Chapter 1

Brief History of Ctenophora

Leonid L. Moroz

Abstract

Ctenophores are the descendants of the earliest surviving lineage of ancestral metazoans, predating the branch leading to sponges (Ctenophore-first phylogeny). Emerging genomic, ultrastructural, cellular, and systemic data indicate that virtually every aspect of ctenophore biology as well as ctenophore development are remarkably different from what is described in representatives of other 32 animal phyla. The outcome of this reconstruction is that most system-level components associated with the ctenophore organization result from convergent evolution. In other words, the ctenophore lineage independently evolved as high animal complexities with the astonishing diversity of cell types and structures as bilaterians and cnidarians. Specifically, neurons, synapses, muscles, mesoderm, through gut, sensory, and integrative systems evolved independently in Ctenophora. Rapid parallel evolution of complex traits is associated with a broad spectrum of unique ctenophore-specific molecular innovations, including alternative toolkits for making an animal. However, the systematic studies of ctenophores are in their infancy, and deciphering their remarkable morphological and functional diversity is one of the hot topics in biological research, with many anticipated surprises.

Key words Ctenophora, Placozoa, Porifera, *Pleurobrachia*, *Mnemiopsis*, Neurons, Muscles, Development, Cell-type evolution, Phylogeny

1 Ctenophores as the Sister Lineage to All Other Animal Phyla

Ctenophores or comb jellies are true wonders of nature! They are the most unusual animals in the marine realm, both from structural and molecular standpoints. "Although it is easy in a given case to determine whether or not a particular animal is a ctenophore, it is equally difficult to establish how closely or distantly ctenophores are related to other forms of animals."—this Krumbach's note (1925) and the challenge [1] was reconfirmed by the leading experts at the beginning of the twenty-first century, with no morphological evidence that could link the phylum Ctenophora to any other extant phylum [2, 3]. This hundred-year enigma started to be uncovered only recently.

Arguably ctenophores are the descendants of the earliest surviving lineage of ancestral metazoans [4–8], predating the branch

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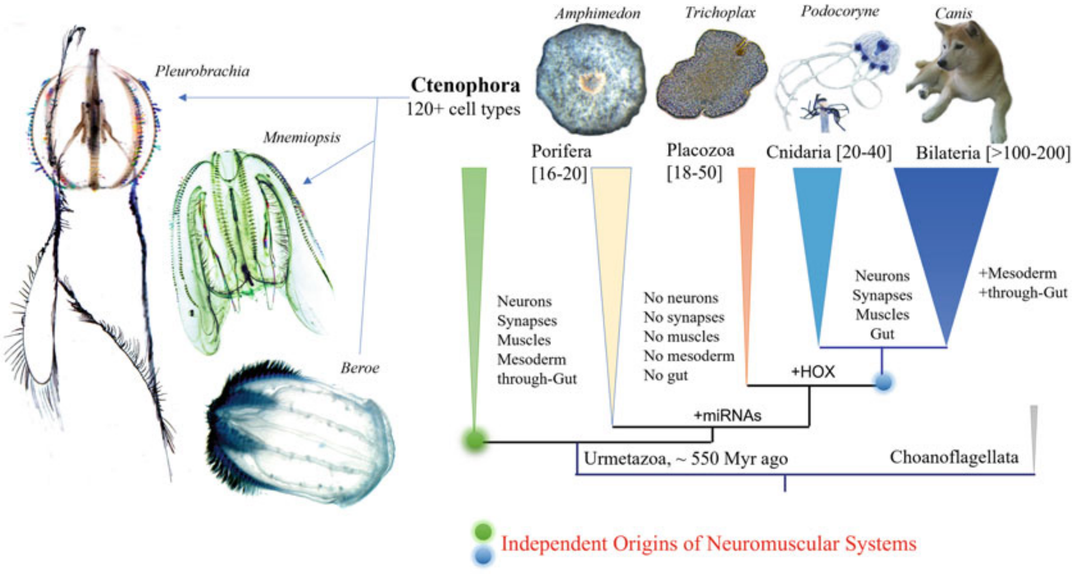

