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Description of *Tottonophyes enigmatica* gen. nov., sp. nov. (Hydrozoa, Siphonophora, Calycothorae), with a reappraisal of the function and homology of nectophoral canals

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Abstract

A new species of calycothoran siphonophore, *Tottonophyes enigmatica* gen. nov., sp. nov., is described. It has a unique combination of traits, some shared with prayomorphs (including two rounded nectophores) and some with clausophyid diphyomorphs (the nectophores are dissimilar, with one slightly larger and slightly to the anterior of the other, and both possess a somatocyst). Molecular phylogenetic analyses indicate that the new species is the sister group to all other diphyomorphs. A new family, Tottonophyidae, is established for it. Its phylogenetic position and distinct morphology help clarify diphyomorph evolution. The function and homology of the nectophoral canals and somatocyst is also re-examined and further clarification is given to their nomenclature.

Key words: Siphonophora, Calycothorae, *Tottonophyes enigmatica*, description, systematics, Tottonophyidae

Introduction

Calycothorae is a sub-order of siphonophores characterized by loss of the gas-filled pneumatophore, reduction in the number of nectophores, and simplification of cormidial structure (Totton, 1965). Calycothorae includes the majority of described siphonophore species, but key questions regarding the evolution of important traits in this clade remain poorly understood. Phylogenetic analyses of siphonophores (Dunn *et al.* 2005) have helped frame and, in some cases, answer these questions. However, there is still a need to characterize the morphology of poorly known members of the group in order to understand the implications of these relationships for trait evolution. Here we describe a new species of siphonophore that has a unique set of characters. Its phylogenetic position sheds important light on one of the still uncertain evolutionary transitions within the Calycothorae – the origin of diphyomorph siphonophores.

Calycothorae has been divided into two groups, referred to as the prayomorphs and the diphyomorphs (Leloup, 1965; Mackie *et al.* 1987). Most prayomorphs have two mature nectophores that are rounded, arranged in apposed positions, and are very similar to each other. The diphyomorphs, in contrast, have one or two angular nectophores. (The sole exception is the family Sphaeronectidae, which belong to the diphyomorph grouping as molecular phylogenetic data (Dunn *et al.*, 2005) have shown, that retains a single rounded larval nectophore in the mature colony.) When two diphyomorph nectophores are present, one is located anterior to the other and they are highly differentiated. The diphyomorphs include many small species that are abundant in shallow waters of the oceans and that are among the most frequently encountered siphonophores.

Molecular phylogenetic analyses (Dunn *et al.* 2005) support the monophyly of Calycothorae. They indicate, though, that the prayomorphs are paraphyletic with respect to the monophyletic diphyomorphs. This indicates that features shared by prayomorphs were present in the most recent common ancestor of Calycothorae, whereas unique features of diphyomorphs are derived traits that are specific to the clade. Those analyses also provided two

other important insights into diphyomorph evolution. Firstly, they suggested that Clausophyidae (of which two species were sampled in the analysis) is sister to the remaining described diphyomorph species. Secondly, they provided strong support for placing an undescribed calycophoran species, tentatively labelled "Clausophyid sp 1" (Dunn *et al.*, 2005) due to several similarities shared with described clausophyids, outside the clade containing all other diphyomorphs. This unique species, which we describe here, has a combination of traits, some shared with prayomorphs (including two almost apposed rounded nectophores) and some with Clausophyidae (including the course of the lateral radial canals of the nectophores, and the presence of a somatocyst in both), that help to clarify the evolution of diphyomorph origins. Because of this uniqueness, we establish a monotypic family for it, the Tottonophyidae.

Terminology. Before describing the new species, and in the light of recent publications, it is necessary to reconsider the terminology for the various parts of the canal system in the nectophores of both physonect and calycophoran siphonophores. Haddock *et al.* (2005) gave a detailed analysis of the morphological nomenclature that is applied to the various axes of orientation, and those definitions are adhered to here. However, with regard to the canal system in the nectophores their discussion mainly considered the arrangement in prayid calycophoran siphonophores, and particularly with the differing usage of the term *pallial canal*, and even *somatocyst*, by certain authors, e.g. Totton (1965) and Pugh (1992). Since then Mapstone (2009) has given another alternative interpretation and, thus, we feel that it is necessary to re-address the matter in order to provide a consistent framework for describing these structures based on the roles that they play.

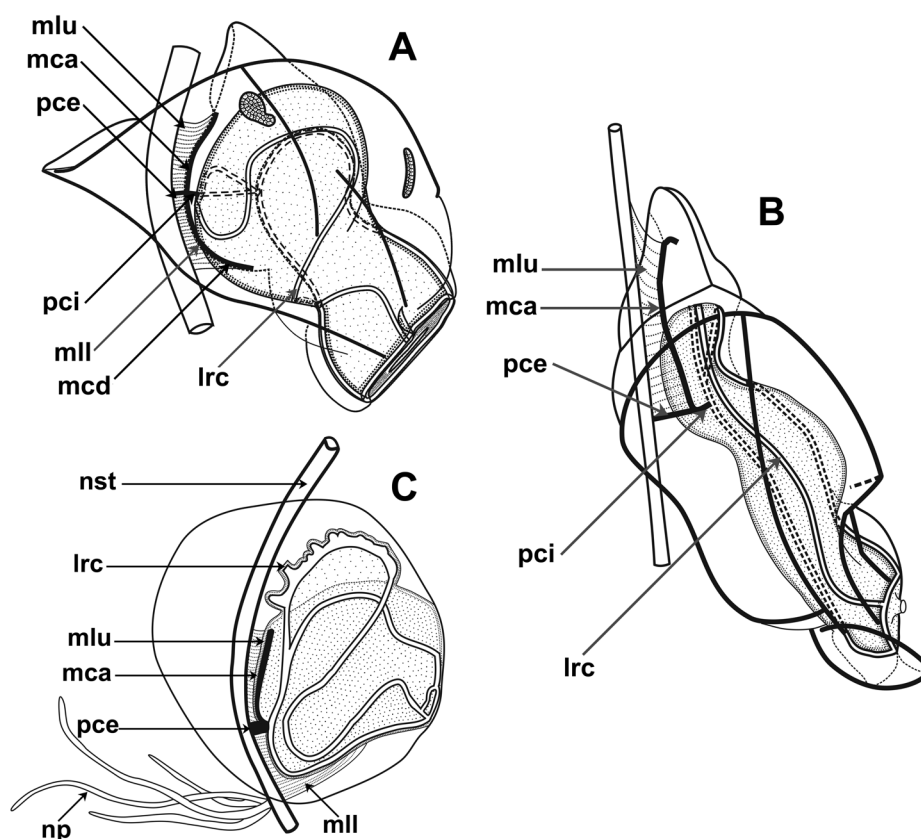


FIGURE 1. Schematic representations of canal systems of physonect nectophores, in lateral view. **A.** *Halistemma* sp. (based on Pugh & Baxter, 2014, Figure 14 *in partim*), **B.** *Bargmannia elongata* (based on Pugh, 1999b, Fig. 2C), **C.** *Apolemia uvaria* (based on Totton, 1965, Fig. 15). **irc.** lateral radial canals; **mca, mcd.** ascending and descending mantle canal, respectively; **mlu, mlu.** lower and upper parts of muscular attachment lamella, respectively; **np.** nectosomal palpons; **nst.** nectosomal stem; **pce, pci.** external and internal pedicular canal, respectively.

Pedicular canal: There is little dispute that the main nectophoral canal, the pedicular canal, is homologous in all siphonophores that possess nectophores. This canal was clearly defined by Haddock *et al.* (2005, p. 705) when they said "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 [*pce*, Figures 1 & 2], while the portion passing through the mesogloea to the nectosac is the internal pedicular canal [*pci*, Figures 1 & 2]". Haeckel (1888) appears to have been the first to use the term pedicular canal, although probably it was just a direct translation of *Stielkanal* or *Stielgefäss* previously used, on occasion, by 19th century German authors.

In all physonect species, the pedicular canal always travels directly from the nectosomal stem to the nectosac of the nectophore (Figure 1), and can be divided into the external portion, between the stem and the nectophore, and the internal portion, within the nectophore, connecting to the radial canals on the nectosac. However, its course is more complicated in some calycophorans. Within the paraphyletic calycophoran family Prayidae, a dichotomy appeared with regard to the presence or absence of what Haddock *et al.* (2005) referred to as a **disjunct portion of the pedicular canal** (Figure 2A, *pcd*). Instead of the pedicular canal running directly to the nectosac, upon entering into the nectophore it first runs longitudinally and posteriorly below the median surface of the hydroecium, for a variable distance, before penetrating into the mesogloea and continuing to the nectosac where it gives rise to the radial canals (Figure 2A). This character basically is coincident with the rough division of the prayine genera into "cylindrical" or "conoid" forms (Pugh & Harbison, 1987), but is more precise. The pedicular canal of the cylindrical forms is disjunct, while in the conoid forms it is direct.

Mantle canals: While it is relatively straightforward to define the pedicular canal, the nomenclature regarding the blind canals that can arise from it is considerably more complicated. Haddock *et al.* (2005, p. 705) noted, "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". Thus, Haddock *et al.* (2005) considered the **ascending branch** and **somatocyst** as homologous, and the **descending branch** as an independent extension of the **pedicular canal**.

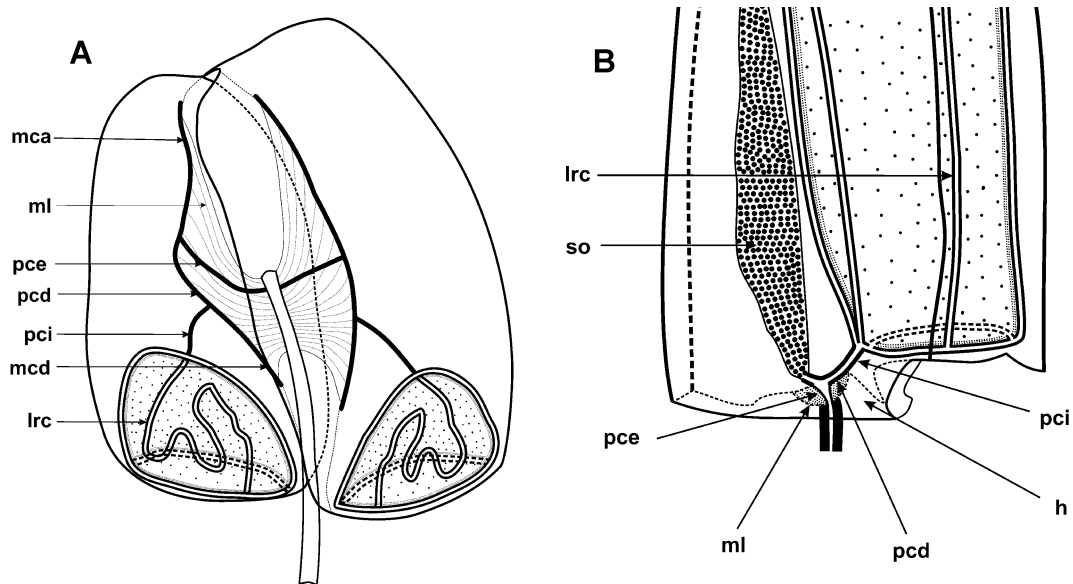


FIGURE 2. Schematic representations of canal systems of calycophoran siphonophores. **A.** *Rosacea* sp., **B.** *Chelophyes appendiculata*. For annotations see Figure 1. **h**, hydroecium; **ml**, muscular attachment lamella; **pcd**, disjunct pedicular canal; **so**, somatocyst.

