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Sequestration of nematocysts by divergent cnidarian predators: mechanism, function, and evolution

Jessica A. Goodheart[1,2,3,a] and Alexandra E. Bely[1]

[1]Department of Biology, University of Maryland, College Park, Maryland 20742, USA
[2]Laboratory of Molecular Evolution, Center for Bioinformatics and Computational Biology, University of Maryland, College Park, Maryland 20742, USA
[3]NMFS, National Systematics Laboratory, National Museum of Natural History Smithsonian Institution, Washington, District of Columbia 20013, USA

Abstract. Animals have evolved diverse mechanisms to protect themselves from predators. Although such defenses are typically generated endogenously, some species have evolved the ability to acquire defenses by sequestering defensive chemicals or structures from other species. Chemical sequestration is widespread among animals, but the ability to sequester entire structures, such as organelles, appears to be rare. Here, we review information on the sequestration of functional nematocysts, the stinging organelles produced by Cnidaria, by divergent predators. Nematocyst sequestration has evolved multiple times, having been documented in Ctenophora, Acoelomorpha, Platyhelminthes, and Mollusca. For each of these phyla, we review the phylogenetic distribution, mechanisms, and possible functions of nematocyst sequestration. We estimate that nematocyst sequestration has evolved 9–17 times across these four phyla. Although data on the mechanism of sequestration remain limited, similarities across several groups are evident. For example, in multiple groups, nematocysts are transported within cells from the gut to peripheral tissues, and certain types of nematocysts are selectively sequestered over others, suggesting convergent evolution in some aspects of the sequestration process across phyla. Similarly, although the function of nematocyst sequestration has not been well documented, several studies do suggest that the nematocysts sequestered by these groups are effective for defense. We highlight several traits that are common to Ctenophora, Acoelomorpha, Platyhelminthes, and Mollusca and suggest hypotheses for how these traits could have played a role in the evolution of nematocyst sequestration. Finally, we propose a generalized working model for the steps that may lead to the evolution of nematocyst sequestration and discuss important areas for future research.

Additional key words: defense, sequestration, convergent evolution, predation, nematocyst, Cnidaria, Ctenophora, Acoelomorpha, Platyhelminthes, Mollusca

Adaptations to avoid predation are extremely common and diverse among animals. Such adaptations can take a variety of forms, including behavioral, physical, or chemical defenses, and can be generated through diverse mechanisms (Adler & Harvell 1990; Pawlik 1993; Cresswell 1994; Stachowicz & Lindquist 2000). Although defenses are often produced endogenously, being entirely encoded by the genome of the organism (Berenbaum 1995), in some cases, animals acquire defenses from exogenous sources. For example, some animals associate with other species that are themselves well-defended, thus garnering protection from their associate's defenses (e.g., Ross 1971). Strikingly, certain animals have evolved the ability to actually sequester the defenses of other species, integrating them into their own tissues (Cronin et al. 1995; Savitzky et al. 2012).

A number of animals can sequester predator-deterring chemicals or structures from other species, such as their symbionts or prey. The ability to

[a]Author for correspondence.
E-mail: jagood@umd.edu

sequester defensive chemicals is widespread among animals, being well documented in Arthropoda (Nishida 2002; Termonia et al. 2002; Opitz & Müller 2009), Mollusca (Pawlik 1993; Cronin et al. 1995), and Chordata (Darst et al. 2005; Hutchinson et al. 2012; Savitzky et al. 2012), among others. Although much less common, some animals have evolved the ability to sequester not just chemicals but entire structures, such as whole organelles or cells, from other organisms (Rowan 1998; Händeler et al. 2009). In most cases investigated, the structures are sequestered from prey, and are inferred to be a source of energy, nutrients, and/or carbon for the sequestering animal. An example of this is kleptoplasty (or chloroplast theft) by some marine gastropods that feed on algae. These gastropods possess the ability to sequester functional chloroplasts from their algal food sources, which are then used as a source of energy (Rumpho et al. 2006). The sequestration of exogenous structures explicitly for defense purposes, however, is rare and much less well documented; the clearest example of this is the sequestration of nematocysts, the stinging organelles of cnidarians.

Several divergent animal lineages have evolved the ability to sequester nematocysts from their cnidarian prey and incorporate these organelles into their own bodies. Nematocyst sequestration has been documented in Ctenophora, Acoelomorpha, Platyhelminthes, and Mollusca (Fig. 1, Karling 1966; Greenwood 2009), indicating multiple origins of this ability. For most species, information on nematocyst sequestration remains limited to a basic description of the location and appearance of nematocysts within the sequestering animal's body. Nematocyst sequestration has been studied further in only a few species, yet such studies provide important insights about the mechanism by which nematocysts are sequestered and the ecological consequences of sequestration.

Here, we review nematocyst sequestration in the four animal groups in which it is known: Ctenophora, Acoelomorpha, Platyhelminthes, and Mollusca. For each group, we review the distribution of nematocyst sequestration across the phylum and, where this is known, the mechanism and potential ecological function of sequestration. We also provide context about the structure and function of nematocysts in Cnidaria. Based on the information available, we highlight similarities among sequestering species, propose a general model for the evolution of sequestration, and highlight important avenues for future research.

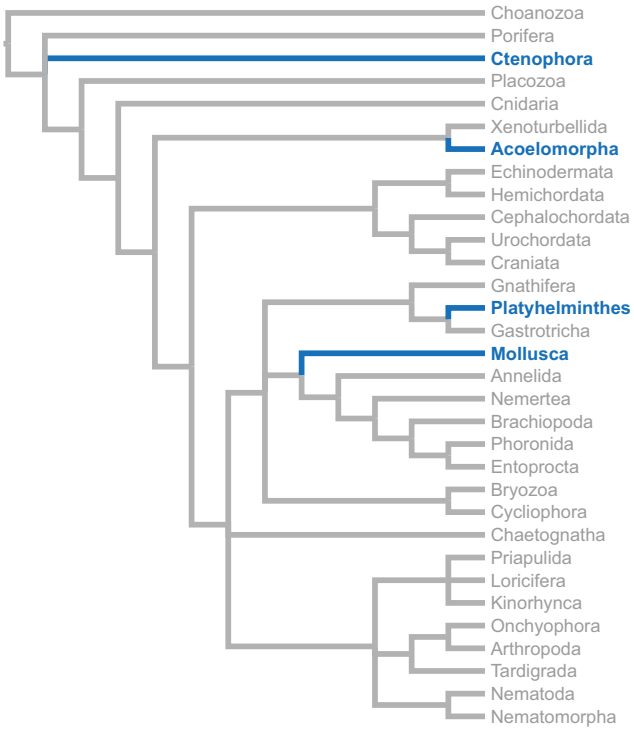

Fig. 1. Phylogeny of Metazoa indicating lineages that are known to sequester nematocysts. Phyla in which sequestering species are known are shown in blue. Relationships are based on Edgecombe et al. (2011) and Dunn et al. (2014).

What are nematocysts?