Fig. 1 Relationships among five basal metazoan clades with Choanoflagellata as the sister group to Metazoa. Three species (*Pleurobrachia bachei*, *Mnemiopsis leidyi*, and *Beroe* sp. from Antarctica) illustrate the phylum of Ctenophora as the descendents of the earliest branching animal lineage. The most recent comparative analyses suggest independent origins and convergent evolution of neurons, synapses, muscles, mesoderm, and through-gut in Metazoa (see text for details). Possible origins of microRNA and HOX gene cluster are indicated. Numbers under each lineage are the author's estimates of the diversity of cell types in basal metazoan clades

leading to sponges (Fig. 1). As a result, virtually every aspect of ctenophore biology, the systemic and molecular organization, as well as ctenophore development are remarkably different from what is described in other representatives of 32 animal phyla. In this respect, comb jellies are indeed *"aliens" of the sea*.

Ctenophores are exclusively marine species—from the surface to the record depth of 10,040 meters [9]. Most of the ctenophores, especially in deep habitats, are bioluminescent. The functional role of bioluminescence is unknown, but it is mediated by a distinct group of photoproteins [10–23] unrelated to the famous green fluorescent protein family.

These beautiful "*aliens of the sea*" (sometimes reaching 1.5 m—*Cestum*) can be easily recognized on a calm day in seawater [24] across the globe, from polar to tropical habitats [25]. Any curious observer can find ctenophores without difficulties (Fig. 2). Ctenophores are unmistakably distinguished from the canonical jellies (which belong to another phylum Cnidaria) by the presence of brightly iridescent [26] fused cilia assembled in eight comb rows [27, 28], hence, the name cteno-phora—comb bearers (Ancient Greek: κτείς *(kteis)* "comb" and φέρω *(pherō)* "to carry"). Fused locomotory cilia are the largest in the animal kingdom and are used to glide animals in the water with minimal disturbance, often as

Fig. 2 Diversity of ctenophore species. (1) Benthic ctenophores (Platyctenida). (2) Tentaculate ctenophores (Cydippida). (3) Atentaculate Beroida or Nuda (*Beroe*). (4) Lobata (*Bolinopsis* and *Mnemiopsis*). (5) Lobata: *Ocyropsis*. (6) *Labatolampea*

stealth predators [29, 30]. Such a mode of locomotion separates *comb* jellies from true jellyfishes that are moved by muscular jet-type propulsions. Most ctenophores are holopelagic, but some are creeping (Platyctenida) and even sessile (*Tjalfiella tristoma*, *Lyrocteis imperatoris*).

The first ctenophore drawing (*Bolinopsis* and *Mertensia*) was provided by a ship doctor Martens in 1671, in the vicinity of Spitzbergen [31]. The relationships of comb jellies with other organisms were unclear. The phylum Ctenophora was formally established in 1889 by Hatschek as a separate group distinct in their organization from cnidarians. However, until recently, their affinity with cnidarians was considered, forming a clade coelenterates. All current phylogenomic reconstructions reject this association.

