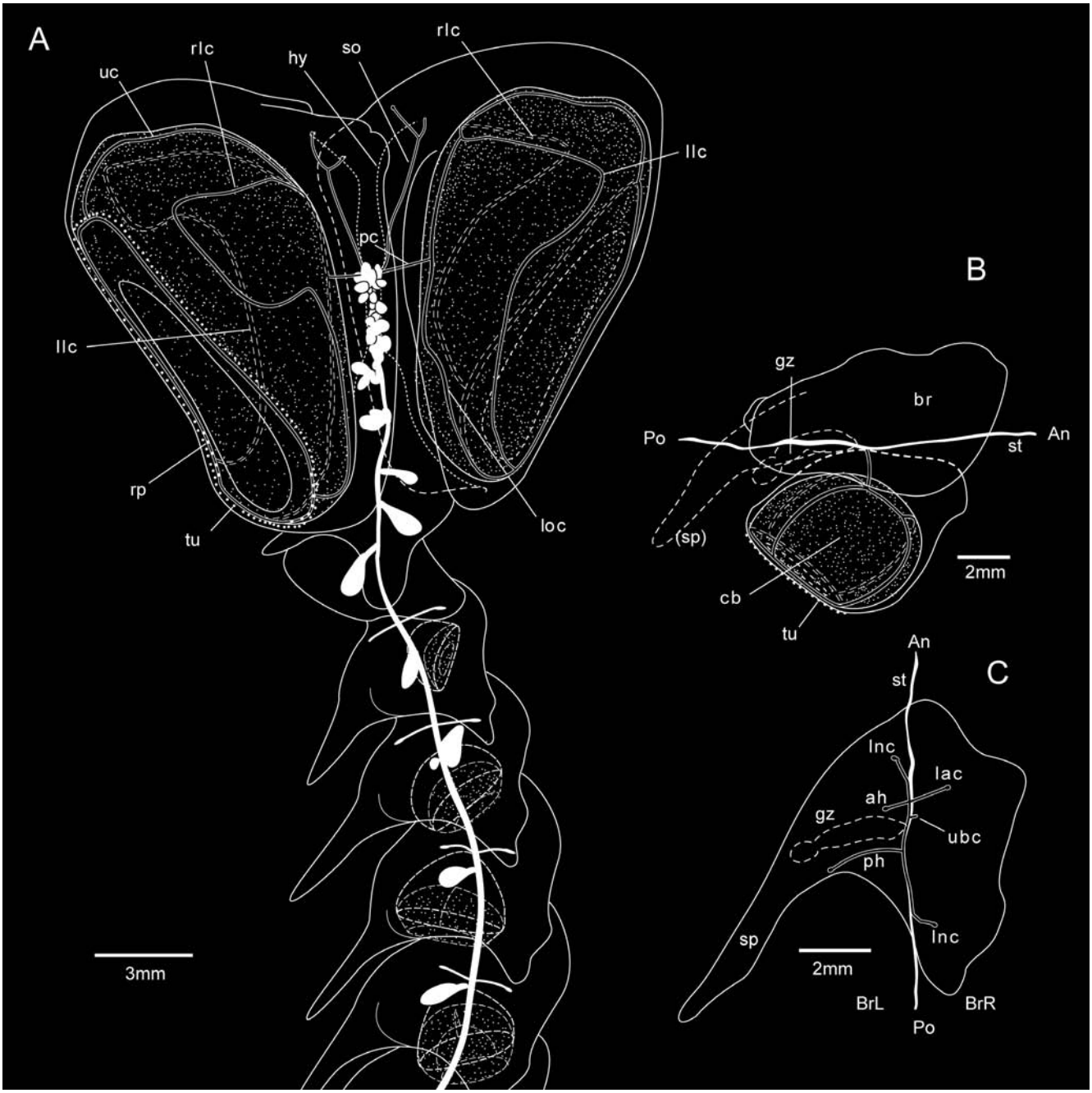


Figure 5. *Lilyopsis fluoracantha* sp. nov. (A) Whole animal in approximately lateral view. The nectophore drawn on the left is pointing slightly out of the page, and the one on the right is pointing into the page; (B) lateral view of a bract and cormidial bell from the bract's right side. Spur and gastrozooid are shown only for orientation, and do not accurately represent their positions; (C) upper view of the bract. Note that the bract comes to sit on the dorsal side of the stem. Abbreviations: anterior (An); posterior (Po); bracteal left (BrL); bracteal right (BrR); somatocyst (so); hydroecium (hy, fine dashed line); pedicular canal (pc); right lateral canal (rlc); left lateral canal (llc); upper radial canal (uc); lower radial canal (loc); red pigment spot (rp); marginal tubercule (tu); bract (br); stem (st); cormidial bell (cb); spur (sp); gastrozooid (gz); longitudinal bracteal canal (lnc); lateral bracteal canal (lac); upper bracteal canal (ubc); anterior hydroecial canal (ah); posterior hydroecial canal (ph).