Animals within Cnidaria, a large, diverse clade of over 13,000 species (Marques & Collins 2004; Collins 2009), sting predators and capture food with complex intracellular organelles called cnidae (Watson 1988). These structures are found within cells called cnidocytes, which are most commonly found in the epithelial lining of tentacles but may also occur in other regions of the body (Fig. 2A). Cnidae are of several forms, the most common being the nematocyst, which is likely the ancestral form given its widespread distribution across the phylum (Collins 2009). Nematocysts are small venom-filled capsules containing an eversible tubule (Fig. 2B), often with spines or barbs, that can be discharged into the tissues of other organisms with very high accelerations, up to 5 million *g* (Nüchter et al. 2006; Oppegard et al. 2009). The discharge of these structures is triggered by the stimulation of the cnidocil (a modified cilium on the outside of the cnidocyte) by chemical and/or mechanical mechanisms (Cormier & Hessinger 1980; Östman 2000; Özbek et al. 2009). Based largely on the shape of the tubule and its shaft, as well as the presence

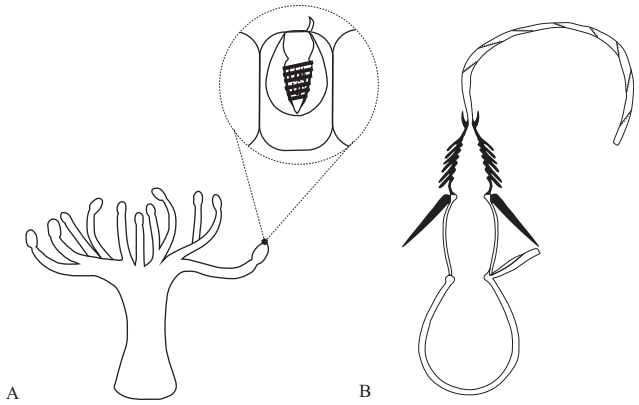

Fig. 2. Location and morphology of cnidarian nematocysts. **A.** A generalized cnidarian polyp showing the location of nematocysts in the tentacle epithelium. Within the enlarged region is a nematocyst located inside an epithelial cell. **B.** An everted nematocyst (specifically a stenotele nematocyst) after it has fired.

and shape of their armaments, several subtypes of nematocysts are recognized, including isorhizas (in which the tubule is of largely uniform thickness across most of its length and does not have a well-defined shaft), mastigophores (in which the tubule extends well beyond a well-defined shaft), and stenoteles (in which the tubule shaft possesses three large spines), among others (Mariscal 1974; Östman 2000).

The efficacy of nematocysts for defense in cnidarians

Although the primary function of nematocysts in cnidarians is thought to be prey capture, a fundamental question is whether they are also effective as defensive structures (Mariscal 1974; Conklin & Mariscal 1977; Purcell 1984, 1997; Harris 1986; Shanks & Graham 1988; Shick 1991; Stachowicz & Lindquist 2000; Bullard & Hay 2002; Greenwood et al. 2004). The few studies attempting to address this question have provided some evidence supporting the hypothesis that nematocysts can indeed have a defensive function. Stachowicz & Lindquist (2000) and Bullard & Hay (2002) showed that several species of predatory fish were deterred by nematocysts, with the fish showing a preference for consuming tissues in which nematocysts were either absent or had previously been discharged. As discussed by Mariscal (1974), it is difficult to experimentally separate the effect of nematocysts from that of other potential defenses such as chemicals that may be present within cnidarian tissues. However, in both of these fish studies, experiments that were designed to address this issue (by testing palatability with and without nematocysts or chemical compounds) identified nematocysts as a major contributor to predation deterrence, with a greater relative effect than alternative chemical defenses. Additionally, in the nudibranch mollusk, *Spurilla neapolitana*, Conklin & Mariscal (1977) noted that nematocysts from cnidarian prey can potentially cause death of the nudibranch if the nematocyst concentration is sufficiently high. Although further experimental studies of this effect are needed, this observation suggests that nematocysts can have a potent effect against this cnidarian predator. Some cnidarian predators also have developed what appear to be counterdefenses to cnidarian nematocysts (e.g., Greenwood et al. 2004; Martin et al. 2007), implying that there is a need for such protection. Together, such studies provide evidence strongly suggesting that nematocysts can provide a defensive function to the cnidarians that synthesize these structures.

Nematocyst sequestration in four metazoan lineages

Although cnidarian nematocysts can serve as defensive structures, a diverse array of cnidarians are preyed on by species from a range of animal phyla (von Salvini-Plawen 1972; Arai 2005). Predators of cnidarians include Chordata (specifically fish, reptiles, and birds), Chaetognatha, Arthropoda, Ctenophora, Acoelomorpha, Platyhelminthes, and Mollusca. Focusing on the last four phyla (these being the focus of this article, Fig. 3), these four groups alone are known to feed on diverse cnidarians representing four of the five major cnidarian clades: ctenophores are known to feed on anthozoans, scyphozoans, and hydrozoans such as narcomedusans; platyhelminths and acoelomorphs are known to feed on scyphozoans and hydrozoans such as *Hydra*; and mollusks are known to feed on anthozoans such as soft corals, hard corals, anemones and sea pens, hydrozoans such as hydroids and siphonophores, scyphozoans, and staurozoans (the stalked jellyfish) (von Salvini-Plawen 1972; Arai 2005; Mills & Hirano 2007).

In addition to simply feeding on cnidarians, some Ctenophora, Acoelomorpha, Platyhelminthes, and Mollusca encapsulate cnidarian nematocysts and sequester these structures within their own body tissues (Greenwood 2009) (Table 1, Fig. 4). These sequestered nematocysts, often called kleptocnidae, are thought to provide a defensive function to the predator, thus representing exogenously produced defenses. Below, we review nematocyst sequestration