With regard to these definitions, Mapstone (2009, p. 10) considered that "*somatocyst along the hydroecium*, the name used by Haddock et al. (2005a) for that diverticulum from the pedicular canal which extends anteriorly along the nectophore surface, could be applied only to calycephorans, since physonects have neither a somatocyst nor a hydroecium". She used that reasoning to suggest that it warranted a new name, *ascending surface diverticulum*, and that there should be a corresponding *descending surface diverticulum*. However, this nomenclature has its own problems that will be discussed further. Huxley (1859, p. 16) originally defined the term *hydroecium*, in *Praya*, as "a sort of chamber or *hydroecium*, into which the cœnosarc can be retracted, as into a house". He then went on to describe its presence in several other calycephorans, except *Hippopodius* it appears. However, he did not confine his definition to calycephoran siphonophores but (*ibid.* p. 80), stated that the overlapping opposed nectophores of the physonect species, *Physophora* sp. "enclose the cœnosarc in a sort of hydroecial canal". Although the structure of the hydroecium is often an important taxonomic character in calycephoran species, it has little such value in physonects and so the term is rarely applied to them, although there are a few instances such as Carré (1971), Grossmann *et al.* (2013), and Pugh (2016). As for referring to the ascending canal as being part of the somatocyst then, if this were proved the case, there would be no reason why the homologous canal in physonects should not be referred to in the same way.

If we are to avoid calling them *pallial canals* then do they need new names? If one considers the literature then clearly this is not the case, as was briefly discussed by Pugh & Baxter (2014). The term *pallial canal* first appeared in Haeckel (1888) and it appears likely that the person who translated the original German text derived it from *Mantelgefässe*, via the Latin *pallium*, meaning mantle. The term *Mantelgefässe* was first used by Leuckart (1853, 1854) who, indeed, was the first person to recognize the presence of these canals in physonect nectophores. Thus Leuckart (1854, p. 322–3¹) noted that "The vascular apparatus of our nectophores presents a very complete state of development, with mantle and nectosac canals. The former, overlooked by Kölliker, as well as Mr Vogt, loop upwards and downwards in the median plane of the nectophore, as we have already found in *Hippopodius* and *Praya*. They arise from the central [pedicular] canal immediately after it enters the mantle and are, above and below, developed in a quite consistent manner".

The term *Mantelgefässe* became widely used by German scientists, e.g. Gegenbaur (1859, Claus (1860), Chun (1891) and Schneider (1896), and was also recognised as an appropriate name by Huxley (1859). The term *mantle canals* has clear precedence over pallial canals, but it fell into virtual disuse after Haeckel's (1888) *Challenger* Monograph, although Totton's (1965, p. 35) definition of these canals as "*Upper and lower diverticula of the pedicular canal at the point of entry into a nectophore or gonophore*" is entirely appropriate. We therefore recommend that the term mantle canals be reinstated to describe those blind canals that can arise from the pedicular canal.

The evolution of the mantle canals: While the ascending and descending mantle canal are both blind-ending diverticula that arise from the pedicular canal, their phylogenetic distribution and, therefore, evolutionary history are quite distinct. Cystonects lack a nectosome, but do possess nectophores within their gonodendra. The arrangement of the pedicular canal in these nectophores is not as simple as in physonect nectophores, as the internal part of the pedicular canal does not connect directly with the external part of the canal, but there is an extensive disjunct section running along the median distal side of the hydroecium, connecting the two (see Totton, 1960, Plate XXIV, fig. 6; PRP personal observation). Nonetheless, neither a descending nor an ascending mantle canal is present. Thus, mantle canal evolution would be a feature of the Codonophora, the clade that includes the paraphyletic Physonectae and the monophyletic Calycephorae. The phylogenetic distributions suggest that all ascending mantle canals are homologous to each other, and that all descending mantle canals are homologous to each other.

The ascending mantle canal has a much broader phylogenetic distribution than the descending mantle canal. It is found in all physonects that possess nectophores, and is present and variously elaborated in prayomorph calycephorans, as well as some clausophyid species (Fig. 3) (see also Table 3 in Haddock *et al.*, 2005). This distribution is consistent with a single gain of the ascending mantle canal along the stem of Codonophora, making

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1. Original text: "Der Gefässapparat unserer Schwimglocken zeigt eine sehr vollständige Entwicklung, Mantelgefässe und Schwimmsackgefässe. Die ersteren, die sowohl von Kölliker, als auch von Herrn Vogt übersehen sind, verlaufen (Fig. 13) in der Medianebene der Schwimglocke bogenförmig nach oben und unten, wie wir es schon bei *Hippopodius* und *Praya* gefunden haben. Sie entspringen aus dem Centralkanale so gleich nach dem Eintritte desselben in den Mantel und sind oben, wie unten, in ganz übereinstimmender Weise entwickelt."

it a synapomorphy of the clade, followed by one or more losses within the diphyomorphs. It is a simple, unbranched caecum in all physonects, but it takes on a much greater variety of forms within the Calycophorae.

The descending mantle canal is found in a subset of those siphonophores that have an ascending mantle canal. For the physonects, it is absent in the Apolemiidae and genera of the "dioecious" group of physonects, as designated by Pugh (2006a), but present in his "monoecious" group. Among the calycophorans, it is present in the Prayidae and some Clausophyidae, but absent from all others. Thus this distribution is consistent with a single gain of the descending mantle canal, roughly coincident with the shift from dioecy to monoecy (Dunn *et al.* 2005; Pugh 2006a), and then a single loss within Calycophorae such that it is missing in the diphyomorphs that are the sister group to the Clausophyidae and to the species herein described.

The distribution of the descending mantle canal within the Clausophyidae is more complicated than in any other siphonophore taxa. In two of the genera, *Kephyes* and *Crystallophyes*, a descending mantle canal is present in the anterior nectophore, while in the three others it is absent. However, for three of the genera a descending mantle canal is present in the posterior nectophore, while in the genus *Clausophyes* it is absent (PRP personal observation). The posterior nectophore of the species of the genus *Heteropyramis* has yet to be described. For the diphyid sub-family Sulculeolariinae, Mapstone (2009) considered that the descending mantle canal had disappeared in both nectophores, while the anterior nectophore retained a remnant of the ascending mantle canal, as Totton (1965) had suggested. However, Haddock *et al.* (2005) have already noted that this was actually not a canal but scar tissue left after the detachment of the muscular attachment lamella. The same applies to her illustration (see figure 5H of Mapstone, 2009) of *Lensia conoidea* (Keferstein & Ehlers).

The function of the various nectophore canals: The phylogenetic distribution and variation in morphology of the mantle canals may provide some insight into their function. In the case of the pedicular canal, it seems clear that its basic function is to provide nutrition to the muscular walls of the nectosac and to the velum surrounding the ostium; thereby allowing the nectophore to fulfil its function of jet propulsive swimming. The function of the mantle canals rarely has been considered in the past. However, Leuckart (1853, p. 12)¹, when first describing them, stated, "The vessels, which are intended for the nutrition of the mantle, develop rather late, after the latter has reached a considerable size". On the other hand, Mapstone (2009, p. 10) stated that: "The present author considers that this 'pallial canal' canal [sic] may fulfill an important function in many species by facilitating the shedding of nectophores during autotomy". Nevertheless, it seems unlikely that a canal should facilitate autonomy, as there is no known mechanism by which its presence would weaken or provide a breakpoint for nectophore attachment. However, Mapstone (2009, p. 10) did draw attention, in her Figure 5, to the "relationship between the nectophoral muscular lamella and the median gastrovascular canal on the proximal surface of the nectophore". We propose that there is a functional connection between the two, in that the canal provides nutrition to the muscular lamella. This refines Leuckart's suggestion that the canals do provide nutrition, but simply to the muscular attachment lamella, rather than the whole mantle. The lamella is a highly contractile structure that plays a critical role in orientating the nectophore during swimming (Costello *et al.*, 2015), and may have a high metabolic activity.

Within the full diversity of physonect and calycophoran siphonophores, it is found that most have mantle canals that lie in close proximity to the attachment lamella, throughout most or all of its length. In species with a long lamella, such as *Rosacea* (see Figures 2A, 3A), these canals are extensive, but in species with a reduced lamella, such as the diphyids, the subtending canals are also reduced. Interestingly, though, the particular canals that lie in close proximity to the lamella vary from species to species. These canals can include the ascending mantle canal, descending mantle canal, disjunct pedicular canal, and even the lower radial canal on the nectosac. Within the Physonectae, as noted above, those species that are included in the "monoecious" group have an attachment lamella both anterior and posterior to the pedicular canal and it is subtended entirely by the ascending and descending mantle canals (Figure 1A). For the species of the "dioecious" group, the attachment lamella is present only anterior to the external pedicular canal and is subtended by the ascending mantle canal, and there is no descending canal (Figure 1B). However, the Apolemiidae is exceptional in that a lamella is present both anterior and posterior to the external pedicular canal but, while the anterior portion is subtended by the ascending mantle canal, the role of nutrition for the part of the lamella posterior to the pedicular canal falls to the lower radial canal that closely subtends it (Figure 1C), as Totton (1965) described.

1. Original text "Die Gefäße, die für die Ernährung des Mantels bestimmt sind, entstehen erst ziemlich spät, nachdem der letztere bereits eine ansehnliche Grösse erreicht hat".

This pattern of canals paralleling the attachment lamellae is even more apparent in Calycophorae, where both the course of the canal systems and the length of the lamella are more variable (Figures 2, 3). Within Prayidae there is great diversity in the extent of both the ascending and descending mantle canals and the pedicular canal. For the mantle canals, there was generally a reduction eventually to nothing; for the pedicular canal, it mainly concerned the extent of the disjunct portion. Thus, in *Rosacea*, the attachment lamella is subtended by the ascending mantle, disjunct pedicular, and descending mantle canals (Figures 2A, 3). As Table 3 in Haddock *et al.* (2005) showed the descending mantle canal was eventually lost and species of only three of the nine known genera of prayine siphonophores retain it. The most extreme reduction in the canal systems occurs in the adult nectophores of *Lilyopsis* and *Gymnopraila* species. There, not only has the descending mantle canal completely disappeared, but also there is no disjunct portion to the pedicular canal. In addition, the ascending mantle canal lies entirely within the mesogloea of the nectophore, such that there are no superficial canals subtending the muscular lamella. The tendency for all, or just the anterior part, of the ascending mantle canal to branch off into the mesogloea of the nectophore is common amongst prayine species, occurring in six out of the nine current genera. It can range from a very short inflection, as in *Desmophyes haematogaster*, that may swell up immensely as in *D. annectens* (Figure 4); through multiple, more extensive and complexly branched apical inflections, together with transverse branches from the mantle canal itself, as in *Praya dubia*; to the situation, as discussed above, where the ascending mantle canal lies entirely within the mesogloea.