specimens or seen in the *in situ* photographs.

Gonophores. Male and female gonophores were present on the type specimen, and the live male gonophores were pale white with a pink core (Figure 3D).

Gastrozooid and tentacle. The gastrozooids were a uniform bright carmine colour with a tubular proboscis (Figure 3D). Tentilla were of the typical prayine sort with an arced cnidoband and a single long terminal filament (Figure 3E).

Distribution

Specimens were observed in the eastern temperate Pacific Ocean between 34°45.0'N 124°34.3'W and 36°43.4'N 122°4.8'W. They were collected using the

ROVs 'Ventana' and 'Tiburon' from depths of 357 m to 520 m (mean = 439 m) (Table 1).

DNA sequences

Gymnopraia lapislazula has been included in a molecular phylogenetic study (Dunn et al., in press b), though its position within the Calycophorae was not well resolved. Sequences of 18s SSU rRNA (no. AY937359) and 16s mtDNA (no. AY935317) from the paratype have been deposited in GenBank.

Remarks

Similar Species

Gymnopraia lapislazula is superficially similar to *Desmophyes haematogaster* Pugh, 1992 in the possession of rounded nectophores and red-pigmented gastrozooids, but *D. haematogaster* is readily distinguished by the presence of bracts, its disjunct pedicular canal, and the fact that a substantial portion of the somatocyst remains in contact with the upper wall of the hydroecium (see Discussion below for an explanation of the features). *Lilyopsis rosea* Chun, 1885 and *L. fluoracantha* sp. nov. have a similar arrangement of the pedicular canal and somatocyst to that in *Gymnopraia*, but they can be distinguished by the presence of bracts, bifurcating somatocyst, path of the radial canals, and the relative depth of the nectosac.

Genus *Lilyopsis* Fewkes, 1883
Lilyopsis fluoracantha sp. nov.
(Figures 5 & 6)

Type Material

Holotype: specimen no. SS5 collected at a depth of 395 m during ROV 'Ventana' dive 2558 (13 August 2004; 37°42.0'N 122°04.8'W). Photographed, preserved in 4% formalin, and stored at the National Museum of Natural History, Washington, DC. DNA sequences (18s rRNA) available as GenBank accession no. AY919607.

Material examined Five specimens observed by video, three of which were collected, near Monterey Bay, California between 1998 and 2004.

Diagnosis

Two definitive nectophores, with looped unbranched radial canals. Red pigment spots around only a portion of the ostium, but not on radial canals. Bracts with a conspicuous elongate spur on the left side, directed posteriorly. Cormidial bell without pigment spots along the circular canal. Nectophores and bracts a uniform fluorescent green in life.

Etymology

The specific name derives from its fluorescent properties and from the Greek ακανθα, meaning 'thorn', and referring to the characteristic protrusion of the bracts.

Description

Nectophores. The two apposed definitive nectophores were nearly identical, without ridges, and measured 15 mm long by 9 mm wide (Figures 5A & 6A,E). The nectosac occupied most of the volume, reaching more than 2/3 the length of the nectophore. The hydroecium was wide and shallow, forming slight wings near the hemispherical apex. The hydroecium did not extend onto the anterior surface. Nectophores and bracts were brightly fluorescent (Figure 6B), with a green emission maximum at 491 nm (Figure 4B).

The portion of the pedicular canal connecting the stem to the nectophore was very short, as there was a bulge in the hydroecial wall at that point. From there the pedicular canal passed directly to the nectosac, where it gave rise to the upper and lower radial canals only. The lateral radial canals branched from the upper radial canal close to the anterior end of the nectosac. They originated together on one nectosac and slightly offset on the other. The upper and lower canals were straight between the pedicular canal and the circular canal, while the left and right radial canals were S-shaped with asymmetrical loops, and they joined the circular canal close to the lower end of the nectosac.