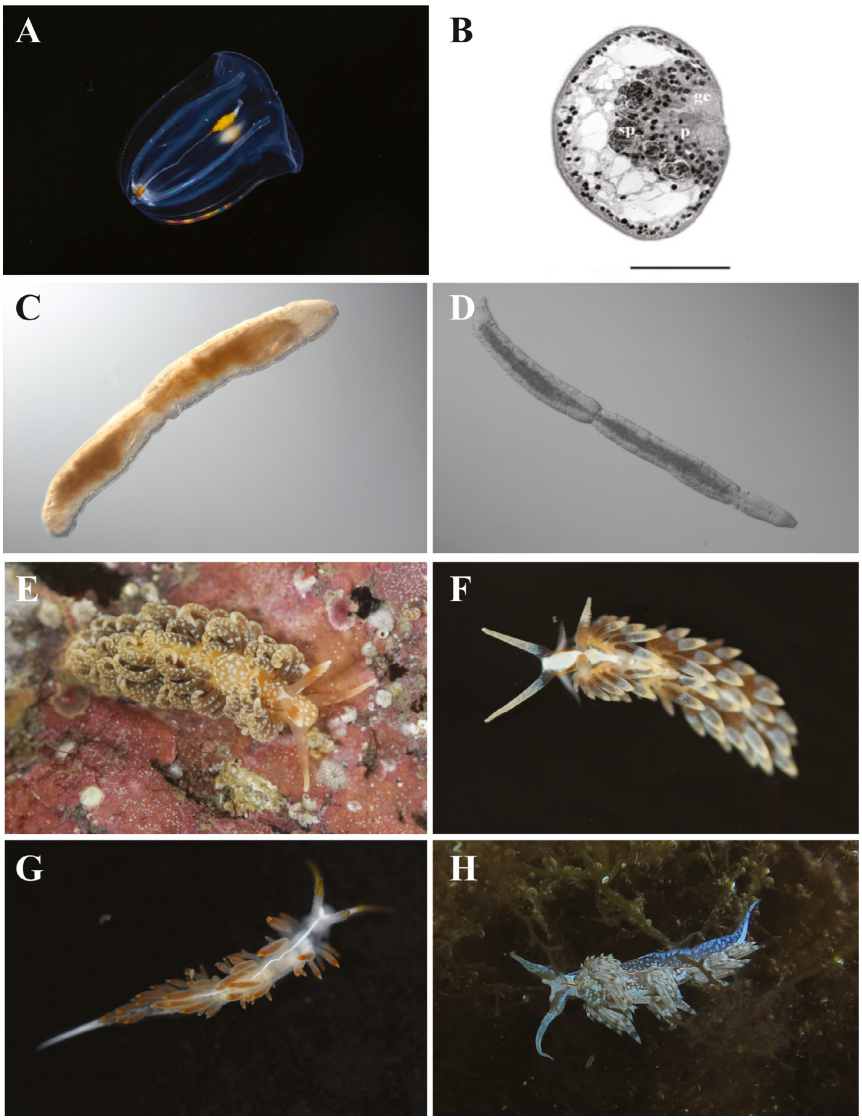

Fig. 3. Photographs of species from the four metazoan phyla known or thought to sequester nematocysts. **A.** Ctenophora: *Haeckelia rubra*. **B.** Acoelomorpha: *Childia dubium* (in cross section; scale bar=250 mm; gc, glandular complex; p, penis; sp, sperm), reproduced from Tekle (2006) with permission from the author. **C,D.** Platyhelminthes: *Microstomum* spp. **E–H.** Mollusca: **E.** *Spurilla braziliana,* **F.** *Berghia stephanieae,* **G.** *Flabellina trilineata,* and **H.** *Dondice occidentalis*. Photo credits: Steve Haddock (*H. rubra*), Yonas Tekle (*C. dubium*), Julian Smith III (*Microstomum* spp.), and Jessica Goodheart (*S. braziliana*, *B. stephanieae*, *F. trilineata,* and *D. occidentalis*).

in animals, focusing on the four phyla in which this phenomenon is known or thought to occur. For each phylum, we provide an overview of the group and its natural, endogenous defenses, review the phylogenetic distribution of sequestration, and where data are available, review what is known about the mechanism of sequestration, the location of sequestered nematocysts in the predator's tissues, and evidence for sequestered nematocysts providing a defensive function.

Ctenophora

Ctenophores, also known as comb jellies, are mostly planktonic marine predators with gelatinous, transparent and relatively fragile bodies, and in some species, tentacles that are used for prey capture (Dunn et al. 2015). To protect themselves, ctenophores primarily employ defensive behaviors, such as intimidation or escape behaviors (Mackie 1995). In addition, the nutritional quality of some

Table 1. Taxonomic distribution of nematocyst sequestration within Metazoa.

Phylum	Order	No of Species	Inferred No of Origins
Ctenophora	Cydippida	3	1
Acoelomorpha	Acoela	1	1
Platyhelminthes	Catenulida	1	6–13
	Macrostomorpha	12	
	Proseriata	5	
	Prolecithophora	4	
	Polycladida	9	
	Rhabdocoela	1	
Mollusca	Nudibranchia	~600	1–2

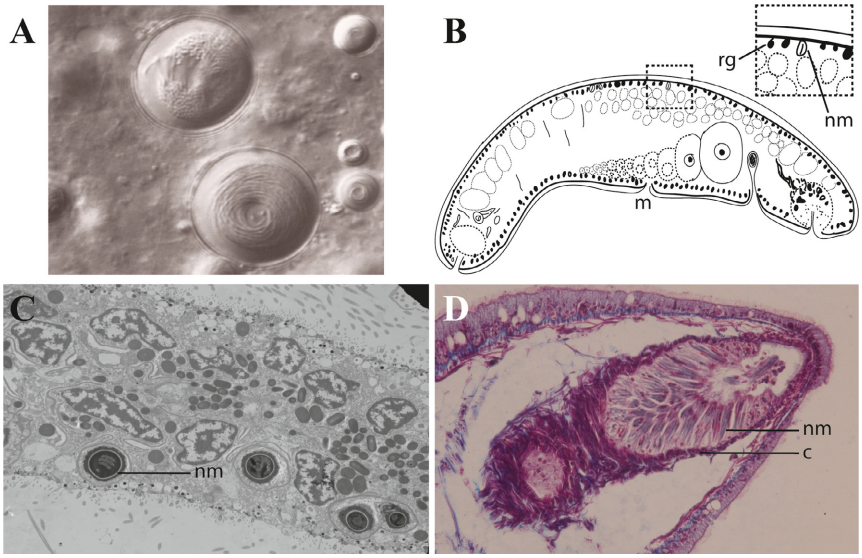

Fig. 4. Nematocysts within the tissues of sequestering species. **A.** Ctenophora, *Haeckelia rubra;* tentacle. **B.** Acoela, *Childia dubium*. Illustration is adapted from Westblad (1942), anterior is to the left. **C.** Platyhelminthes, *Theama* sp.; epithelium. **D.** Mollusca, *Aeolidia papillosa;* cerata tip. Photo credits: Claudia Mills (*H. rubra*), Julian Smith III (*Theama sp.*), and Jessica Goodheart (*A. papillosa*). c, cnidosac; m, mouth; nm, nematocyst; rg, rhabdoid gland.

ctenophores is known to be low (Bullard & Hay 2002), which may make them undesirable prey. Nematocyst sequestration has been suggested in only one genus in the phylum, *Haeckelia*. Nematocysts have been found in the tissues of three of the four species in this genus, *H. rubra* (Gegenbauer 1856), *H. bimaculata* (Carré et al. 1989), and *H. beehleri* (S. H. D. Haddock, Unpubl. data), while nematocyst storage in the fourth (*H. filigera*) has not been described.

Although the presence of nematocysts within *Haeckelia* has never been significantly questioned, the source of these nematocysts (specifically within *H. rubra*) was for a while debated in the literature. Some authors regarded the nematocysts as endogenous to the ctenophore, and cited this as a useful phylogenetic character supporting Coelenterata, a clade containing Cnidaria and Ctenophora (Komai 1942; Komai & Tokioka 1942; Picard 1955; Hand 1959; Hyman 1959; Rees 1966). Other authors, however, viewed nematocysts as exogenous structures, likely originating in cnidarians (Komai 1951, 1963; Hadži 1953).