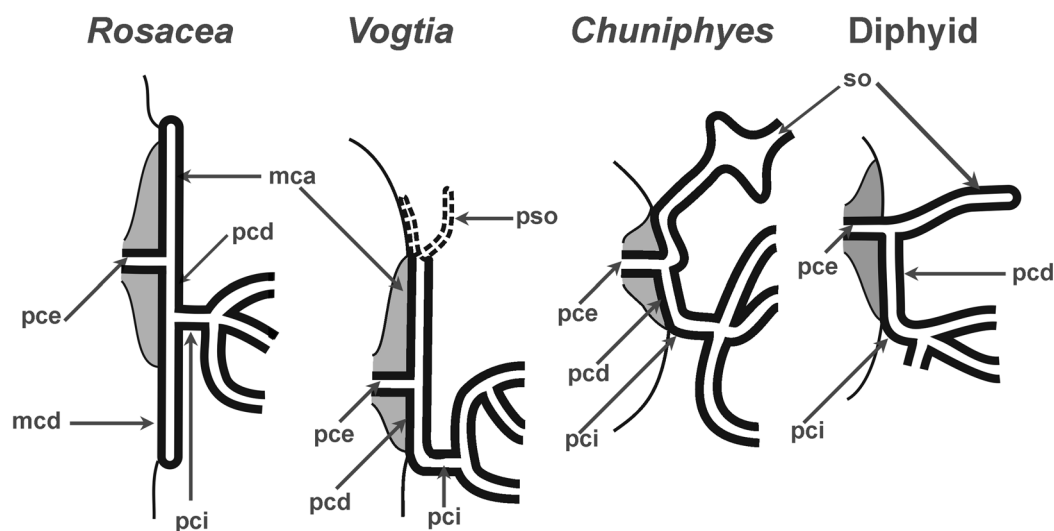


FIGURE 3. Schematics showing the diversity in the arrangement of the various canals in the nectophores (*Rosacea* and *Vogtia*) or anterior nectophore (*Chuniphyes* and a diphyid), and the region of attachment of the muscular lamella (shaded). Annotations as for Figures 1 & 2; **abs**, "ascending branch of somatocyst". The three canals arising from the internal pedicular canal are, from top to bottom the upper, lateral and lower radial canals on the nectosac, except for the diphyid where the bottom canal is the ostial ring canal as the lower canal is virtual. It remains to be determined whether the disjunct or internal portion of the pedicular canal is always present in diphyomorphs. Modified and adapted from Mapstone (2009) Figures 5 & 6.

There is also great diversity in the internal branching of the ascending mantle canal. While the lamella, nectosac, and ostium are the primary muscular structures of the nectophore and likely have the greatest metabolic needs, the region where the ascending mantle canal is found varies greatly in volume and the canal system may covary so that no part of this structure is too far from the nutritional supply of a canal (see Mapstone, 2009, fig. 28D). In the Prayidae, there is much variation in this branching but, with one major exception, the branches are usually simple canals and are not swollen. In this regard, *Amphicaryon*, *Rosacea* and *Craseoa* are the simplest, as the ascending mantle canal does not penetrate into the mesogloea, and it is neither branched nor swollen (Totton, 1965). In *Maresearsia*, the ascending mantle canal is enlarged considerably, but it does not penetrate into the mesogloea. In *Desmophyes haematogaster*, *Mistopraila*, *Prayola* and *Gymnopraila*, the ascending canal does penetrate the mesogloea, but it is neither swollen nor branched. In *Praya*, *Stephanophyes*, *Lilyopsis*, *Nectopyramis* and *Nectadamas* the canals are branched but not extensively swollen, although their distal ends may be slightly so

(Pugh, 1992; Haddock *et al.*, 2005). However, for all siphonophores, there is bound to be at least one exception to the rule and that is the case in *D. annectens* where the internal extension of the ascending mantle canal is greatly inflated (Figure 4). In some hippopodiid species there is a short apical extension of the ascending mantle canal within the mesogloea, which Mapstone (2009, e.g. Fig. 40) referred to this as an "*ascending branch of the somatocyst*". We consider that, as in other prayomorphs, it represents an extension of the mantle canal system, with the same nutritive function.

Within the Calycophorae, there was a shift from apposed to superimposed nectophores, *i.e.*, one nectophore came to be located to the anterior of the other (see Figure 4 in Mapstone, 2009). This coincided with a reduction in the extent of the attachment lamella and its associated canals. The descending mantle canal, already lost in many prayids, disappeared completely in Hippopodiidae and all diphyomorph calycophorans, with the exception of some members of Clausophyidae, e.g. *Kephyes* (Pugh, 2006b). The ascending mantle canal also became reduced to the point of total elimination so that in many diphyid species the attachment lamella is only partially subtended by a canal, the disjunct portion of the pedicular canal (Figure 3 Diphyid). However, the area of attachment is relatively small and, presumably, sufficient nutrition is derived from the external and disjunct portions of the pedicular canal.



FIGURE 4. *Desmophyes annectens* Haeckel, 1888.

Thus, our basic contention is that the mantle canals arose at separate stages in the evolution of the Codonophora in order to provide nutrition to the muscular lamella attaching the nectophore to the stem and were subsequently lost in some clades where the extent of the lamella was greatly reduced. Among physonect siphonophores, with the exception of the Apolemiidae, the presence of a descending mantle canal is directly associated with the attachment lamella extending posterior to the external pedicular canal. In the Apolemiidae, as Totton (1965) points out, it is the lower radial canal that fulfils the same function. Within the Calycophorae, it would appear that both the ascending and descending mantle canals were originally present and that there was an extensive attachment lamella between the two apposed nectophores extending both to the anterior and posterior of the external pedicular canal.

Defining the somatocyst. The term somatocyst has been applied inconsistently by different authors, although it has always been applied only to calycophorans and never to physonects. It has variously been defined as the entire ascending mantle canal system (regardless of structure), or portions of that canal system, or only to the anterior, swollen portion that lies entirely within the mesogloea.

Huxley (1859, p. 5) was the first to define the term *somatocyst*, as "In the *Calycophorid* ... the proximal end of the cœnosarc dilates a little, and becomes ciliated internally, forming a small chamber, which gives off the ducts, by whose intermediation the systems of canals, which embrace the cavities of the organs of locomotion, are brought

into communication with the somatic cavity. At its upper end, this chamber is slightly constricted, and so passes by a more or less narrowed channel into a variously shaped sac whose walls are directly continuous with its own, and which will henceforward be termed the *somatocyst*. The endoderm of this sac is ciliated, and it is generally so immensely vacuolated as almost to obliterate the internal cavity and give the organ the appearance of a cellular mass.

"The somatocyst very commonly contains large, strongly refracting globules of an apparently albuminous matter, of precisely the same character as those which may be observed occasionally to pass through the pyloric valves of the polypites, into the somatic cavity; and I do not doubt that the globules result from the accidental accumulation of such products of digestion." Thus Huxley (1859) considered that the somatocyst could function as a storage organ. Although, he also applied the term *somatocyst* quite broadly, including the longitudinal median, i.e. mantle, canals of a prayid species.

Haddock *et al.* (2005, p. 705) defined the **somatocyst** as "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". They noted (*ibid*) that "this terminology, as opposed to the previous terminology used in prayines, is consistent with that of diphyid and abyloid calyphorans, in the sense that our definition of a somatocyst accommodates the way that term is usually applied in those groups".

Mapstone (2009, p. 12) disagreed with Haddock *et al.* (2005) in that she did not regard her "ascending surface diverticulum" to form part of the somatocyst, and considered that a new definition of the **somatocyst** was required; namely "those diverticula from the pedicular canal or its ascending surface diverticulum that penetrate the mesogloea". This definition, thus, includes all the internal branches of the mantle canals found in some prayomorph species as well as the somatocyst in diphyomorphs. For the prayomorphs, Mapstone (2009, e.g. fig. 6) then refers to the anterior canal, penetrating into the mesogloea, as the "ascending branch of the somatocyst". This would, thus, appear to indicate that the somatocyst and the ascending surface diverticulum are one and the same, thereby reflecting the original definition given by Haddock *et al.* (2005).

The great variation in the structure of the ascending mantle canal system makes such distinctions difficult to define and apply universally, and they may be somewhat arbitrary. Thus, we have decided to refine our definition, somewhat along the line suggested by Mapstone, and now consider the somatocyst as an extended portion of the ascending mantle canal that lies entirely within the mesogloea of only diphyomorph calyphorans. We chose this definition because it is consistent with most historical usage; it is relatively simple; and it probably corresponds to novel evolutionary function (see below).

For all other diphyomorphs, where the descending mantle canal and the portion of the ascending mantle canal along the hydroecium are absent, the somatocyst arises directly from the external pedicular canal that penetrates into the anterior, or larval in the case of the Sphaeronectidae, nectophore. The arrangement in Clausophyidae is more complicated. In some clausophyid species, the ascending mantle canal runs along the hydroecium and then penetrates into the mesogloea as the somatocyst, in both the anterior and posterior nectophores (PRP personal observation). However, for the posterior ones, although it is difficult to be certain of the point of insertion of the external pedicular canal, it appears that the latter canal gives rise directly to the somatocyst and to its disjunct portion within the nectophore. Thus, the ascending mantle canal along the hydroecium already has been suppressed completely. For four of the clausophyid genera the descending mantle canal is still present, but it is absent from *Clausophyes* species. Similarly, a short ascending mantle canal along the hydroecium appears to be retained in the anterior nectophore, with the exception of *Clausophyes* species, where the somatocyst arises directly from the external pedicular canal. However, for *Chuniphyes moserae*, the ascending mantle canal along the hydroecium is very short.