Evenly spaced red pigment spots were arranged adjacent to the circular canal, but only on the lower portions where the lateral canals joined. There were no red pigment spots on the radial canals. Numerous whitish tubercles (= *tentacules pyriformes* in Carré, 1969) bordered the ostium, both on the nectophores and the cormidial bells (Figure 5A,B).

A narrow somatocyst arose from the pedicular canal and ascended along the hydroecial bulge for a short distance before penetrating into the mesogloea at about 1/9th of its total length (Figure 5A). It bifurcated near the anterior end of the nectophore and each branch terminated in a minute swelling. There was no descending branch of the pedicular canal.

Siphosome. *In situ* video showed the siphosome of the holotype to be 12 cm long, bearing 35 closely connected cormidia with their bracts in an overlapping sequence. Aside from the green bracts, no siphosomal elements were coloured.

Bracts. The main body of the bract ran along the axis of the stem (Figure 5B,C). The lower surface of the bract was concave and draped over the stem, partially enclosing the cormidial elements. The right posterior portion of each bract overlaid the anterior portion of

Table 2. *Observations of* Lilyopsis fluoracantha *by ROVs, including specimen data where applicable.*

ROV Dive-Specimen	Date	Depth (m)	Lat./Lon.
Ventana 1522-D2	03 Nov 1998	327	36°42'N 122°04'W
Ventana 1522-D3	03 Nov 1998	330	36°42'N 122°04'W
Tiburon 110	25 Feb 2000	–	36°35'N 122°31'W
Ventana 1860	02 Nov 2000	393	36°43'N 122°03'W
Ventana 2558-SS5	13 Aug 2004	395	36°42'N 122°04'W
Ventana 2625	09 Feb 2005	476	36°42'N 121°03'W

the next bract to its posterior. The left side of each bract bore a distinct elongate spur, which extended posteriorly (Figure 6C). The bracteal canals had the same general arrangement as those of *Lilyopsis rosea*: the anterior and posterior tips of the longitudinal bracteal canal extended into the mesogloea, with the lateral bracteal canal arising opposite the anterior hydroecial canal. The anterior hydroecial bracteal canal was much shorter than the posterior one, and the upper hydroecial canal was short and bent.

Gonophores. No gonophores were found in the collected specimens.

Cormidial bells. Each cormidium possessed a single cormidial bell (=asexual nectophore). The ostium of each bell was ringed with small tubercles. However, we did not observe any red pigment spots around the periphery of the ostium like those of the nectophore, or on any of the radial canals. The canal arrangement was the same as that of *Lilyopsis rosea*, with the pedicular canal giving rise to an anterior and two latero-posterior canals. The anterior canal then divided into two equivalent canals before joining the circular canal.

Gastrozooid and tentacle. Gastrozooids were clear or whitish and cylindrical with a short rounded proboscis (Figure 6A,D). They often contained oil droplets. Tentacles were fragile and broke off easily. *In situ* images show that tentilla appeared yellowish and were widely spaced along the tentacle. Tentilla were typical prayine form, with short and slightly curved cnidobands.

Distribution

Specimens of *L. fluoracantha* were rather rare and were only observed on six occasions (Table 2). They were seen at depths ranging from 327 m to 476 m, (mean = 384 m) and located between 36°35'N 122°31'W and 36°42'N 122°04'W. Of the observed individuals, three specimens were collected.

DNA sequences

The 18S SSU rRNA sequences for the holotype of *L. fluoracantha* have been deposited to GenBank as accession AY919607. *Lilyopsis fluoracantha* grouped with *L. rosea* in both parsimony and likelihood searches when they were added to the dataset of Dunn et al. (in press b), and differed in five of the 1799 nucleotides examined.

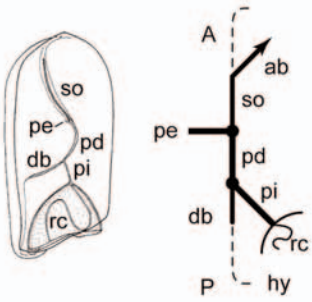
Remarks

The shape and arrangement of the nectophores in *Lilyopsis fluoracantha* are virtually identical to the definitive nectophore of *Lilyopsis rosea*, except that they are about twice the size (15 mm long in the former, 7–8 mm in the latter). (The definitive nectophore is designated N_2 in Carré (1969), although the labels were inadvertently transposed in his Pl.1 figure 1.)