The exogenous origin of nematocysts in the ctenophore is now well established for this genus. Carré & Carré (1980a,b) were the first to provide solid evidence for this, describing in *H. rubra* the sequestration of nematocysts from the primary prey of this species, the hydrozoan narcomedusa *Aegina citrea*. The nematocysts were described from the ctenophore's tentacles, and the distribution of nematocysts within tentacles was interpreted as indicating

that nematocysts are integrated there by way of the tentacular canals, which are connected to the stomach where the prey would be located (Carré & Carré 1980b). Furthermore, it has been shown that the eggs of *H. rubra* are surrounded by nematocysts, and that these structures are ingested by the larvae during development (Carré & Carré 1989).

Within the tentacles, the nematocyst capsules are transported through the tissue of the ctenophore within endocytotic vesicles (Carré et al. 1989). However, the nematocysts that reach the tentacles are no longer within a cnidocyte and are without a cnidocil according to Carré & Carré (1980a, 1989); the lack of a cnidocil raises the question of how nematocyst discharge is triggered in ctenophores (Cormier & Hessinger 1980). Sequestered nematocysts appear to be associated with a sensory cell of the ctenophore that might serve the purpose of controlling nematocyst firing (Carré & Carré 1980a). Nematocysts incorporated in the tissues of individuals of *H. rubra* appear to be primarily a subset of the nematocysts present in the prey. Specifically, the prey organisms of *H. rubra* (*A. citrea*, and other similar narcomedusans) possess microisorhizas (small isorhizas with a mean diameter of ~4 μm) and macroisorhizas (large isorhizas with a mean diameter of ~8 μm), but most sequestered nematocysts are microisorhizas (Carré et al. 1989); this finding suggests that particular subtypes of nematocysts are selectively sequestered by individuals of *H. rubra*. Because very few macroisorhizas appear to be sequestered, it is assumed that most of the larger nematocysts present in *A. citrea* are not sequestered but are instead digested or passed through the gut undigested, although there is no direct evidence for this.

Beyond the information described above, there is virtually no information regarding the process of sequestration in *Haekelia,* and no studies have attempted to determine the actual function of the nematocysts in these ctenophores (Haddock 2007). However, the finding that nematocysts are present solely in the tentacles of these ctenophores is consistent with the hypothesis that sequestered nematocysts are used for prey capture rather than defense.

Acoelomorpha

Acoelomorpha is a clade of soft-bodied, primarily marine worms comprised of two subgroups, Acoela and Nemertodermatida (Edgecombe et al. 2011; Ruiz-Trillo & Paps 2015). The most well-characterized defensive structures of acoelomorphs are sagittocysts, needle-shaped secretory structures that can be ejected from the epidermis (Gschwentner et al. 2002), although rhabdoids (secretory inclusions which may release protective coatings) and mucous-producing frontal glands may also provide a defensive function within acoelomorphs (Rieger et al. 1991). A single species of Acoelomorpha, the acoel *Childia dubium* (Mecynostomidae) from the Mediterranean Sea, appears to possess the ability to sequester nematocysts from its cnidarian prey.

Westblad (1942) originally identified structures within specimens of *C. dubium* as "cnidocytes," although he provided no details regarding the structures other than their location just below the epidermis. Additional information was provided by Karling (1966), who identified them as nematocysts and indicated that the number of nematocysts sequestered within individuals of *C. dubium* is small (although he gave no actual numbers). Karling also identified undischarged nematocysts in the syncitial gut, within both the central and peripheral parenchyma; these nematocysts were not enclosed in any special cells or cysts, in contrast to where these organelles occur in cnidarians. Interestingly, no nematocysts have been described from the epidermal epithelium (Westblad 1942; Karling 1966), unlike nematocysts sequestered within ctenophores and platyhelminths.

Platyhelminthes

Platyhelminthes is a large phylum (roughly 20,000 species) of soft-bodied worms, often referred to as flatworms, that include both free-living and parasitic species (Riutort et al. 2012). Free-living platyhelminths are typically small aquatic worms and are known from nearly every body of water on the planet (Appeltans et al. 2012; Laumer et al. 2015), while parasitic worms live on or within the tissues of a wide range of hosts. Platyhelminths have a relatively simple body organization, lacking a coelom, hemal system, and cuticle (among other traits). Among free-living platyhelminths, primary defensive strategies are based on exocytic organelles including both paracnids (refractive glands that sometimes also have an eversible tubule) and rhabdites (rod-like structures in the epidermis that release mucous or potentially repellant substances) (Martin 1978; Sopott-Ehlers 1981; Smith et al. 1982).

Nematocyst sequestration has been described in multiple species from a variety of groups within Platyhelminthes, suggesting multiple origins of this feature within the phylum. The first mention of nematocysts in Platyhelminthes appears to have been by Lang (1884), who described the presence of nematocysts and bundles of needle structures in the

dorsal epidermis of specimens of *Anonymus virilis* (Polycladida). Several additional studies have identified nematocysts in other groups of Platyhelminthes (Laidlaw 1906; Martin 1908; Kepner 1911; Bock 1922; Poulter 1975; Snyder 1980; Martens & Schockaert 1986; Holleman 1998; Rawlinson et al. 2011), and a comprehensive overview was published by Karling (1966). In total, 33 species from 13 families are known to sequester nematocysts, and we estimate, based on the distribution of this trait, that nematocyst sequestration likely evolved between 6 and 13 times within Platyhelminthes (Table 2, Fig. 5). In all but the monotypic or very small genera, nematocyst sequestration is indicated in only some, but not all, of the species in the genus, suggesting that most if not all origins of this ability are relatively recent. Interestingly, one sequestering species, *Wahlia macrostylifera* (Rhabdocoela), is a commensal parasite within a holothurian host (Snyder 1980), representing the only known instance of nematocyst sequestration in a commensal organism.

The acquisition and storage of nematocysts show some similarities across sequestering platyhelminths. First, nematocysts from the consumed cnidarian prey are taken up by the gastrodermal phagocytes, and some of these nematocysts remain both unfired and undigested. Nematocyst-bearing gastrodermal cells, which are known as cyst cells or cnidophages, then move away from the gastrodermis, passing through the parenchyma. Finally, the undischarged nematocysts make their way into the epidermis and come to reside among the epidermal cells. Usually nematocysts are located above the basement membrane, but in some species, such as those in *Microstomum* and *Archimonocelis*, nematocysts can also be found subepidermally (beneath the basement

Table 2. Taxonomic distribution of nematocyst sequestration within Platyhelminthes.