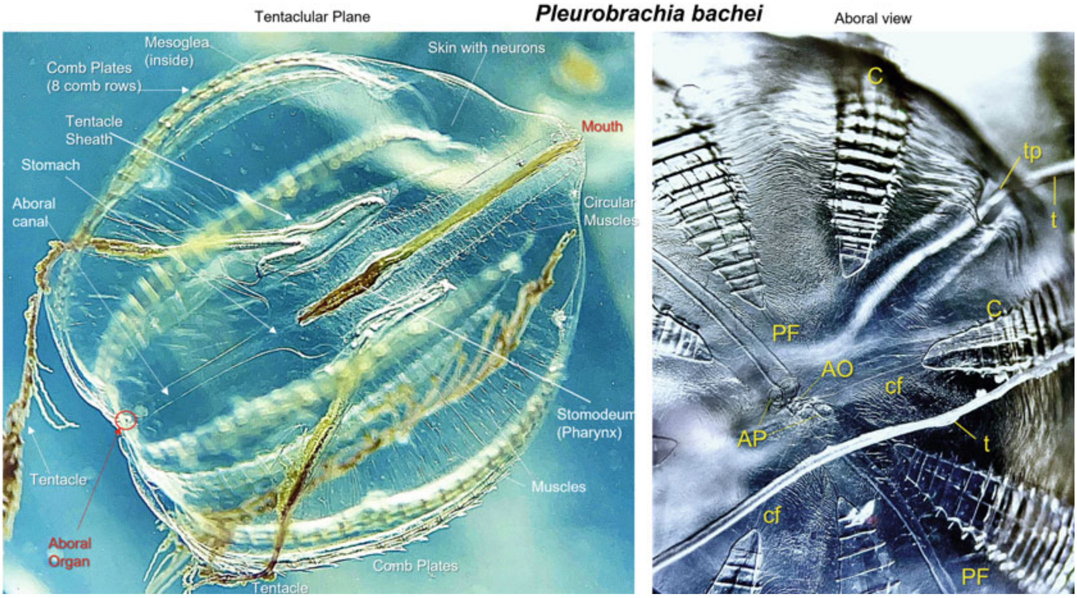

Fig. 3 Illustrative anatomy of *Pleurobrachia bachei* as the representative species for Cyddipida. Abbreviations: *AO* the aboral organ, *AP* anal pores, *C* comb plates, cf ciliated furrows, *PF* polar fields, *t* tentacles, *tp* tentacle pocket

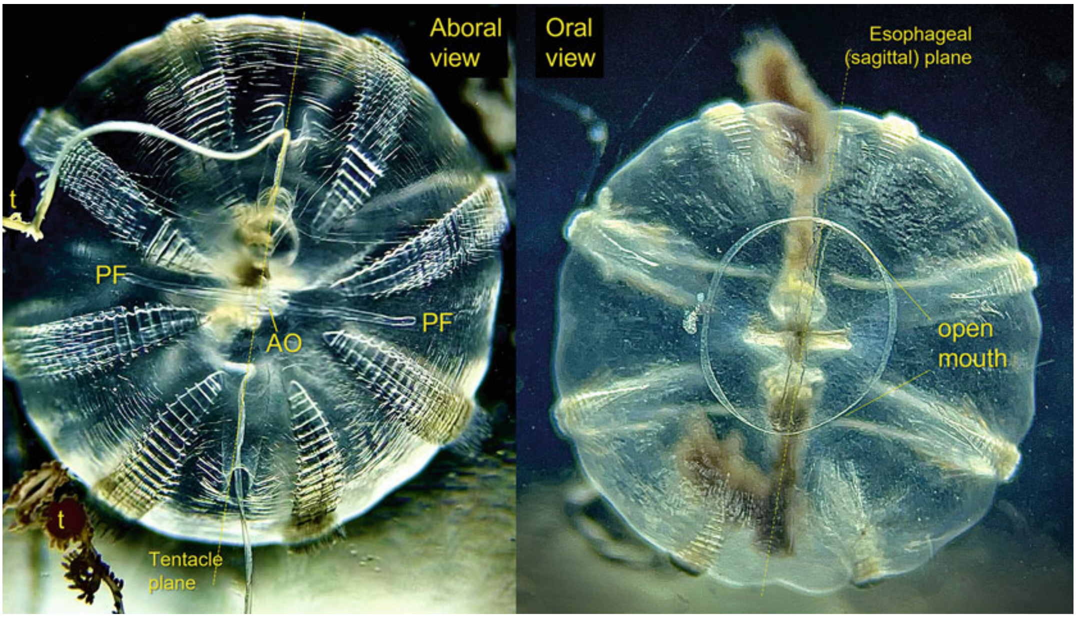

Fig. 4 Two symmetry plans in ctenophores: tentacle and sagittal/esophageal axes (*Pleurobrachia bachei*). Abbreviations: *AO* the aboral organ, *PF* polar fields, *t* tentacles

Four ctenophore genomes have been sequenced, annotated, and published: two closely related cydippid species, *Pleurobrachia bachei* [5] (Figs. 3 and 4) and *Hormiphora californensis* [32], and