Carré noted that the larval nectophore, which he called the N_1 nectophore, was retained in the adult colony, but could eventually be dropped and replaced by another (definitive) nectophore that was essentially identical to the so-called N_2 nectophore. Because the two nectophores of *Lilyopsis fluoracantha* were nearly identical, and did not have any of the distinctive features found in larval nectophores of *L. rosea*, we believe that such a replacement has occurred in our specimens of *L. fluoracantha*. However, we do not know if a larval nectophore is ever retained in the adult colony. In *L. fluoracantha*, there are no red pigment spots on the lateral canals of the nectophores, as there are in *L. rosea*, and the pigment spots on the ostium are restricted to the region where the lateral radial canals connect with the circumostial canal. Pigment spots are also absent on the ostium and the radial canals of the cormidial bell of *L. fluoracantha*, while they are present on two of the radial canals and around the ostium in *L. rosea*. The two species differ most notably in the morphology of the bracts, with *L. fluoracantha* bearing a pronounced spur.

Presently *Lilyopsis fluoracantha* is only known from Monterey Bay, California. *Lilyopsis rosea*, which was described from the Mediterranean Sea, has been collected there on several subsequent occasions (Carré, 1969). It has also been seen in the North Atlantic and in warmer Pacific waters off California, Australia, and Malaysia (S.H.D.H. personal observation; Bedot, 1896; Bigelow, 1911), with some records from other regions (Alvariño et al., 1990) which we consider dubious. It should be noted that Fewkes' (1883) specimen came from Villefranche-sur-Mer, Mediterranean

Table 3. *Distinguishing characteristics of the genera of prayine siphonophores. Diagnosis is based mainly on the following features: the pedicular canal, which can include external (pe), disjunct (pd), and internal (pi) segments, as well as a descending branch (db); the somatocyst (so), which may have an ascending (ab) branch; and the radial canals (rc) of the nectosac. Schematics, summarizing all states, are oriented with the anterior-posterior (A,P) axis vertical and the stem attachment at the left. The hydroecium (hy) is shown only in the legend. Circles represent points where extensions of the pedicular canal originate. A divided ascending branch may designate either a simple bifurcation or complex branching. Query marks and dashed lines indicate instances where two different conditions may occur in species of the same genus, or states that cannot be determined based on the literature or examination of preserved specimens. In the case of genera with both larval and definitive nectophores, this table presents only features of the presumed definitive one.*



LEGEND

Genus	Praya	Desmophyes	Rosacea	Craseoa	Prayola	Mistoprayina	Stephanophyes	Lilyopsis	Gymnopraia	
pedicular canal, stem to nectosac	disjunct	disjunct	disjunct	disjunct	direct	?direct	direct	direct	direct	(pd)
somatocyst along hydroecium	present	present	present	present	?present	present	present	absent	absent	(so)
ascending branch	divided	short	absent	absent	absent	short	divided	divided	long	(ab)
descending branch	present	absent	present	absent	absent	absent	present	absent	absent	(db)
lateral radials	branched	straight	recurved	curved	slightly curved	straight	recurved	recurved	straight	(rc)
Schematic										

Sea, and not from the western Atlantic as Bigelow (1911) suggested.

DISCUSSION

Coloration

Most siphonophores have transparent nectophores and bracts, and some have red or other colours of pigments in their gastrovascular system. The two species described here have marked coloration of their nectophores. *Gymnopraia lapislazula* achieves bright blue iridescence (Figures 3B,C & 4A) through structural coloration. Structural colouring results from optical interference produced by a variety of physical mechanisms such as thin films, diffraction gratings, scattering, photonic crystals, and interaction between structures with different refractive indices. It occurs in many marine taxa as well as birds, butterflies, lizards, and mammals (reviewed by Parker, 2000). These forms of coloration are distinct from pigments, which produce colour through differential absorption of particular wavelengths. There are a few other examples of blue-coloration in organisms found at similar depths as *G. lapislazula*. From the ROV, for example, a new species of salp (Madin & Madin, unpublished data) and the hydromedusa *Colobonema sericium* are often noticed first by their conspicuous blue colour (S.H.D.H. personal observation). Blue iridescence in octopods and nudibranchs has been attributed to Rayleigh (wavelength-selective) scattering by particles of 10 nm (Parker, 2000). In order for this mechanism to be at work with *G. lapislazula*, the 12 μm inclusions would be required to contain smaller particles embedded within them to achieve their coloration.