Order	Family	Genus	Species	Reference
Catenulida	Stenostomidae	*Stenostomum*	*sieboldi*	Martin (1914)
Macrostomorpha	Microstomidae	*Microstomum*	*lineare*	Martin (1914)
			papillosum	Martin (1908)
			rubromaculatum	Martin (1914)
			mundum	Karling (1966)
			mortenseni	Karling (1966)
			gabriellae	Karling (1966)
			jenseni	Karling (1966)
			ulum	Marcus & Marcus (1951)
			breviceps	Marcus & Marcus (1951)
			spiriferum	Karling (1966)
			hamatum	Karling (1966)
			caudatum	Kepner & Barker (1924)
Prolecithophora	Ulianiniidae	*Ulianinia*	*mollissima*	Martin (1914)
			westbladi	Karling (1966)
	Pseudostomidae	*Pseudostomum*	*klostermanni*	von Graff et al. (1908)
	Cylindrostomidae	*Cylindrostoma*	*monotrochum*	Martin (1914)
Proseriata	Archimonicelidae	*Archimonocelis*	*mediterranea*	Karling (1966)
			bathycola	Karling (1966)
			koinocystis	Karling (1966)
			semicircularis	Karling (1966)
			coronata	Karling (1966)
Polycladida	Stylochoplanidae	*Stylochoplana*	*tarda*	Martin (1914)
			inquilina	Poulter (1975)
	Anonymidae	*Anonymus**	*kaikourensis*	Holleman (1998)
			multivirilis	Holleman (1998)
			virilis	Karling (1966)
	Chromoplanidae	*Chromoplana*	*bella*	Karling (1966)
	Prosthiostomidae	*Amakusaplana*	*acroporae*	Rawlinson et al. (2011)
	Amyellidae	*Amyella**	*lineata*	Bock (1922)
	Theamatidae	*Theama*	sp.	J. P. S. Smith III, Unpubl. data
Rhabdocoela	Umagillidae	*Wahlia*	*macrostylifera*	Snyder (1980)

*Genera in which all of the species are known to sequester nematocysts.

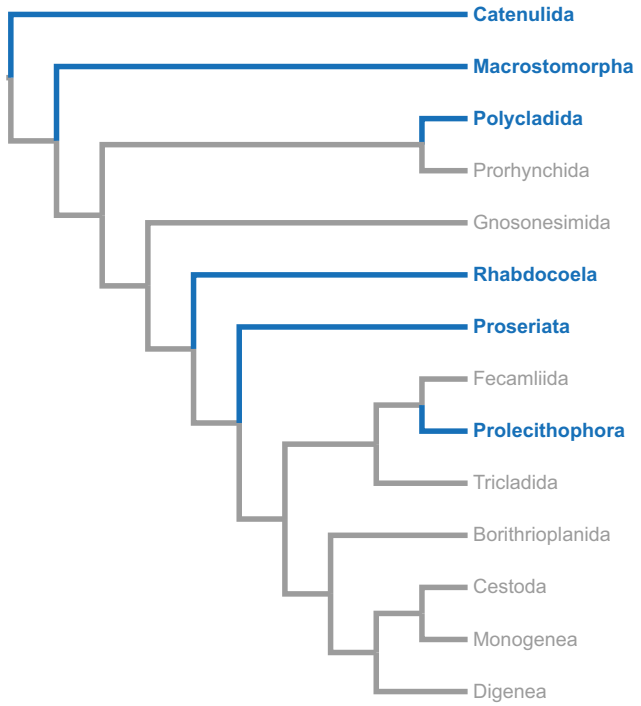

Fig. 5. Phylogeny of Platyhelminthes indicating lineages that are known to sequester nematocysts. Taxa in which sequestering species are known are shown in blue. Relationships are based on Laumer et al. (2015).

membrane) and/or in the digestive epithelium (Karling 1966).

In many cases, including in *Microstomum, Archimonocelis*, and *Anonymus,* the nematocysts in or near the epidermis are grouped into clusters (often with two to three nematocysts per cluster). In both *Archimonocelis* and at least two species of *Microstomum* (*M. hanatum* and *Microstomum* cf. *lineare*), the membrane around the bundle of nematocysts is surrounded by musculature (Karling 1966), which could potentially be used to actively and rapidly expel the organelles. In the case of *Microstomum* cf. *lineare*, the musculature involves a single-celled muscle sheath basally and laterally and is associated with parenchymal muscle cells (Etheredge & Smith 2006). In each animal, there are typically multiple such muscular bundles of nematocysts, which Karling (1966) refers to as cnidosacs.

Although nematocyst sequestration is well documented in several platyhelminths, nothing is known about how nematocyst-sequestering flatworms protect themselves against the firing of nematocysts within the gut or about the ultimate function of sequestered nematocysts. Karling (1966) suggested that mucus secretions may provide some protection from nematocysts, and Bock (1922) suspected that sequestered nematocysts help to protect the worms, but these hypotheses have not been tested. Some further discussion of nematocyst sequestration is available in Kepner (1951) and Kepner & Barker (1924), who also provide evidence, primarily anecdotal, suggesting that sequestered nematocysts are used for both defense and prey capture in *Microstomum*. The behavior of sequestering species of *Microstomum* also suggests that nematocysts are in some way useful, as these species appear to only feed on *Hydra* when the supply of undischarged nematocysts in their own tissues is low (Kepner & Barker 1924). Given that nematocyst sequestration appears to have evolved many times within this phylum (Fig. 5), Platyhelminthes is a particularly useful group in which to further investigate the evolution of this ability.

Mollusca

Mollusca is a highly diverse and species-rich group, with approximately 100,000 extant species described and including organisms with a wide diversity of body forms. This group is composed of seven classes: Aplacophora, Polyplacophora, Monoplacophora, Cephalopoda, Scaphopoda, Bivalvia, and Gastropoda (Kocot et al. 2011; Smith et al. 2011). Of these, Gastropoda (snails and slugs) is the most diverse group, making up approximately 80% of the species richness within the phylum (Appeltans et al. 2012).

Although gastropods are known for their coiled shell, which is retained in most species, multiple gastropod lineages have lost the shell (Wägele & Klussmann-Kolb 2005). One of these lineages is Nudibranchia, an order of shell-less gastropods known for their bright coloration and charismatic patterns. Species within this clade use several types of defensive strategies, including the synthesis or uptake of biochemically active compounds in tissues (Barsby 2006; Paul & Ritson-Williams 2008), warning coloration to deter predators (Tullrot 1994), cryptic coloration to avoid detection, and the use of nematocysts acquired from their cnidarian prey (Greenwood 2009). These defensive strategies are widespread throughout Nudibranchia, with one exception: nematocyst sequestration in this group is found only within some species of Cladobranchia, a group of nudibranchs known for their characteristically branched digestive glands (Pola & Gosliner 2010). More specifically, the sequestration of cnidarian nematocysts occurs primarily in one group of cladobranchs, Aeolidida, a group that appears to be monophyletic (Goodheart et al. 2015). Additional species within the cladobranch genus *Hancockia* are

also known to sequester nematocysts, but the position of this genus in the cladobranch tree is still uncertain. Thus, nematocyst sequestration appears to have evolved at least once, and possibly twice, within Mollusca.