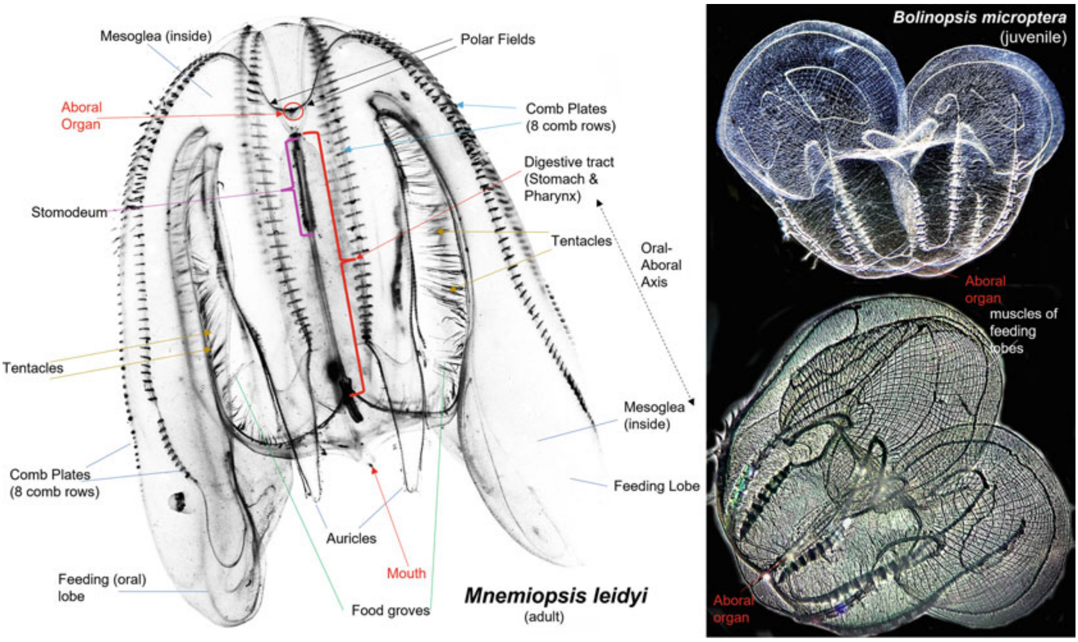

Fig. 5 Illustrative anatomy of *Mnemiopsis and Bolinopsis* as representative species for Lobata

two closely related lobates (Fig. 5), *Mnemiopsis leidyi* [33] and *Bolinopsis microptera* [6]. Three of them (*Hormiphora*, *Pleurobrachia*, and *Bolinopsis*) have chromosome-scale resolution [6, 34] with about 13 chromosomes, suggesting that a common $n = 13$ karyotype is ancestral to this cydippid-lobate group. These sequenced genomes are quite small, with estimated 1C sizes of 100–254 Mbp. Two additional genomes from atentaculate ctenophores (*Beroe forskalii* and *B. ovata*) were recently sequenced and deposited to NCBI (Bioprojects: PRJNA421807, PRJEB23672). The representatives of Beroida are active swimmers (Fig. 6) and often prey on other ctenophores (such as *Bolinopsis*, Fig. 7) and diverse pelagic invertebrates.

The sequencing of these ctenophore genomes and functional/developmental data provided convincing arguments that the ctenophores form the first branch of the animal tree of life, sister to the rest of all metazoans (Figs. 1 and 8). This conclusion is based on two compelling lines of evidence. First, integrative, interdisciplinary analysis of multiple traits and genes encoding neural, muscular, immune, mesoderm, and intracellular signaling components, combined with phylogenomics, revealed a reduced representation in each of these toolkits compared to sponges and the rest of metazoans [5]. This discovery led to the scenario that neurons, muscles, and mesoderm, systemic gut with two anuses, and sensory organs evolved more than once and independently in the ctenophores vs. Cnidaria+Bilateria clade [5, 35–37].