While nourishment of the lamella provides a clear hypothesis for the function of the canals that branch from the ascending mantle canal in prayomorph siphonophores, it does not provide a functional explanation for the ascending structures that penetrate into the mesogloea in many diphyomorph calyphorans, which are unbranched, but vary greatly in the degree to which they are swollen. There are at least two possible functional implications: They may provide for nutritional storage, as previously hypothesized by Huxley (1859). Alternatively, they may serve for buoyancy control. Buoyancy would be further refined into two categories – *gross buoyancy*, which could influence location in the water column, and *trim*, which could influence the resting orientation of the nectophore in the water column. For instance, the nectophores of *Lensia conoidea*, *in situ*, are

seen to be horizontal when at rest, with the somatocyst in the anterior nectophore uppermost. However, in several diphyids the somatocyst is greatly reduced and it would be difficult to ascribe a buoyancy function to it. Buoyancy in the prayomorph calycophorans is mainly provided by the large volumes of mesogloea, and active exclusion of heavy sulphate ions (Bidigare & Biggs, 1980) also would help to reduce the overall of the nectophore. Thus, we suggest that a change in function of the internal extension of the apical end of the ascending mantle canal occurred during the evolutionary transition from apposed to superimposed nectophores.

Description

Family *Tottonophyidae* fam. nov.

Tottonophyes gen. nov.

Monotypic genus for *Tottonophyes enigmatica* sp. nov.

Tottonophyes enigmatica sp. nov.

Diagnosis. With two rounded, almost spherical, dissimilar nectophores with one slightly larger and slightly to the anterior of the other. Extensive nectosac and hydroecium occupying most of each nectophore. No flaps on lateral walls of hydroecium; no mouth plate on posterior nectophore; ascending and descending mantle canals present, with internal somatocyst forming a conical structure. No disjunct portion of the pedicular canal in either nectophore.

Material examined. Four specimens of *Tottonophyes enigmatica* sp. nov. have been examined. All were collected by the MBARI ROVs, namely *Tiburón* (T), *Ventana* (V), or *Doc Ricketts* (DR), whose dive details are shown in Table 1. They were preserved in 5% buffered formalin in sea water. Unfortunately, only one of the nectophores from the DR0105 and DR0552 dives was preserved. A fifth specimen (T0398) was collected and photographed in the ship-borne laboratory, then part was frozen for molecular analyses. Five other specimens (unboldened) in Table 1 have been identified from *in situ* frame grabs.

The specimen from Tiburón Dive 897 has been designated as the holotype, and will be deposited at the Smithsonian National Museum of Natural History. The remainder will be deposited with the Peabody Museum of Natural History at Yale University.

TABLE 1. List of observed specimens of *Tottonophyes enigmatica* sp. nov. For the specimens collected (emboldened), "D" means that they were collected with a "Detritus sampler". * Specimens not examined for present study.

ROV Dive	Depth of collection/ Observation (m)	Date	Latitude	Longitude	Water Depth (m)
T0364	3092	2–Oct–2001	36°20.25'N	122°53.96'W	3496
T0398–D1*	2836	23–Feb–2002	36°34.37'N	122°31.20'W	3036
T0438	2368	12–Jun–2002	36°34.81'N	122°25.18'W	2421
T0762	3384	16–Nov–2004	36°19.25'N	122°53.29'W	3451
T0897–D4	1342	23–Sep–2005	36°39.31'N	122°09.52'W	1356
V3005–D8*	963	15–May–2007	36°41.90'N	122°03.93'W	1741
DR0026	3281	27–May–2009	36°06.88'N	122°45.09'W	3415
DR0105–D3	2469	13–Dec–2009	36°04.08'N	122°17.89'W	2476
DR0327	3539	4–Dec–2011	35°55.97'N	122°55.99'W	3612
DR0552–D1	2222	20–Nov–2013	36°4.11'N	122°17.77'W	2481
DR0965–D1	910	14–Jun–2017	36°41.96'N	122°01.98'W	1555

Description: An *in situ* frame grab from *Doc Ricketts* Dive 105 (Figure 5A) shows that the larger of the two nectophores of *Tottonophyes enigmatica* sp. nov. is only partially superimposed over the smaller one. This superimposition (i.e. positioning of one nectophore to the anterior of the other) is much greater than seen in prayomorphs (Figure 2A), where they are apposed, and much less than for other diphyomorphs, even the clausophyids (Figure 5B, C). However, unlike prayids, there are clear differences in the two nectophores of *T. enigmatica* sp. nov., and for ease of comparison with the diphyomorph species the nectophores will be referred to as anterior (larger) and posterior (smaller).

The holotype specimen from *Tiburón* Dive 987 was chosen as the best preserved of all the specimens collected up until the time that this description was begun. Very recently a further specimen was collected during *Doc Ricketts* Dive 965. This proved to be the largest specimen so far collected, with the anterior nectophore measuring 13.5 mm in length and 9 mm in width, and the posterior one 11.5 by 7 mm. Nonetheless, the general structure of both nectophores showed no major variations from the norm, and there was no evidence there to suggest the unlikely eventuality that we were dealing with a different species. However, there appeared to be a minor difference in the special nectophores in the cormidia that is discussed below.

Anterior nectophore: (Figures 6 & 7). The larger, anterior nectophore of the holotype specimen measured, in its preserved state, 7.6 mm in height, 7.6 mm in width and 4.6 mm in breadth. The proximal two-fifths of the nectophore was almost entirely occupied by the hydroecium (Figure 7), which was open at its lower end, but at its upper end its median wall curved over and ran to the proximal wall of the nectophore a short distance from its apex. The distal three-fifths of the nectophore were mainly occupied by the extensive nectosac; its ostial opening, which included a large velum, was directed obliquely toward the upper side of the nectophore, such that outer side of the nectophore was only about two-thirds the height of the inner or proximal side.

All four radial canals arose from the short and direct internal pedicular canal. In its preserved state the upper part of the nectosac was more extensive laterally than in the mid-line, such that the upper radial canal ran through a median depression. However, in life this depression was less marked (Figure 6A). The upper and lower radial canals ran directly to the ostial ring canal. The lateral radial canals, however, followed a course somewhat similar to that found in *Clausophyes* species. From their origin, they extended across the nectosac, with only a very slight upward curve. Close to the outer side of the nectosac they curved through 90° and ran directly toward the ostium, before again bending through 90° and running back across the nectosac, with a slight upward curve as they approached the proximal side. A further curve through 90° again directed them toward the ostium, but before reaching it, they curved through c. 60° and then ran obliquely down to join with the ostial ring canal.

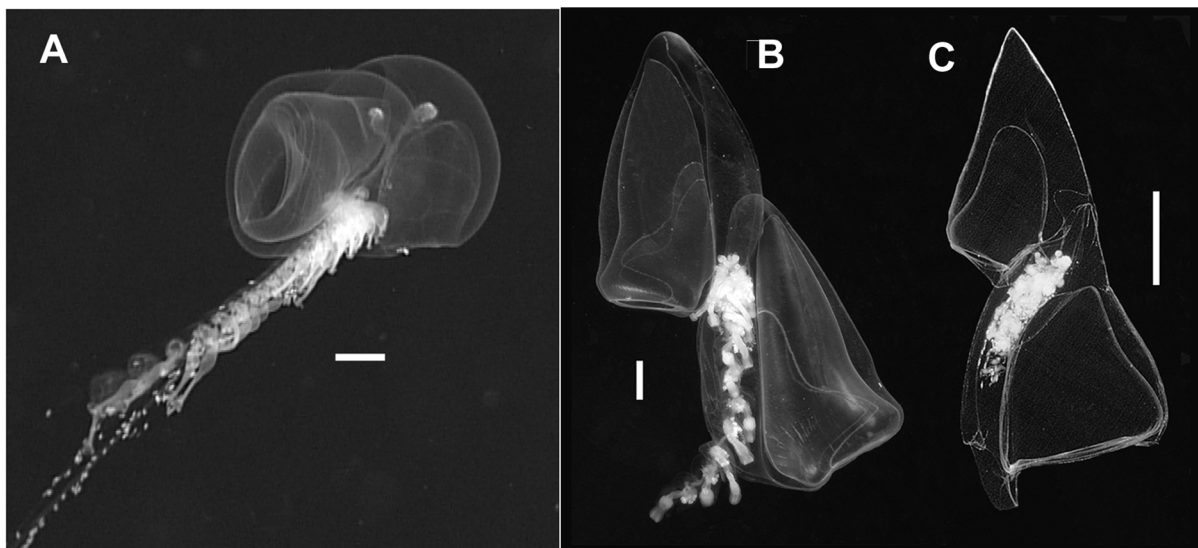


FIGURE 5. A. *In situ* Frame Grab of *Tottonophyes enigmatica* sp. nov. taken during *Doc Ricketts* Dive 105; Specimens of (B) *Kephyes hiulcus* Grossmann & Lindsay, 2017. and (C) *Clausophyes moserae* from Pugh, 2006b. Scale bars 2 mm.

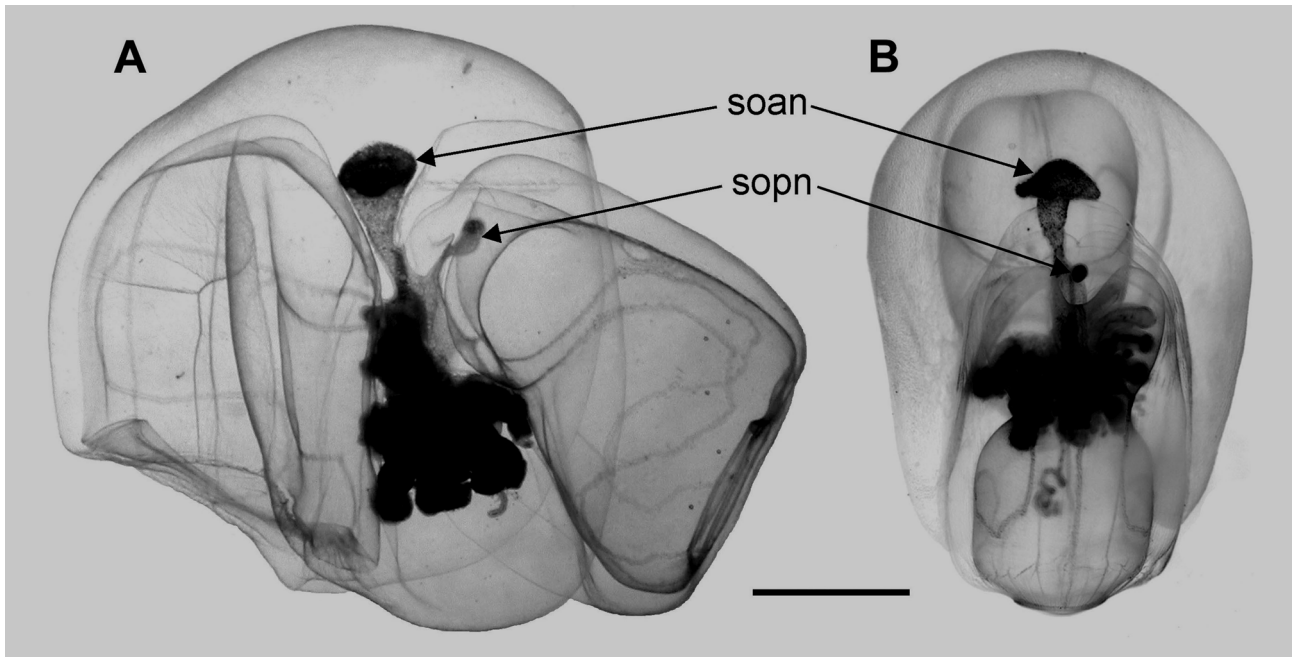


FIGURE 6. Photographs showing **A.** lateral and **B.** from distal side of posterior nectophore views of *Tiburon* Dive 897 holotype specimen of *Tottonophyes enigmatica* sp. nov. **soan** and **sopn** somatocyst of anterior and posterior nectophores, respectively. Scale bar 2 mm.