Nematocyst sequestration in aeolid nudibranchs has been relatively well studied and is far better characterized than in the three phyla previously discussed here. A detailed review of nematocyst sequestration in aeolids has recently been published by Greenwood (2009). Below, we present a relatively brief overview of this phenomenon, referring the reader to this earlier review for more thorough coverage of the topic.

Aeolids feed on a variety of cnidarians, including corals, anemones, and hydroids, and must protect themselves from nematocysts of their prey. Two forms of protection have been proposed to be present in aeolids. The first is a physical protection: a chitinous cuticle covers the epithelium of the buccal cavity and the esophagus (Edmunds 1966; Martin et al. 2007). The second is a chemical protection: certain chemicals present in nudibranch mucus appear to prevent nematocysts from firing (Greenwood et al. 2004).

Once cnidarian tissue containing nematocysts is ingested by a nudibranch, some nematocysts (both discharged and undischarged) are excreted as waste. Other nematocysts are retained and passed through the branched digestive tract to diverticula of digestive glands located within dorsal body outgrowths, named cerata. These dorsal appendages, including the nematocysts within them, can be autotomized (released) if the animal perceives danger (Miller & Byrne 2000).

Once inside the digestive gland of a ceras, nematocysts are moved to a muscular sac, the cnidosac, located at the tip of each ceras, and are stored there until release or digestion (Conklin & Mariscal 1977; Greenwood 2009). During this process, nematocyst packaging and transport appear to occur in different ways in different species. In most aeolids, individual nematocysts are passed through the digestive gland and are encapsulated in cells called cnidophages as they are moved into the cnidosac (Grosvenor 1903; Greenwood 2009). In others, such as *Cratena peregrina*, nematocysts have been found within large vacuoles inside the digestive cells of the lining of the lumen of the digestive gland (Martin 2003). In *Hancockia*, nematocysts appear to be encapsulated in cnidophages within the lumen of the digestive gland and transported to the cnidosacs within the cnidophages (Martin et al. 2009). In the aeolid *Spurilla neapolitana*, the release of nematocysts is triggered by non-motile, sensory cilia on the external surface of the ceras. Once the signal is received, nematocysts are extruded through an opening at the tip of the ceras called the cnidopore. When the nematocysts reach the external environment, they are then fired from their capsule (Conklin & Mariscal 1977).

In some nudibranch species, immature nematocysts are sequestered from cnidarian prey and these nematocysts continue to mature within the nudibranch itself. In *S. neapolitana*, only immature nematocysts are sequestered, and it has been proposed that this strategy is less dangerous to the nudibranch because nematocysts being moved to the cerata would be less likely to fire, and thus less likely to cause damage, within the animal (Greenwood & Mariscal 1984). Recently, the mechanism of nematocyst maturation was investigated in *Berghia stephanieae* by Obermann et al. (2012), who determined that an accumulation of protons, causing a decrease in pH, is involved in initiating maturation of sequestered nematocysts.

Although it is clear that aeolid nudibranchs retain and store nematocysts from their cnidarian prey, there is much less evidence for the use of sequestered nematocysts for defense. It is known that aeolids will release stored nematocysts when they are threatened, and that these nematocysts can sting and damage predators (Edmunds 1966; Aguado & Marin 2007). Another study found that several potential predators of nudibranchs, including several fish and a shrimp, fed more quickly on aeolids that had had their cerata removed than on those that were intact, suggesting that the cerata and their nematocysts may have some defensive value (Ohkawa & Yamasu 1993). However, it has been difficult to distinguish between the defensive effects and relative contributions of nematocysts fired from the cerata from that of other defenses, such as chemical defenses, that are present in some species (Edmunds 2009). For this reason, the defensive function of sequestered nematocysts is still viewed as tentative by some authors (Marin 2009; Penney 2009), and it has even been suggested that cnidosacs may simply be excretory organs rather than defensive ones (Streble 1968). However, it does appear that the particular combination of nematocyst types within a species, referred to as the cnidome, can differ between the nudibranch and its prey. Specifically, the nudibranch cnidome often comprises only a subset of the nematocyst types present in a particular prey item (Edmunds 1966; Conklin & Mariscal 1977; Martin 2003; Frick 2005), and the nudibranch cnidome can even change depending on the

particular predator that is threatening the individual (Frick 2003). This suggests that there may be selectivity in nematocyst sequestration and that the selection of particular nematocysts could be a type of inducible defense. These findings lend further support to the hypothesis that nematocysts within nudibranchs have a defensive role.

In spite of the potential defensive benefits of sequestration ability, the evolutionary loss of nematocyst sequestration is suggested within at least one group of aeolids. Species in the genus *Phyllodesmium* have switched to feeding on octocorals, a group of corals known to have less potent nematocysts than other cnidarians. Species of *Phyllodesmium* do not appear to sequester nematocysts from their food, as their cnidosacs are consistently devoid of nematocysts, suggesting they are no longer functional (Carmona et al. 2013; Bogdanov et al. 2014). Thus, although nematocyst sequestration may be an important defense mechanism across most of Aeolidida, it is not present in all species in this group and appears to have been lost at least once.

The evolution of nematocyst sequestration in Metazoa

Similarities in nematocyst sequestration across disparate groups

Although nematocyst sequestration has evolved independently in the four groups discussed above, several similarities in the process are apparent across these different groups, suggesting some convergent evolution. Specifically, there are similarities regarding the mode of transport of nematocysts, selectivity of particular nematocyst types in the sequestration process, and the storage of groups of nematocysts in larger muscular sacs.

First, in sequestering ctenophores, platyhelminths, and mollusks, nematocysts are transported from the gut to a storage destination and may be transported in similar ways. Specifically, in each of these groups available data suggest that in some cases nematocysts are transported within cells often called cnidophages (in endocytotic vesicles) from tissues near the gut to their storage location, namely the tentacles (*Haeckelia*), epidermis (Platyhelminthes), and cerata (Nudibranchia) (Conklin & Mariscal 1977; Carré et al. 1989). In nudibranchs, these cnidophages continue to house the nematocysts until they are ejected from the muscular storage sac (the cnidosacs) or digested (Greenwood et al. 2004).

Second, species from two groups appear to preferentially sequester certain nematocyst types over others from their prey. Nudibranchs in the genera *Spurilla, Aeolidia,* and *Aeolidiella* appear to selectively retain the mastigophores from their anemone prey (Edmunds 1966; Conklin & Mariscal 1977), and species in other nudibranch genera, such as *Catriona*, *Cuthona*, *Eubranchus*, *Godiva,* and *Tergipes*, appear to preferentially sequester long isorhizas from their hydroid prey (Edmunds 1966). It is important to note, however, that these preferences might be dependent on the particular prey item. Research on the cnidome of the nudibranch *Flabellina verrucosa* indicates a preferential uptake of a particular type of mastigophore (microbasic mastigophores) when feeding on *Obelia*, but this nudibranch does not appear to selectively sequester particular types of nematocysts when feeding on *Tubularia* (Frick 2005). In ctenophores, *H. rubra* appears to consistently sequester the smaller microisorhizas over macroisorhizas from their cnidarian prey (Carré et al. 1989), although no data exist on the other species within *Haeckelia*. The selectivity of sequestration of particular nematocyst types is strongly suggestive of sequestration having a defensive function, especially considering that in at least some species (e.g., *F. verrucosa*), the cnidome of the sequestering species is sensitive to the type of predator (Frick 2003). Currently, it is not known how the cnidome of acoels and platyhelminths compares to that of their prey; such information would be particularly interesting to obtain so that it can be compared to that from the other groups.