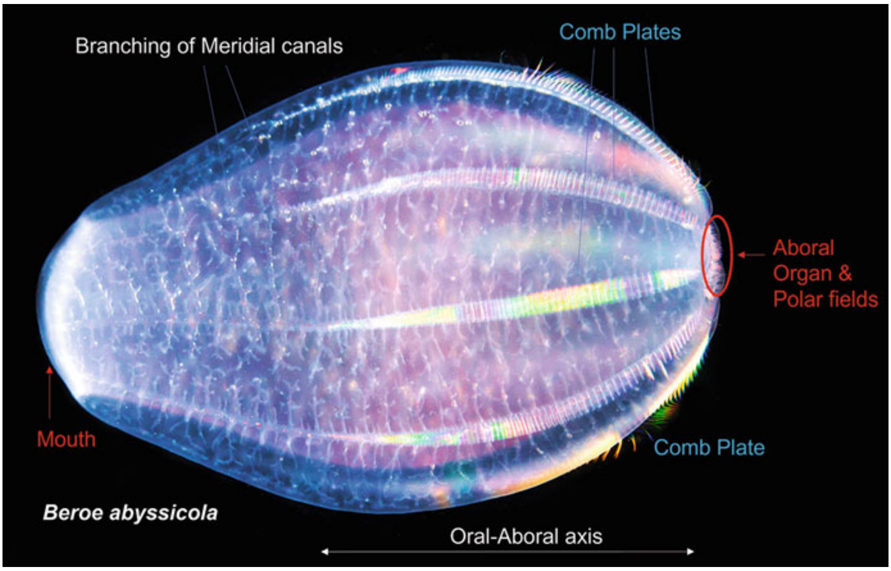

Fig. 6 Illustrative anatomy of *Beroe* as the representative species for Nuda

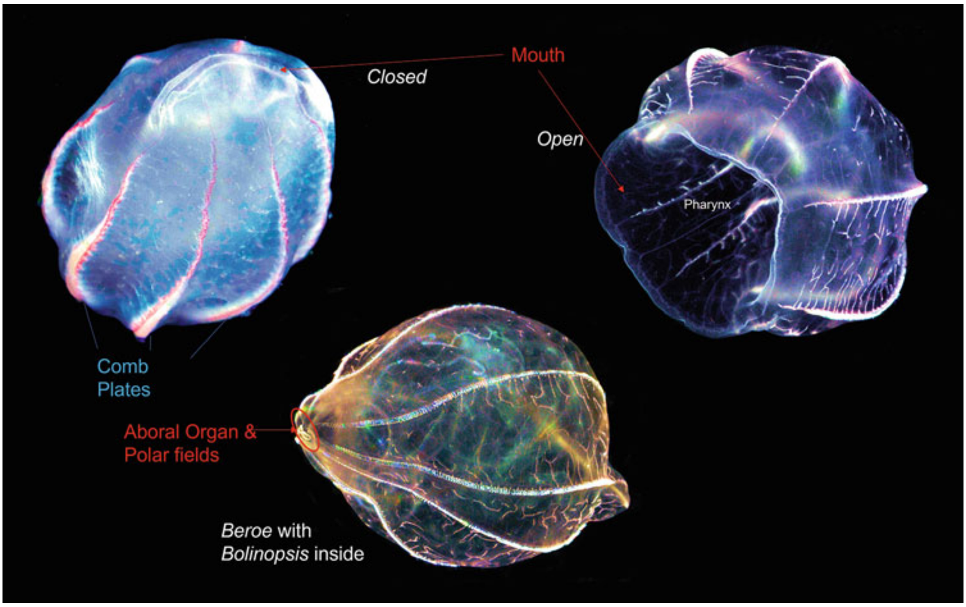

Fig. 7 *Beroe* anatomy and feeding on *Bolinopsis* (Florida Keys)

Second, the chromosome-level synteny analyses across Metazoa showed that ctenophores and unicellular eukaryotes share ancestral metazoan patterns, whereas sponges, bilaterians, and cnidarians share derived chromosomal rearrangements [6]. Schultz and colleagues pointed out: "the patterns of synteny shared by