Lilyopsis fluoracantha displays an equally dramatic coloration (Figure 6), but in that case it is achieved through fluorescence: blue ambient light is absorbed and re-emitted with an emission maximum of 491 nm (Figure 4B). For *L. fluoracantha*, the association with a bioluminescence system may account for the presence of a fluorescent moiety; other gelatinous plankton use fluorescent proteins in direct association with photo-

proteins to modify their emission wavelengths (Haddock & Case, 1999).

Downwelling light is present but dim at depth ranges of these species, so there is the potential for an ecological function to their coloration. In a monochromatic environment there are few ways to modify visibility. In such conditions, pigments can only darken the appearance, while fluorescence can provide a colour palette. On the other hand, structural colours, much like reflective surfaces, provide a way to appear brighter in the dimly lit ocean regions. There is presently insufficient information to speculate on ecological functions of this coloration, but the discovery of such dramatic examples provides excellent incentive for further examination.

Prayine characters

In an effort, again, to clarify our terminology, we present the following interpretation of the canals of calycophoran nectophores, with special regard to prayines. When considering these canals, it is important to note that they are all evaginations of the same contiguous gastrodermal layer. Viewing their relationships in a developmental context helps to indicate which traits might be fundamental, and which are of secondary importance.

Pedicular canal has been consistently used in the literature to refer to the canal that gives rise to the radial canals of the nectosac. However, beyond that, there is a range of opinions about its extent. Totton (1965, p. 35) considered that it 'arises from the point of origin in the stem,' while Margulis (1995) believed that there are two separate pedicular canals, one from the stem to the somatocyst, and one from the somatocyst to the nectosac. We believe the former view is more reflective of the true nature of the gastrovascular system, because the pedicular canal must be continuous in all stages of development in order for it to give rise to the canals of the nectosac. Any nomenclature that implies that the pedicular canal is not a continuous entity does not accurately reflect its significance. Thus in our usage, the *pedicular* is considered to be the entire canal that runs from the stem to the hydroecial wall, penetrates the mesogloea, and connects to the radial canals of the nectosac. The portion of the pedicular canal from the stem to the nectophore can be termed the *external* pedicular canal, (Table 3, pe) while the portion passing through the mesogloea to the nectosac is the *internal* pedicular canal (Table 3, pi). In some prayines there is an intervening segment running along the hydroecial wall between these two parts of the canal, and we call this the *disjunct portion* of the pedicular canal (Table 3, pd). For example, in *Rosacea*, the external pedicular canal runs from the stem to the hydroecial wall and then the disjunct portion runs posteriorly along the hydroecium, before it bends and the internal portion runs through the mesogloea to the nectosac.

This disjunct portion of the pedicular canal, running longitudinally along the hydroecium, has often been described as part of the somatocyst, especially since the attachment point of the external pedicular canal is rarely noted. However, here we restrict the usage of *somatocyst* (Table 3, so) to refer only to any blind branch of the gastrovascular system that runs *anteriorly* from the external pedicular canal at the point it reaches the hydroecial wall. The somatocyst may penetrate into the mesogloea, either immediately or after extending along the hydroecial wall. This portion of the somatocyst within the mesogloea has been called an *ascending branch* (Table 3, ab), and it may also bifurcate or ramify more complexly. Note that this terminology, as opposed to the previous terminology used in prayines, is consistent with that of diphyid and abylid calycophorans, in the sense that our definition of a somatocyst accommodates the way that term is usually applied in those groups.

Because we consider the pedicular canal to be the essential feature from which other endodermal structures arise, we define a *descending branch* (Table 3, db) as an independent extension of the pedicular canal, rather than as a continuation of the somatocyst as it has been interpreted previously. Specifically, it is a blind canal which originates at the point where the pedicular canal bends toward the nectosac, and which extends posteriorly along the lower wall of the hydroecium.