Third, in both nudibranchs and some platyhelminths, sequestered nematocysts are stored in muscular sacs, referred to as cnidosacs (Karling 1966; Conklin & Mariscal 1977; Etheredge & Smith 2006; Greenwood 2009). Although these structures appear somewhat similar morphologically, in platyhelminths, the precise function of the cnidosac, including the associated musculature, remains uncertain and should be studied further so that it can be better compared to that in nudibranchs.

Common features of sequestering taxa

Many groups are known to prey upon cnidarians, including other cnidarians, ctenophores, acoelomorphs, platyhelminths, mollusks, arthropods, chaetognaths, fish, reptiles, and birds (Arai 2005). Among these, only species of the four phyla discussed above (Ctenophora, Acoelomorpha, Platyhelminthes, and Mollusca) have evolved the ability to sequester nematocysts from their cnidarian prey. Below, we highlight several similarities among these four groups that we hypothesize may have

contributed to the evolution of their ability to sequester nematocysts.

One obvious similarity among sequestering groups is that they are all soft-bodied, without any hard exteriors. Although this feature is common within Metazoa, the fact that sequestering organisms are soft-bodied implies that they have little in the way of external physical protection from predators. Soft-bodied species without a hard exterior often evolve alternative modes of protection, such as physical weapons (e.g., rhabdites, sagittocysts), chemical defenses, aposematism, and crypsis (Martin 1978; Tullrot 1994; Gschwentner et al. 2002; Barsby 2006; Paul & Ritson-Williams 2008; Greenwood 2009). This is particularly apparent in the Mollusca, where loss of the shell is quite tightly associated with gain of alternative defenses (Pawlik 1993). This general pattern suggests the hypothesis that nematocyst sequestration is favored in soft-bodied taxa via selection for defense from predators.

A second character shared by many nematocyst-sequestering species is a branched (i.e., diverticulated) digestive system. Such a feature is present in *Haeckelia* in Ctenophora (Mills & Miller 1984), in Polycladida within Platyhelminthes (Jennings 1957), and in Cladobranchia within Mollusca (Wägele & Willan 2000). In *Haeckelia,* the gut includes a system of multiply branched gastrovascular canals that connect to the tentacular canals, which extend into the tentacles; in polyclads, the gut consists of multiple branched tubes each extending toward the surface of the body (Newman & Cannon 2003); and in Cladobranchia, the gut includes branches which extend dorsally from the main body into the cerata. The sequestering acoel *C. dubium* has a central mass of digestive tissue rather than a true gut cavity (Achatz et al. 2013), but this digestive mass is typically in close proximity to the epidermis (Rieger et al. 1991). Thus, in the digestive systems of each of these groups, there is the possibility for contents of the gut to be passed relatively easily into tissues close to the body surface where nematocysts are held, such as the tentacles, epidermis, or dorsal cerata. Although a branched gut occurs in many nematocyst-sequestering taxa, it is important to note that not all sequestering species possess this gut feature; for example, many Platyhelminthes, including some species that sequester nematocysts, do not have a branched digestive system (Rieger et al. 1991). However, collectively, these observations lead to the hypothesis that a branched gut may facilitate nematocyst sequestration by providing a relatively easy way for gut contents, including nematocysts, to be distributed to other body regions.

A third feature that is shared across many sequestering taxa is the ability to regenerate. Regeneration of structures and/or tissues has been documented in all four phyla known to sequester nematocysts (Bely & Nyberg 2010; Bely et al. 2014), and more specifically, the particular structures in which sequestered nematocysts are stored are known to be able to regenerate in at least some species of these phyla. Many adult tentaculate ctenophores can regenerate tentacles and other tissues or structures that have been damaged or lost (Henry & Martindale 2000); some acoels and many platyhelminths can regenerate every part of the body and also continually regrow the epidermis from stem cells (Bely & Sikes 2010; Rink 2013; Bely et al. 2014); and nudibranchs can regenerate cerata that have been autotomized (Marin et al. 1991; Miller & Byrne 2000). The ability to regenerate tissues, and specifically tissues in which nematocysts are stored, could be of considerable advantage to nematocyst-sequestering organisms. This is because most sequestering species do not appear to be capable of controlling the firing of nematocysts, and thus predators will likely be affected by nematocysts only after attempting to eat tissue of sequestering species. The ability to replace these lost body parts would therefore be advantageous. Thus, another hypothesis to consider is that, because structures and tissues that sequester nematocysts are vulnerable to predation, selection may favor both nematocyst sequestration and regeneration of these body regions.

Although many animal groups possess one or more of the features we highlight as being associated with sequestration (namely, a soft body, a diverticulated digestive system, and the ability to regenerate), to our knowledge no groups other than the four phyla discussed here both consume cnidarians and possess all three of these features. The only exceptions are cnidarians that prey on other cnidarians, yet these already possess nematocysts (or other types of cnidae) and thus would not be expected to derive a benefit from sequestering nematocysts from their prey. It is worth noting, however, that studies aimed at assessing whether all nematocysts within a cnidarian are made endogenously (by the species possessing them) are rare, and thus that detection of nematocyst sequestration in cnidarians could easily be overlooked.

Proposed steps in the evolution of sequestration

The evolution of nematocyst sequestration is expected to be a process involving multiple evolutionary steps, with intermediate steps each

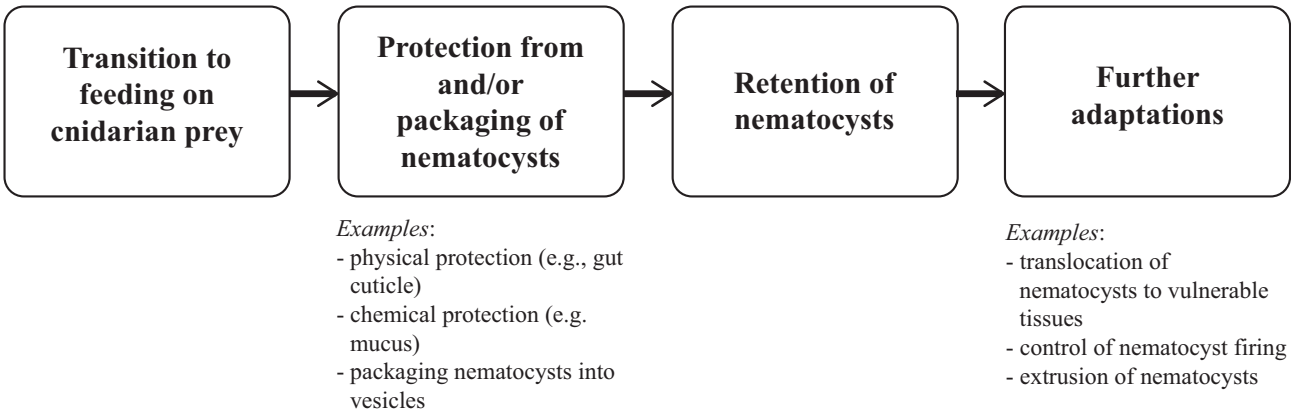

Fig. 6. Hypothesized evolutionary steps leading to the ability to sequester nematocysts. The third step, retention of nematocysts, is viewed as the step that establishes nematocyst sequestration in a species. Sequestering species in all four phyla reviewed here have features of the first three steps; species of Aeolidida (Mollusca) and some sequestering species within Platyhelminthes additionally have features of the fourth step.