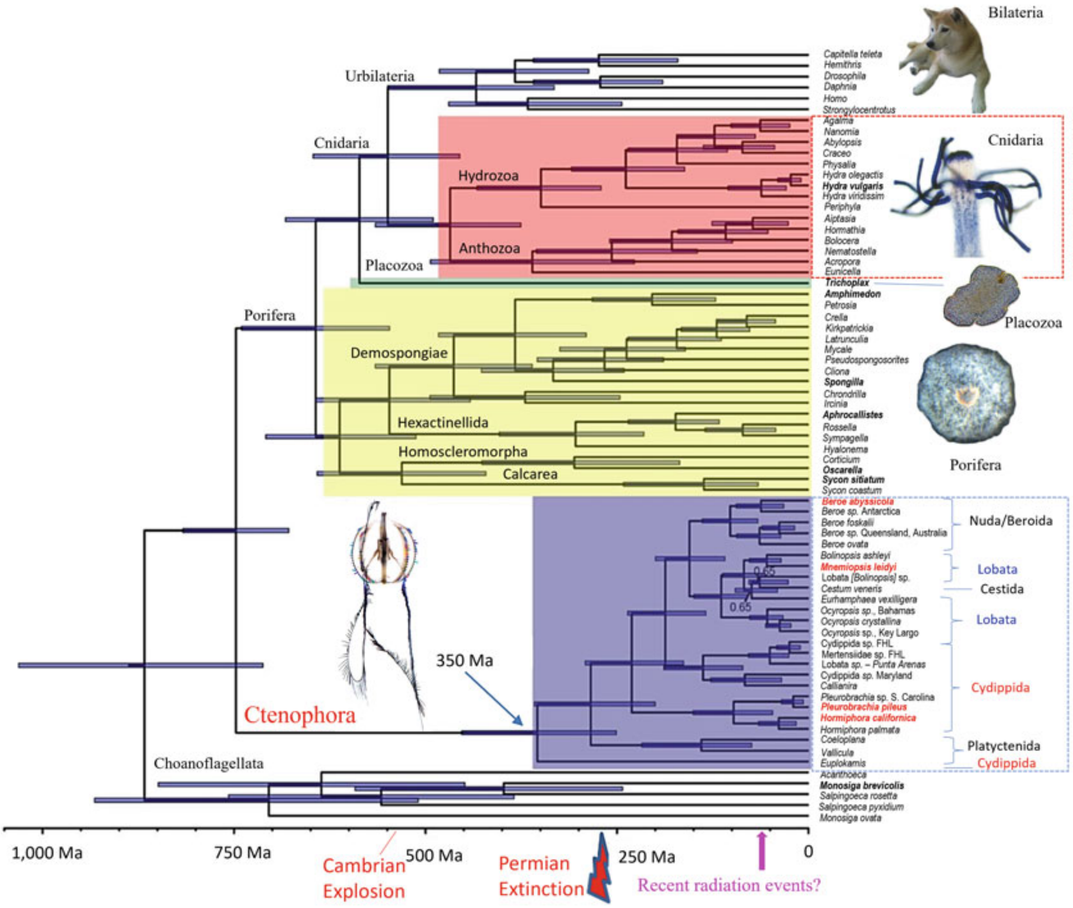

Fig. 8 Ctenophora as sister to the rest of Metazoa. The tree shows relationships among basal metazoan clades and within species of the phylum Ctenophora (Adapted and modified from Ref. [8]). Of note, this phylogeny does not support the classical ctenophore systematics and indicates the polyphyly of Lobata and Cydippida and the placement of Nuda/Beroida within Lobata

sponges, bilaterians, and cnidarians are the result of *rare and irreversible chromosome fusion-and-mixing events* that provide robust and unambiguous phylogenetic support for the ctenophore-sister hypothesis" [6]. More than 30 ctenophore transcriptomes were obtained in parallel, leading to the same conclusion and the ctenophore-first hypothesis [5, 7, 8] (Fig. 8).