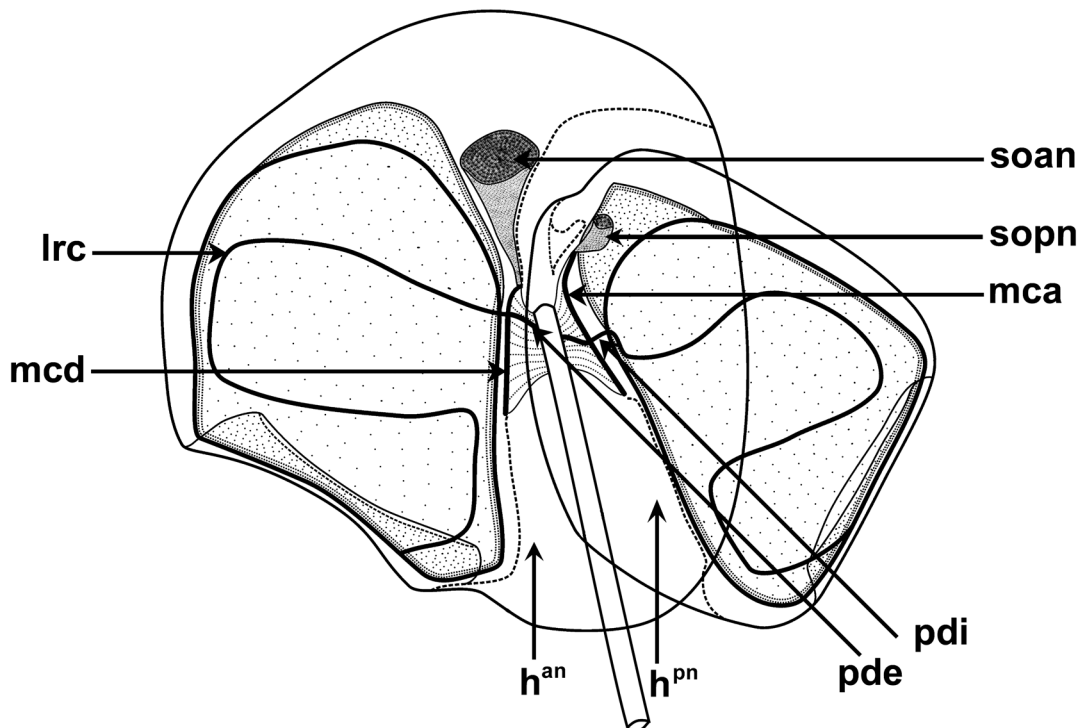


FIGURE 7. Nectophores of *Tottonophyes enigmatica* sp. nov. **A.** anterior, and **B** posterior. **h^{an}**, **h^{pn}**: hydroecium of anterior and posterior nectophore, respectively; **mca**, **mcd**, ascending and descending mantle canal, respectively; **ns**: nectosac; **pde**, **pdi**, external and internal pedicular canal, respectively; **soan**, **sopn**: somatocyst of anterior and posterior nectophore, respectively. Scale bar 2 mm.

As well as the short internal pedicular canal, which reached the nectosac approximately at the mid-height of the nectophore, there were two short median longitudinal canals running along the wall of the hydroecium: the

ascending and descending mantle canals. The ascending canal was shorter than the descending one, and soon gave rise to the internal somatocyst, within the mesogloea. The somatocyst was a funnel-shaped, laterally compressed structure (Figures 6 & 7, **soan**). Its apex, which was covered with large globular cells, was expanded laterally so that, from this angle, the somatocyst looked like a mushroom (Figure 6B **soan**). In life, the apex of the somatocyst was deep brown in colour, while the remainder of the cone was suffused with a lighter tan colouration. For the DR0965–D1 specimen the fluid within the somatocyst, particularly that of the anterior nectophore, in life, was a pinkish-red in colour.

Posterior nectophore: The smaller posterior nectophore (Figures 6 & 7) measured, in its preserved state, 5.6 mm in height and 4.5 mm in width. Its breadth was not measured as the nectosac had collapsed and the whole nectophore narrowed. However, the posterior nectophore from *Doc Ricketts* Dive 105 remained in good condition and its dimensions were: height 7.6 mm, width 8.2 mm, breadth 6.2 mm. Thus, it was considerably larger than the posterior nectophore of the type specimen and, commensurately one would have expected the anterior nectophore to be considerably larger. However, as noted above, the anterior nectophore was unfortunately lost during collection.

The hydroecium occupied less than a third of the total width of the nectophore (Figure 7) and was open throughout almost the entire height of the nectophore, but faded out shortly before reaching its lower edge. At its upper end, just above the swollen part of the internal somatocyst, the hydroecium formed a small median cavity. The remainder of the nectophore was almost entirely occupied by the nectosac, with its ostial opening and a slightly smaller velum directed obliquely toward the upper side of the nectophore. No mouth plate was present. The courses of the radial canals were similar to those of the anterior nectophore. In the preserved state, the upper canal likewise ran through a median furrow in the apical part of the nectosac, whose lateral wings apically came to a more or less pronounced point. This arrangement was also apparent in the living specimen. The lower canal ran directly down to the ostial ring canal. The lateral radial canals, after arising from the short internal pedicular canal, almost immediately curved basad and then after a short distance ran outwards across the nectosac. As they approached its outer wall they gradually curved to run basally again, before turning to run obliquely upward toward the proximal side. Then they curved basally again before turning distad and running obliquely down to the ostial ring canal.

Both descending and ascending mantle canals were present (Figure 7 **mcd, mca**), although their relative lengths were the reverse of those in the anterior nectophore. The ascending mantle canal was much longer than that in the anterior nectophore and, below the median cavity in the hydroecium, it penetrated into the mesogloea and formed the internal somatocyst. This was relatively short and without lateral extensions (Figures 6 & 7 **sopn**). It also had a small brownish patch of cells at its distal end, while the remainder contained light-brown pigmentation, after preservation.

Cormidium: The siphosomal gastrovascular lumen, like that of the somatocysts, contained a pinkish-red fluid. Much of the siphosome of the type specimen of *Tottonophyes enigmatica* **sp. nov.** was lost during collection and so only the very youngest immature cormidia remained (Figure 6). Fortunately, more of the siphosome was retained with the posterior nectophore collected during *Doc Ricketts* Dive 105, and the terminal cormidia, although still immature, can be described. Photographs of the oldest one of these are shown in Figure 8. It consisted of a bract, a gastrozoid and tentacle, and what appears to be an asexual swimming bell, together with two small buds (Figure 8 **b**). The specimen from *Doc Ricketts* dive 552, received after the major part of the manuscript had been written, possessed a longer piece from the anterior end of the siphosome, despite the fact that the anterior nectophore was lost. Some of the cormidial groups appeared to be fully developed and as the swimming bells, at all stages of development, bore no trace whatsoever of a manubrium, then the presumption that they were special nectophores appeared to be correct.

Bract: The largest bract *Doc Ricketts* Dive 552 specimen (Figure 9, left) measured 3 mm in length and 1.85 mm in width. It was a very simple, leaf-like structure, being concave on the lower side, convex on the outer. There was a small notch (Figure 10 A) toward the proximal end. The phyllocyst was reduced to a central, thickened part overlying the stem, and two hydroecial canals, which in the youngest bracts ended in slight swellings. In the younger bracts (Figure 10 B) the two hydroecial canals were of almost equal length, but as they enlarged they became distinctly asymmetrical.

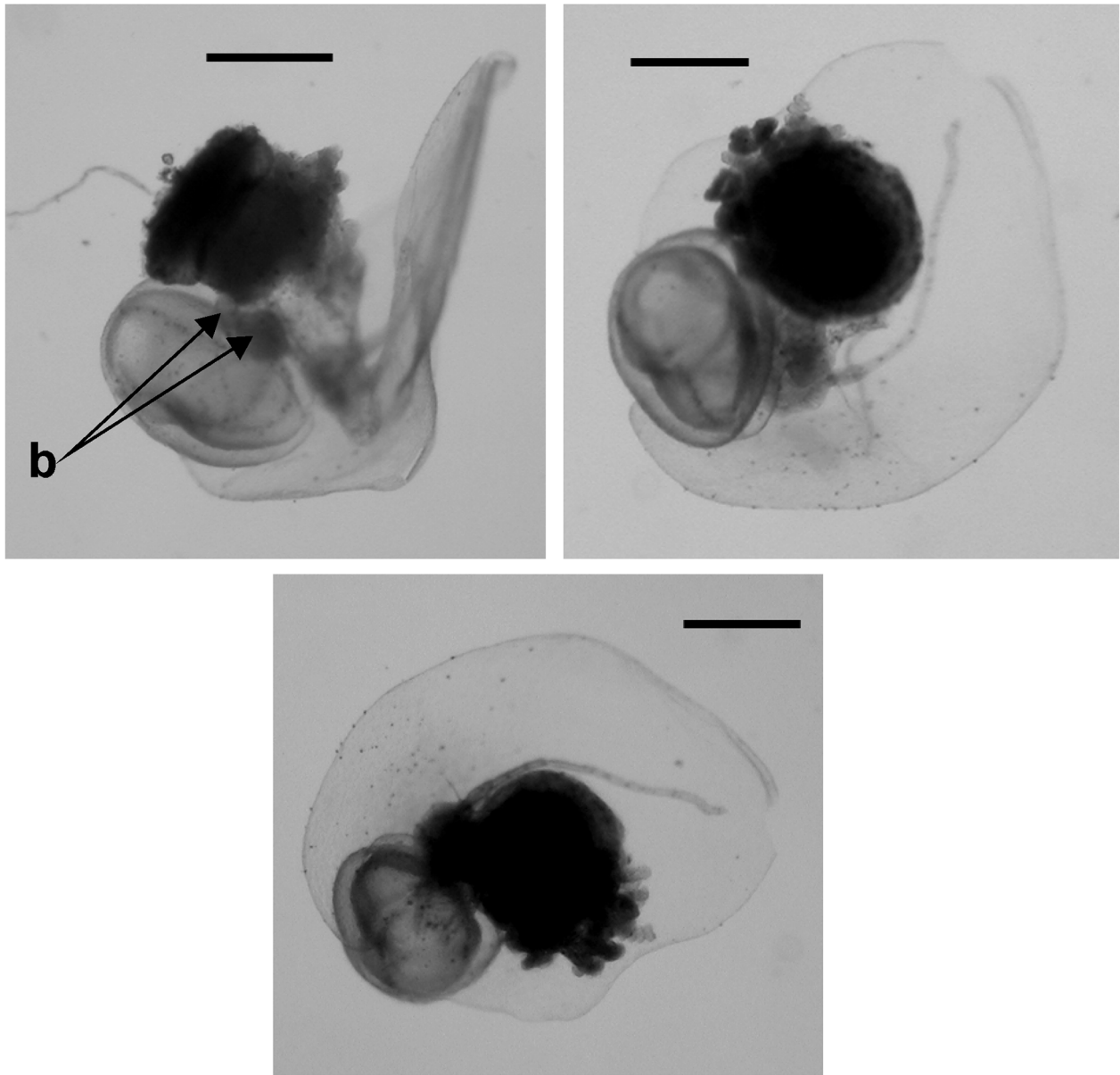


FIGURE 8. Three views of an individual cormidium from *Doc Ricketts Dive 105* specimen of *Tottonophyes enigmatica* **sp. nov.** The two buds (**b**) are marked by arrows, and are referred to in the text. Scale bars 0.5mm.