Historically, the term *pallial canal* has been used to describe a variety of gastrovascular extensions in siphonophore nectophores. In calycophorans, particularly prayines, it has been used to describe various parts of the somatocyst and segments of the pedicular canal, including, perhaps mistakenly, the portion giving rise to the radial canals (Totton, 1965; Pugh, 1992). In physonects it has consistently referred to the ascending and descending branches of the pedicular canal that run along the proximal surface of the nectophore. It is probable that the pallial canals of physonects are homologous to the somatocyst and descending branch of the pedicular canal in calycophorans. Nonetheless, because of these uncertainties and the many ways that the term has been applied, we have avoided using *pallial canal* in the present manuscript, and await detailed examination of the homology of these structures between calycophorans and physonects.

Pugh & Harbison (1987) emphasized three principal characters for distinguishing nectophores of prayine siphonophores, which they arranged in the following

order of importance: (a) their general shape, whether roughly cylindrical, with the nectosac occupying less than half their volume; or conoid, with the nectosac occupying more than half their volume; (b) the arrangement of the canals, particularly whether *ascending* or *descending* branches are present; and (c) the course of the lateral radial canals on the nectosac. In our attempts to apply this system to *Gymnopraia*, however, we encountered two difficulties.

First, re-examinations of preserved specimens and of species descriptions have revealed some potential discrepancies in existing knowledge of prayine features. For instance, although the original description suggests that the nectophore of *Mistoprayina* has a descending branch (Pugh & Harbison, 1987), we now interpret this apparent feature as a scar left by the attachment lamella. The same is true of a so-called pallial canal described in *Sulculeolaria biloba* (figures 85, 86 in Totton, 1965), and likely many others. A similar scar looked deceptively like a descending branch in detached nectophores of *Gymnopraia lapislazula*, but the true origin became apparent in examinations of intact specimens. Although attachment lamellae often run along the hydroecial wall adjacent to gastrovascular canals, it is possible to discern the presence of an independent descending branch, as seen in *Rosacea*. In another example, the thickened somatocyst depicted for *Prayola tottoni* Carré, 1969 might also be attributed to the attachment of the lamella around the pedicular canal, leading to the surprising conclusion that that species has no true somatocyst, although the other member of this genus does (Pugh & Harbison, 1987).

The second difficulty of applying the framework established by Pugh & Harbison (1987) is with the scheme itself. Although some siphonophores are clear examples of a conical or cylindrical morphology, there is also a gradation between the two nectophore types, so it may be difficult to categorize a species such as *Gymnopraia lapislazula* whose nectosac occupies close to one-half of the nectophore volume. Furthermore, with the addition of a new genus, the diagnostic features that the scheme emphasizes are no longer sufficient to separate all prayines.

In view of these considerations, we have re-examined the known prayine genera, and tabulated the characters that we consider most important in distinguishing them (Table 3). Although we have removed the conical/cylindrical diagnostic, most of the features emphasized by Pugh & Harbison (1987) are still highly informative. To their basic list, we have added a trait describing whether or not there is a portion of the somatocyst running anteriorly along the hydroecium (Table 3, so). In addition, we feel that the presence of a disjunct portion of the pedicular canal is an important feature.

Presence or absence of a disjunct portion cleanly separates the prayines into two groups, in a manner similar to the original cylindrical/conical dichotomy: in species where the nectophores are elongated to a cylindrical form, the pedicular canal is substantially disjunct. Unfortunately, it is often difficult to determine the initial attachment point of the pedicular canal in isolated, fixed nectophores, so this character and others are best scored on living specimens with the nectophores still attached to the stem. It is therefore important that future examinations include living material, ideally at various stages of development. In conjunction with further molecular phylogenetic work, such observations will help resolve the uncertain aspects of siphonophore classification and test the organizational framework that we have proposed.

We thank Craig Dawe, D.J. Osborne, Knute Brekke, and the crews of the ROV 'Ventana', RV 'Point Lobos', ROV 'Tiburon', and RV 'Western Flyer' for expert specimen collection. We would also like to acknowledge the assistance of Lynne Christianson, Rob Sherlock, Bruce Robison, Lonny Lundsten, Christine Schnitzler, Christen Herren, Peter Girguis, and Gunter Wagner. The manuscript was improved by the insightful and expeditious comments of the anonymous referees. This work was supported by the David and Lucile Packard Foundation, as well as a NSF Doctoral Dissertation Improvement Grant and NSF Graduate Research Fellowship to C.W.D. Additional support was provided by NSF Grant no. DEB-9978131 A000 (the Hydrozoan PEET grant) and the Society for Systematic Biologists mini-PEET grant.

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Submitted 19 February 2005. Accepted 27 March 2005.