Giant mitochondria [38] and compact mitochondrial genomes in ctenophores are also unique and highly derived due to their rapid evolutionary dynamics [39–47]. These findings prevent the use of mitogenomics for macrophylogeny. In contrast, mitogenomics is highly valuable for deciphering divergent evolution within the phylum [41, 42, 48, 49]. In addition, the diversity of mobile elements in ctenophores might support the origins of certain innovations and even facilitate transcription factors' evolution [50, 51];

many of transcription factor families (e.g. BHLH) resulted from ctenophore-specific difersification events, supporting complex tissue and organ specification.

The outcome of this ctenophore-first hypothesis is that most cellular and system-level components associated with the animal organization result from convergent evolution. In other words, the ctenophore lineage independently evolved such high level of animal complexities with the astonishing diversity of cell types and structures as bilaterians and cnidarians. Parallel and early evolution of complex metazoan traits is associated with a broad spectrum of ctenophore-specific molecular, cellular, developmental and feeding innovations, including novel toolkits for making an animal.

2 Recent Diversification and Bottlenecks in Ctenophore Evolution

Ctenophores are animals with exceptional rotational-type symmetry [52, 53] (Fig. 4), not recognized in other metazoans. There are 185 described species of Ctenophora (See Moroz, Collins, Paulay, Chapter 2, this book [198]), and likely this number could be doubled to incorporate recently discovered (but not formally described) and mostly unknown deep-water species.

The existing classical ctenophore taxonomy recognizes two established classes [2, 31], 9 orders, 32 families, and >50 genera (see also Fig. 2). Traditionally, the class Tentaculata includes ctenophores with tentacles, such as illustrated here representatives of the two largest orders: Cydippida (Figs. 3 and 4) and Lobata (Fig. 5). The class Nuda includes ctenophores without tentacles, with one order (Beroida) and two genera, *Neis* and *Beroe* (Figs. 6 and 7), which secondarily lost tentacles both in their larval and adult stages. The presence of tentacles in adults and larval ctenophores (cydippid larva) is likely the ancestral trait.

However, the emerging molecular phylogeny challenges the classical taxonomy [5, 7, 8], uncovering the polyphyly of Lobata and Cydippida. The parallel evolution of multiple traits (Figs. 8 and 9) includes two independent transitions to benthic lifestyles in Platyctenida or benthic ctenophores and *Lobatolampea*, respectively (Fig. 9, red arrows). Furthermore, the comparative phylogenomic analysis, using more than 30 ctenophore transcriptomes and molecular clock estimates, indicated that the ctenophore lineage went through a significant bottleneck about ~350–250 million years ago [8], with a possibility of the most recent diversification events that occurred around 100–60 million years ago (Fig. 8), which correlates with the Cretaceous–Tertiary (K–T) extinction at the end of the Mesozoic era, also ending the dinosaurs' epoch.

These evolutionary bottlenecks explain the loss of some distinctive features of ancient ctenophores found in fossils of about 20 species. Indeed, some Cambrian ctenophores possessed 16–80