Special cormidial nectophore: The large medusoid (Figure 9, 10 C) structure measured about 2.5 mm in height and 2 mm in maximum width. It was a ridgeless, simple structure, largely occupied by the subumbrella cavity. All the radial canals were located laterally and originated from the short pedicular canal. One pair ran directly to the ring canal, while the other pair described broad curves, first upward then downward, before running directly to the ring canal. As none of these medusoids, at any stage in development, showed any signs of sexual products they were, as noted above, presumed to be special nectophores. For the more mature cormidial groups, as well as the large medusoid there were one or two small buds (Figure 8B) attached to the stem. These were presumed to be developing male and/or female gonophores but, in all cases, they were too small to be identified positively as such.

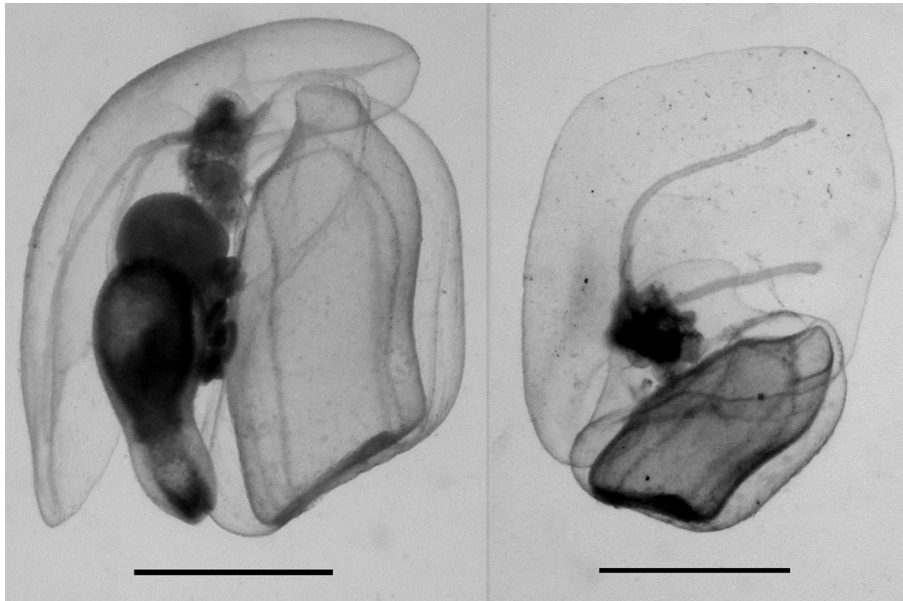


FIGURE 9. Two individual cormidia from *Doc Ricketts* Dive 552 specimen of *Tottonophyes enigmatica* **sp. nov.** Scale bars 1 mm.

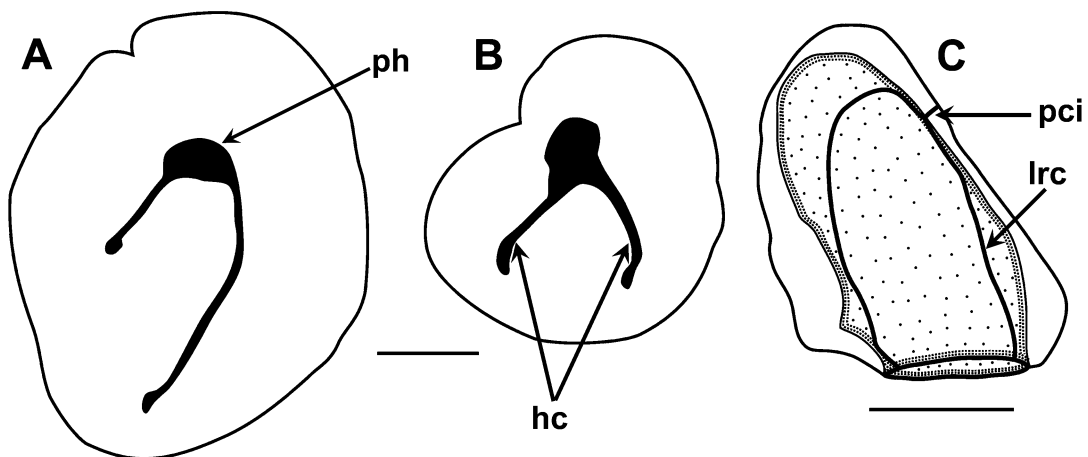


FIGURE 10. Bracts (A, B), and gonophore or special cormidial nectophore (C) of *Doc Ricketts* Dive 105 specimen of *Tottonophyes enigmatica* **sp. nov.** **hc**, hydroceal canals; **lrc**, lateral radial canal; **pci**, internal pedicular canal; **ph**, phyllocyst. Scale bar 0.5mm.

As noted above, the special nectophores of the largest, recently collected specimen, from *Doc Ricketts* Dive 965, showed a slight difference from the norm in that the lateral radial canals did not follow the simple course as shown in Figure 10C. Instead their courses more closely resembled those of the lateral canals on the nectophores. On the other hand, the bracts showed no differences, with the two hydroceal canals and the thickened longitudinal one. As none of the other specimens had even partially developed cormidia, it is not, as yet, possible to say whether this difference is significant.

Gastrozoid and tentacle: The small gastrozooids (Figure 11 A) measured about 12.5 mm in length and 7 mm in diameter, with the conspicuous basigaster occupying the proximal third of the whole. The tentilla (Figure 11 B) were of the typical calycophoran form with a C-shaped cnidoband, orange-red in colour, and a long terminal filament. The cnidoband bore two types of nematocyst. There were about 10 pairs of large nematocysts, measuring c. 95 x 20 µm, which appeared to be the microbasic mastigophores normally found for calycophorans; and several rows of smaller ones, measuring 28 x 5 µm, which were anisorhizas. The terminal filament contained desmonemes, 11 x 9 µm, and anisorhizas, but their exact arrangement was not investigated.

Eudoxid stage? Recently, a strange eudoxid (Figure 12) was found, as bycatch, in a suction sample collected during Doc Ricketts Dive 918 (17th December; 2016; 36°35.5'N, 122°25.57'W; water depth c. 2900 m) in Monterey Canyon, at a depth of 550 m. The eudoxid appeared to belong to a clausophyid species as there were two long hydroecial canals, extending down into the extensive neck shield of the bract. The young bracts of *Tottonophyes enigmatica* **sp. nov.** (see. Figures 9, 10) also showed these canals. The only non-clausophyid species whose eudoxid bracts show that character is *Gilia reticulata* (Totton) (see Pugh & Pagès, 1995) that, for the present, is placed in its own sub-family, Giliinae, within the family Diphyidae, until some phylogenetic analyses can prove otherwise. Unfortunately, although several attempts have been made, molecular analyses of *G. reticulata* have always failed.

In its preserved state, the eudoxid bract measured 6.1 mm in length, of which the neck shield occupied about $\frac{2}{3}$ rd. The headpiece was ridgeless and smooth, and was largely filled by the phyllocyst, which was globular in shape, measuring 1.35 mm in height, and 1.1 mm in width, with a narrow apical extension, 0.35 mm in length. The two hydroecial canals, which arose from the longitudinal canal, extended almost to the base of the neck shield. The longitudinal canal was well-defined and curved around the base of the phyllocyst, on the opposite side to the gonophore, and extended for a short distance beyond the point of origin of the hydroecial canals.

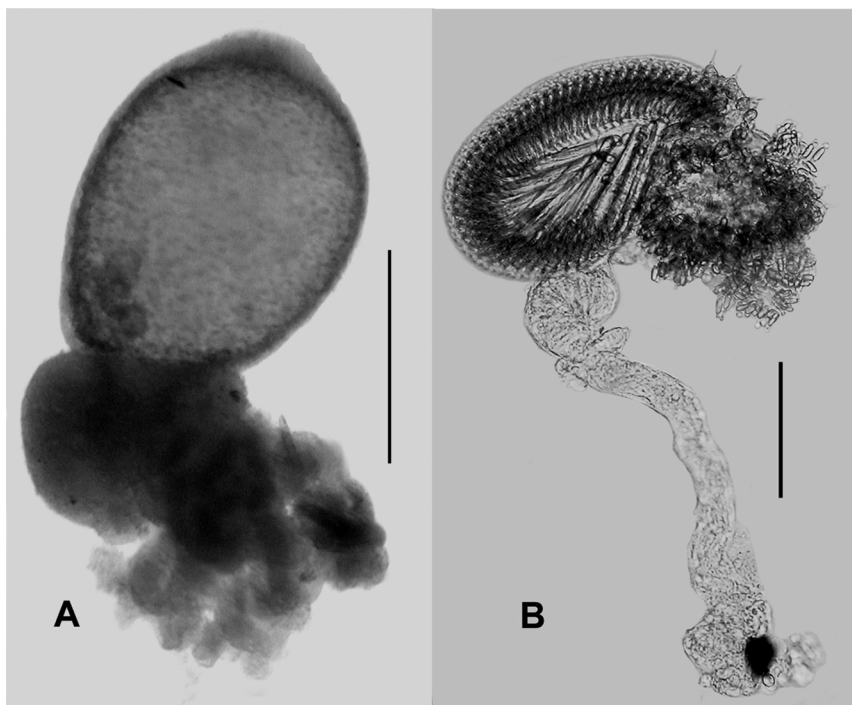


FIGURE 11. **A.** Gastrozoid and **B.** tentillum of specimen *Tottonophyes enigmatica* **sp. nov.** collected during *Doc Ricketts* Dive 552. Scale bars **A.** 0.5 mm and **B.** 100 μ m.