presenting some advantages. We propose a possible model for this process, hypothesizing plausible intermediate steps in the evolution of nematocyst sequestration (Fig. 6). A necessary initial step is the transition to feeding on cnidarian prey. As soon as a species transitions to feeding on cnidarians, the potential arises for protection by nematocysts; this is because, regardless of whether nematocysts are actively sequestered, a predator of the cnidarian predator may still be harmed by ingesting tissue in which active nematocysts are present. Subsequently, likely steps would be the evolution of chemical or physical mechanism(s) to protect the species from the nematocysts that are ingested during feeding and/or the ability to package ingested nematocysts. Evolved forms of chemical or physical protection could involve, for example, mechanisms to prevent nematocysts from firing or a cuticle barrier to protect the lining of the gut from nematocyst firing. Packaging of nematocysts into vesicles by cells in contact with gut contents could involve newly evolved cellular processes or could result from modifications of previously existing digestive processes, and, regardless of the mechanism, could provide strong protection from the nematocysts. The retention of intact (undigested) nematocysts could then evolve, which would establish nematocyst sequestration in the species. Once retention of nematocysts has evolved, further adaptations associated with nematocyst sequestration could then be acquired, such as mechanisms to transport vesicles or cells containing nematocysts to body regions vulnerable to predator attacks, mechanisms to control nematocyst firing, mechanisms to actively excrete sequestered nematocysts even from undamaged tissues, and the evolution of specific structures for nematocyst storage. Evaluating this model will require a combination of comparative analyses to evaluate the order of possible steps, as well as functional studies to evaluate the possible selective advantage of each step.

Conclusions and future directions

The ability to sequester nematocysts from cnidarians appears to be rare, and certainly is much less common than chemical sequestration, which is widespread across the Metazoa. However, nematocyst sequestration has clearly evolved multiple times and in divergent groups of animals, likely as a form of defense. Nematocysts have been described from four metazoan phyla (Ctenophora, Acoelomorpha, Platyhelminthes, and Mollusca) and sequestration appears to have evolved 9–17 times. The literature on nematocyst sequestration is relatively small, but some similarities in the mechanism of sequestration among divergent groups are apparent, such as the transport of nematocysts within cnidophages from the gut to more distal locations of the animal. We have presented hypotheses regarding traits that may be associated with the evolution of this ability as well as proposed a model of possible steps leading to the evolution of nematocyst sequestration. We hope that by reviewing existing literature on nematocyst sequestration and by proposing these hypotheses and this model, this review will stimulate further research into the evolution of nematocyst sequestration.

In addition to testing the broad hypotheses and the general model we propose, future studies should focus on filling a number of important knowledge

gaps regarding the evolutionary patterns and specific processes of nematocyst sequestration. In particular, important questions remain regarding the phylogenetic distribution of predation on cnidarians, the phylogenetic distribution and mechanism for preferential sequestration of certain nematocyst types, the mechanisms of controlling nematocyst firing, and the possible function of sequestered nematocysts.

First, what is the phylogenetic distribution of predation on cnidarians within the four phyla in which nematocyst sequestration has evolved? Knowledge of feeding habits and preferences within these clades will allow evaluation of how prevalent sequestration is among groups feeding on cnidarians, and can elucidate the relative timing between a transition to feeding on cnidarians and the evolution of nematocyst sequestration.

Second, is there preferential sequestration of particular types of nematocysts? If so, in which species and by what mechanisms does this occur? Selectivity in sequestration could be an adaptive mechanism for effective defense in nature. Testing whether the nematocysts that are selectively sequestered are more effective against particular types of predators would provide further insight into the evolutionary function of sequestration.

Third, what are the molecular and cellular mechanisms involved in controlling nematocyst firing? Specifically, what mechanisms are involved in preventing the firing of nematocysts following ingestion and, in certain cases, controlling the later firing of sequestered nematocysts? Answers to these questions are needed to assess the diversity of sequestration and defense mechanisms used by sequestering animals. Even in non-sequestering species, mechanisms to protect the predator from ingested cnidae are known. For example, planktonic larvae of smooth fan lobsters encase jellyfish cnidae inside an additional membrane within their digestive tract, providing protection from the cnidae (Kamio et al. 2016). Among sequestering taxa, some species encapsulate nematocysts within special cells (cnidophages), providing a possible mechanism for controlling nematocyst discharge. Some sequestering species are also known to strip the nematocyst capsules from the cnidocyte, in which cases the nematocysts become devoid of the cnidocil, the structure that typically affects nematocyst firing in cnidarians (Carré & Carré 1980a). In such organisms, if nematocysts are used for defense, how do they control the discharge of nematocysts? Such questions will need to be investigated in other sequestering groups in order to understand the similarities and differences between the mechanisms for controlling nematocysts.

Finally, what is the ultimate function of sequestered nematocysts, and how does sequestration affect the ecology of sequestering species? Answering these questions is critical for evaluating the evolutionary advantages of nematocyst sequestration, yet such questions have received scant attention. Only a few studies have focused on these questions in Nudibranchia (e.g., Edmunds 1966; Aguado & Marin 2007), and these studies have suggested that a defensive function is possible, yet no further effort has been made to address the possible selective advantage of sequestration. Nematocysts are clearly powerful weapons for the cnidarians that produce them, and the use of nematocysts by non-cnidarian species suggests they can provide a selective value to such species, even in the absence of sequestration. For example, *Dardanus* hermit crabs which have *Calliactus* anemones living commensally on their shells have been shown to be protected from octopus predation (Ross 1971), and the cephalopod *Tremoctopus violaceus* has been found to use *Physalia* tentacles as offensive weapons for prey capture (Jones 1963). These examples do not involve actual sequestration, yet they demonstrate the potential of nematocysts for both defensive and offensive functions in organisms that did not actually produce these structures. Elucidating the proximate and ultimate mechanisms leading to sequestration of nematocysts will thus broaden understanding of the many uses of these potent biotic weapons.

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Conflict of interest

The authors have no conflicts of interest to declare.

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