As with the young cormidia, there appeared to be a special nectophore, 7 mm in length, because no sexual products were observed within its subumbrella cavity, which occupied almost the full length of the structure. The lateral radial canals arose from the internal pedicular canal at some distance below the apex of the nectophore. There was a distinct, rounded mouth plate. The deep hydroecium extended for almost the entire length of the zooid, with its lateral edges petering out on the mouth plate. The right-hand wing formed an extensive fold that overlapped the left-hand one for most of its length. Slightly above the ostial level, the right-hand fold was abruptly truncated, except on its outer margin where it protruded basally to form a distinct tooth-like structure

The gastrozoid remained attached to the stem and at its base the tentacle was attached on one side while on the other were a few small buds, measuring up to 0.28 mm, that could be developing gonophores.

Because of the presence of a pair of hydroecial canals, it would appear that this eudoxid belonged to a clausophyid species but, despite the similarities, we cannot be certain for the moment whether it belongs to *Tottonophyes enigmatica* **sp. nov.** However, given that the eudoxid stages, where present, of all clausophyid species

are known, there remains a distinct possibility that it does. The eudoxid stages in clausophyid species vary considerably from the highly complex "fuseudoxid" of *Crystallophyes* (Pagès & Pugh, 2002) to, apparently, the complete absence of bracts, and hence eudoxids, in *Clausophyes* species. For the other genera, the eudoxid bract may be ridged (*Chuniphyes* and *Heteropyramis*) or smooth (*Kephyes*), but always the phyllocyst has an apical extension, and two distinct hydroecial canals.

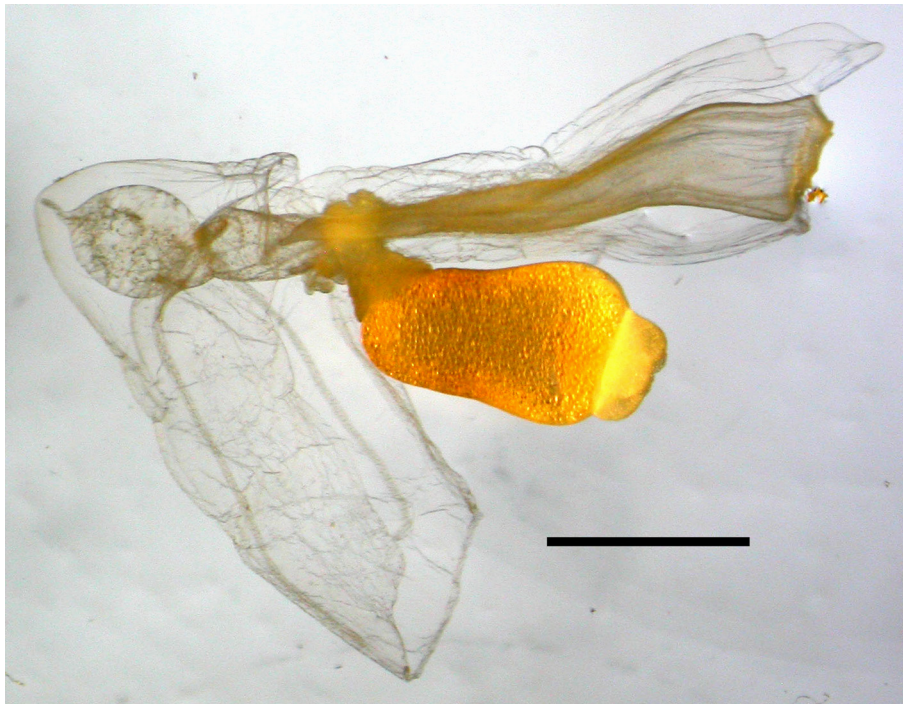


FIGURE 12. Eudoxid stage that possibly belongs to *Tottonophyes enigmatica* **sp. nov.** Scale bar 2 mm.

Distribution. *Tottonophyes enigmatica* **sp. nov.** is known from only a small area of the North East Pacific Ocean in the vicinity of Monterey Bay, California (see Table 1). Seven of the specimens, either collected or identified from video frame grabs, were found at depths >2400 m, with a maximum of 3539 m while two of the others was collected at the much shallower depths of 963 and 919 m. The depth distribution of *Tottonophyes enigmatica* **sp. nov.** may have been affected by the actual depth of the water column, particularly at the deepest depths where specimens were usually found within 400 m of the sea bed. When the water depth was slightly shallower, as during *Doc Ricketts* Dive 105 and *Tiburón* Dive 897, the specimens were found very close to the seabed; being just 7 m above at the former, but the two shallowest specimens were collected at depths more than 600 m above the sea floor. Thus, it appears that *T. enigmatica* **sp. nov.** is largely restricted to deeper depths (>2500 m), but may be upwelled to shallower depths within the complex canyon system in Monterey Bay. Little can be said about the relatively shallow depth of 550 m (water depth c. 2900 m) at which the strange eudoxid was collected until we can be sure that it actually belongs to *T. enigmatica* **sp. nov.**

Molecular phylogenetics. The updated phylogenetic analyses for the Calycophorae presented here (Figure 13) builds directly on the early analysis of Dunn *et al.* (2005), and includes several additional calycophoran taxa. *Tottonophyes enigmatica* **sp. nov.** was included in the previously published analyses under the name Clausophyid sp. 1. The new analyses provide strong support for the monophyly of Calycophorae (Figure 13, branch 1) and also suggest that Prayomorpha is paraphyletic with respect to Diphyomorpha. Support for Diphyomorpha is moderate (Figure 13, branch 2). *Tottonophyes enigmatica* **sp. nov.** is placed as the sister taxon to the remaining Diphyomorpha, for which there is strong support (Figure 13, branch 3). Clausophyidae is recovered, but with very weak support. This indicates that Clausophyidae is either sister group to, or paraphyletic with respect to, the strongly supported clade indicated by branch 4 (Figure 13). This branch 4 clade consists of *Sphaeronectes*, *Abylopsis*, and all sampled taxa that have been assigned to Diphyidae.

Etymology. We dedicate the family and hence the generic names to that great siphonophorologist Arthur Knyvett Totton who strove, in his *Synopsis of the Siphonophora*, to sort out many, but unfortunately not all, of the

taxonomic problems that haunt this group. The specific name refers to the enigmatic combination of traits, some of which are unique to prayomorphs and others unique to diphyomorphs that until now had not been found in the same species.

Discussion. The phylogenetic position of *Tottonophyes enigmatica* sp. nov. (Figure 13) and its unique combination of characters make it possible to postulate more specific hypotheses for the evolution of diphyomorph calyphorans than were possible in the past. These hypotheses can be described in terms of the character changes that occurred along four particularly interesting branches in the siphonophore phylogeny (Figure 13, branches 1–4).

Changes along branch 1 in Figure 13, the stem of Calyphorae, are responsible for the very distinct morphology of that clade, particularly with regard to the great modifications to the nectosome and associated structures. Most species of Physonectae, the paraphyletic group within which Calyphorae are nested, have, starting at the anterior end, a pneumatophore (a gas filled float); followed by a nectosomal growth zone; a length of stem along which the mature nectophores or swimming bells are attached; and then the siphosomal growth zone and siphosome itself (Totton, 1965). The, often numerous, identical nectophores are attached along one side of the stem that, according to the family, can be either dorsal or ventral. However, they are usually displaced laterally into a biserial configuration. As noted above, the physonect species in the "dioecious" grouping possessed only an ascending mantle canal in their nectophores, while those of the "monoecious" group possessed both that canal and a descending mantle canal.

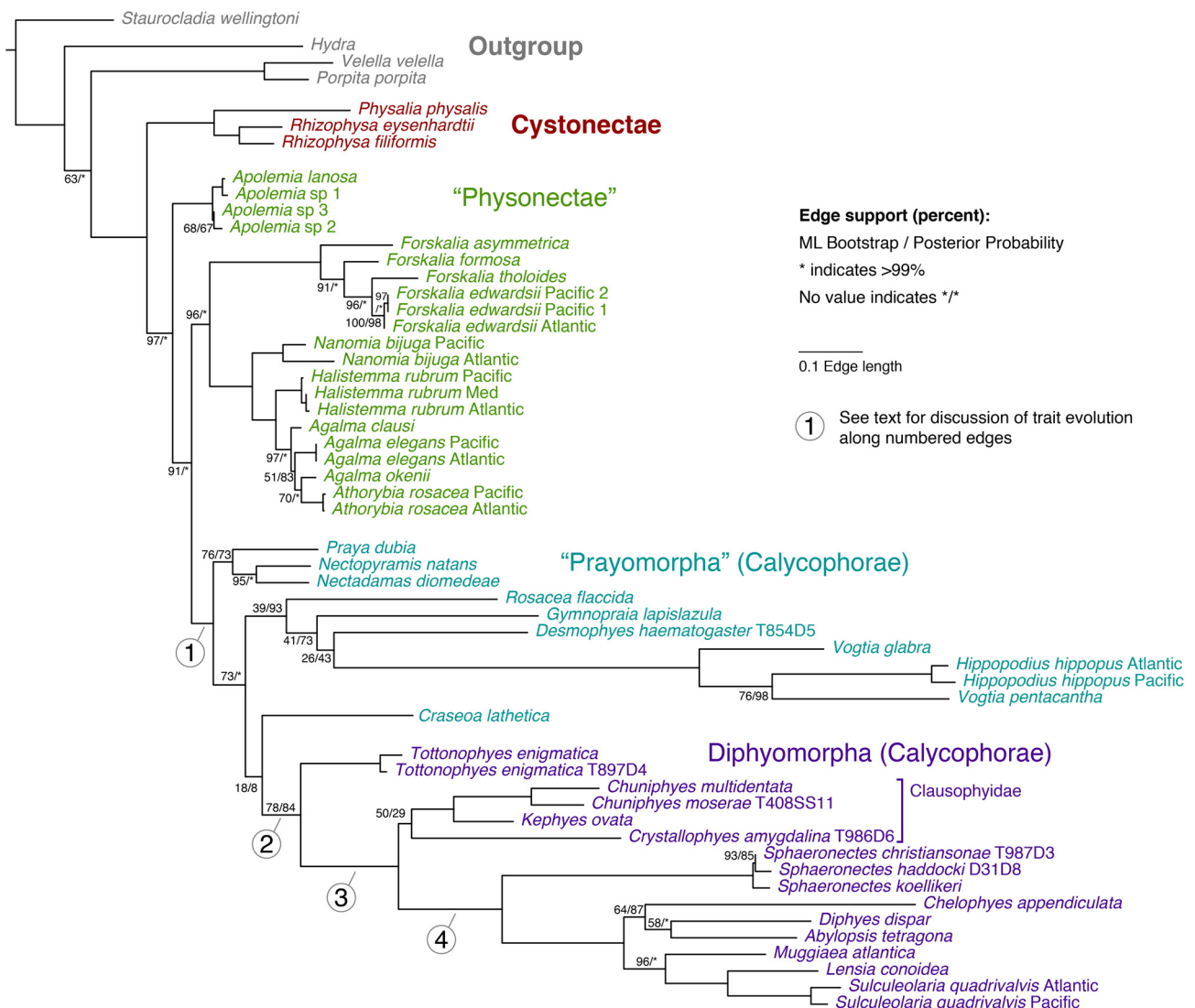


FIGURE 13. Phylogram of Siphonophora, including *Tottonophyes enigmatica* sp. nov. Bootstrap support values are shown at internal nodes. Unlabelled notes have 100/100 support. See methods for more information.

Along branch 1 the pneumatophore was lost and the nectosomal stem greatly reduced, so that the ancestral calycophoran colony possessed just two nectophores placed opposite each other at the anterior end of the stem. The growth zones of the nectosome and siphosome became situated very close to each other. Indeed, according to Bigelow's (1911) illustration (Figure 14), the siphosomal growth zone, or horn, now lay anterior to the point of attachment of the nectophores. However, as the ancestral prayomorphs almost certainly retained the potential to produce replacement bells, it is not exactly certain where, in this situation, the nectophoral buds arose. All calycophorans are monoecious and so, presumably, evolved from the monoecious clade of physonects. This is consistent with the retention of both the descending and ascending mantle canals, as seen in the less derived prayomorphs. However, whereas many monoecious physonect species have nectophores with distinctive ridge patterns, the two relatively large nectophores of "Prayomorphs" are usually smooth and rounded (Mackie *et al.*, 1987).

The changes along branch 1 (Figure 13) were not limited to the nectosome. The composition of the siphosome was simplified in several regards. Palpons were lost (assuming the non-feeding mouthless polyps in *Stephanophyes superba* are vestigial gastrozooids). The tentilla of Calycophorae are less variable than those of Physonectae, with the majority having a crescent shaped cnidoband and single terminal filament, and a consistent cnidome. Exceptions to this tentillum structure are most pronounced in nectopyramids, especially *Nectadamas* Pugh, 1992.

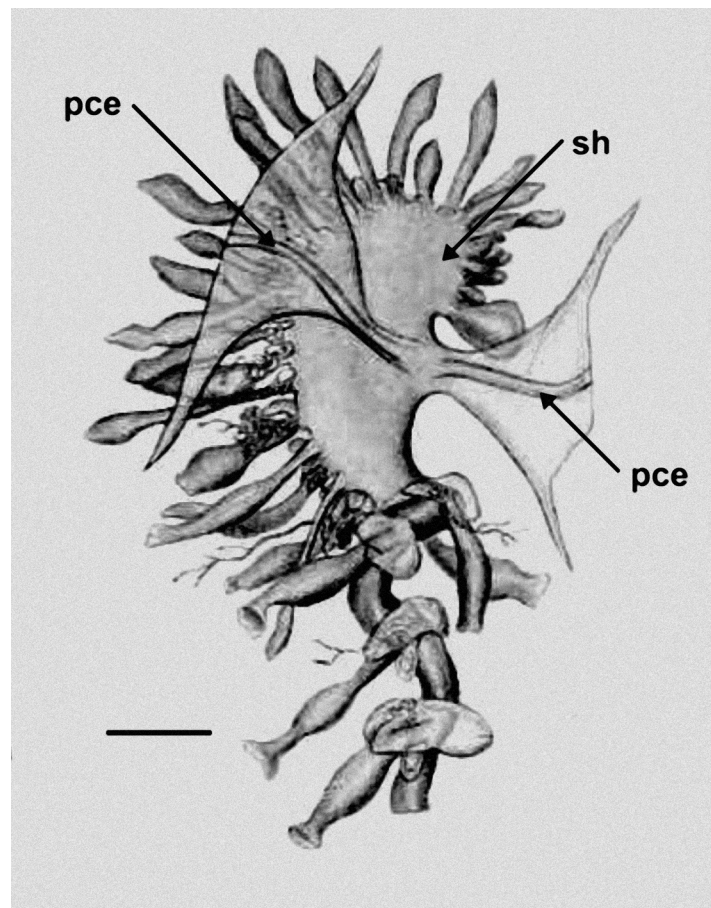


FIGURE 14. Proximal portion of the stem of *Rosacea cymbiformis* delle Chiaje, with buds for siphons, bracts, and gonophores, and the muscular lamellae that bore the nectophores. Reproduced from Bigelow (1911), Plate 2, figure 3. **pce.** external pedicular canal running through the attachment lamella of the nectophore; **sh.** siphosomal horn. Scale bar 5 mm.

Along branch 2 (Figure 13), the stem of Diphyomorpha, the most pronounced changes are again in the nectosome. The nectophores transition from being similar and apposed to becoming heteromorphic and superimposed, with one designated as the anterior and the other as the posterior nectophore (see Fig. 4 in Mapstone, 2009). As noted above, the change in arrangement is associated with a lessening of the extent of the lamella connecting the nectophores to the stem, and a reduction in the extent of both the ascending and descending mantle canals. The disjunct portion of the pedicular canal is retained, with the notable exception of *Tottonophyes*

enigmatica **sp. nov.** The somatocyst may have also originated along this branch, though it is not certain. As described above, we define the somatocyst as any extended portion of the ascending mantle canal that penetrates the mesogloea in diphyomorphs. The ascending mantle canal also penetrates the mesogloea in some, but not all, prayomorph calyophorans, including *Praya*, *Desmophyes*, *Mistopravina*, *Stephanophyes*, *Lilyopsis*, and *Gymnopraia* (Haddock *et al.* 2005 Table 3). The incomplete taxon sampling of the phylogeny presented here (Figure 13), as well as poor support at relevant nodes, mean that it is not clear if any of the prayomorphs with a penetrating ascending mantle canal are sister groups to the diphyomorphs. If they are, then these penetrating canals would be homologous in diphyomorphs (where it is called the somatocyst) and these prayomorphs. Alternatively, the penetrating ascending mantle canal of the diphyomorphs may have arisen independently and is not homologous to other penetrating ascending mantle canals of the prayomorphs.

A general characteristic of diphyomorph species is that the terminal (oldest) cormidium on the siphosomal stem is released to live a free existence as a eudoxid. If our conjecture with regard to the strange eudoxid described above is correct then this applies to *Tottonophyes enigmatica* **sp. nov.** Eudoxid release seems to be a relatively labile trait, though. It appears that some clausophyid species, such as *Clausophyes*, *Chuniphyes* and *Kephyes* do not release eudoxids, although *Crystallophyes* and *Heteropyramis* species almost certainly do (PRP personal observations). Other diphyomorphs also lack eudoxids, notably the Sulculeolariinae (Carré 1979). The species of the highly unusual prayomorph group Nectopyramidinae also have eudoxids, though they may have independently gained this ability to shed cormidia.

Along branch 3 (Figure 13) there is a further superimposition of the anterior and posterior nectophores, with the anterior nectophore generally becoming more streamlined and pointed, while the posterior one becomes more cylindrical. There is a further reduction in the attachment zones of the nectophore lamellae and an associated reduction or complete disappearance of either of the mantle canals. A remnant of the disjunct pedicular canal appears to be always retained. The data for Clausophyidae are somewhat inconclusive, but clearly show the distinctiveness of the three, of five, genera for which data are available. With regard to the mantle canal system in the anterior nectophore, *Kephyes* and *Crystallophyes* both have a descending canal, while it is absent for the other three genera. For the posterior nectophores, *Clausophyes* species are the only known ones that lack a descending mantle canal (PRP personal observations).

Along branch 4, the superimposition of the nectophores becomes further pronounced. *Tottonophyes enigmatica* **sp. nov.** possesses some traits that, to date, were only found in prayomorphs, as well as others found only within diphyomorph calyophorans. Considering the phylogenetic placement of *T. enigmatica* **sp. nov.**, this suggests that multiple diphyomorph traits arose before some prayomorph traits were lost. The traits shared with prayomorphs that persist in *Tottonophyes enigmatica* **sp. nov.** but that have been lost in other diphyomorphs include several that are critical to understanding calyophoran evolution.

The basic canal system of the bracts of prayids consists of five canals (see Haddock *et al.*, 2005), but these can be reduced to varying degrees. This reduction is particularly pronounced in the prayid subfamily Amphicaryoninae where the median longitudinal canal is reduced to a central thickening from which arise only the two hydroecial canals (Pugh, 1999). Almost the exact same pattern is found in the bracts of *Tottonophyes enigmatica* **sp. nov.**, while *Kephyes* and *Chuniphyes* species also retain to a varying degree a vestige of the lateral canal. Thus, like the nectophores, the bracts of *T. enigmatica* **sp. nov.** also show intermediate characters between the prayids and the clausophyids.

In conclusion, *Tottonophyes enigmatica* **sp. nov.**, whose description is given here, is a calyophoran species that has retained certain morphological features of the Prayomorpha, such as rounded nectophores and well-developed mantle canals, while also having features of the Diphyomorpha of the family Clausophyidae. The course of the lateral radial canals is very similar to that of *Clausophyes* species, and the differentiation of a somatocyst in both nectophores is typical of all clausophyids, where both nectophores are known. The molecular phylogenetic data verify these peculiarities and establish that *T. enigmatica* **sp. nov.** cannot be placed in any extant calyophoran family and justify the establishment of a new family to contain it.

Methods. New 16s and 18s molecular sequence data were collected for seven siphonophore specimens. The 18s nuclear ribosomal gene was amplified using the universal primers MitchA (AACCTGGTTGATCCTGCCA-GT) and MitchB (TGATCCTTCTGCAGGTTACCTAC) as described in Medlin *et al.* (1988) and the new data were deposited at NCBI with accession numbers KX421847–KX421854. The mitochondrial 16s gene was amplified as described in Cunningham and Buss (1993) using their primer 16s-SHB (GACTGTTTACCAAAAACATA) and

16s–BR (CATAATTCAACATCGAGG) from Schroth *et al.* (2002). These 16s data were deposited at NCBI with accession numbers KX374464–KX374469.

All analysis source code and associated files are available in a git repository at https://github.com/caseywdunn/pugh_etal. The analyses published here correspond to commit tagged as Revision 1. The new data were aligned with the sequences from Dunn *et al.* (2005) with mafft version v7.130b under default options. Maximum likelihood phylogenetic analyses were conducted on the combined partitioned dataset under the GTR+ Γ model with RAxML version 8.0.22 and Bayesian analyses were conducted under the GTR+I Γ model with revbayes commit7d556ef (Höhna *et al.* 2014). Leaf stability was assessed with phyutility version 2.2.6 (Smith and Dunn 2008) and the seven taxa with stability below 80%, all physonects from Dunn *et al.* (2005), were removed from the alignment. RAxML analyses were then rerun.

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