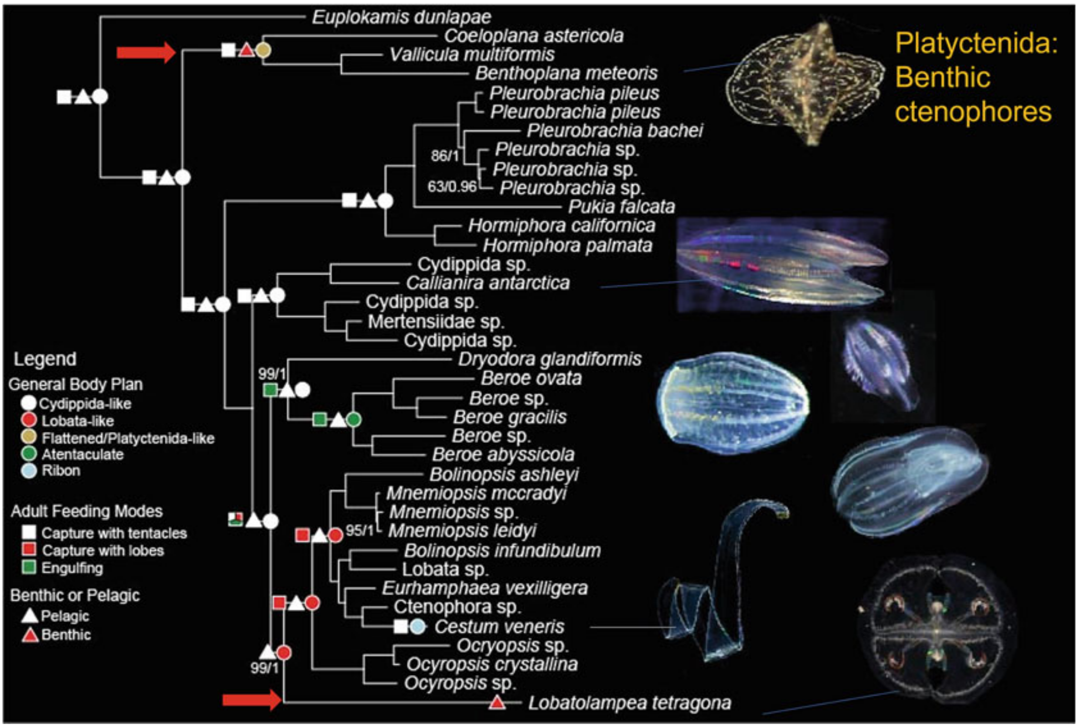

Fig. 9 Ctenophore phylogeny reveals parallel evolution of adaptive strategies in Ctenophora (Adapted and modified from Ref. [8]). Red arrows indicate two independent transitions from pelagic to a benthic lifestyle in Ctenophora

comb rows (vs. only eight comb rows in all extant ctenophores) [54]. There are also speculations that some ancestral ctenophores had sclerotized skeletons and could be secondarily sessile, forming a now-extinct clade Scleroctenophora [55]. Some Ediacaran fossils, such as *Eoandromeda*, were interpreted as an early stem-group ctenophore [56]. Zhao and colleagues also suggested that the earliest ctenophores were suspension feeders [57], implying that tentacles and predation occurred later. The earliest tentaculate ctenophores were found in the early Cambrian [58] and Devonian [59, 60]. Nevertheless, it isn't easy to reconstruct their history due to the poor preservation of ctenophores in fossil records.

3 Ctenophores as Predators

Ctenophores are carnivores (active or ambush predators), feeding on a broad range of animals [61–63]: from zooplanktons to other ctenophores (e.g., *Beroe*, Fig. 7; see also [64]), narcomedusae (e.g., *Haeckelia* [65–67], or larvaceans for *Dryodora* (see also [68–75]. As a result, ctenophores exhibit a remarkable diversity of behaviors [76–89], which are little investigated. Tentacles and their small branches (tentillae/tentilla) contain specialized sticky glue cells or colloblasts [90, 91], facilitating prey capture and performing other functions.

Ctenophores have highly elaborated digestive systems with well-developed tripartite **through-gut** [31]: mouth, pharynx, stomach, and a pair of anal pores with rhythmic contractions, often associated with defecation [92]. Such distinctive through-gut evolved in ctenophores independently from the rest of metazoans. Absorption of digested nutrients is transported to a branching gastro-endodermal canal system (meridional canals) and delivered to the rest of the body.

4 Ctenophore Life Is Based on Cilia and Alternative Neural Systems

It would be proper to say that virtually all ctenophore organization and their life is based on cilia [27, 93]. The diversity, complexity, and control of cilia in ctenophores are greater than that observed in other animals. In contrast to other animals, cilia, not muscles, are the primary effectors in many ctenophores. Muscles in ctenophores are usually involved in pray catching rather than in locomotion. Only a few species evolved muscular jet-like propulsion (e.g., *Ocyropsis crystalline*) and sinusoidal undulations of the whole body (e.g., *Cestum veneris*) during swimming escape responses. Some muscles are giant and well-characterized electrophysiologically [94–100]. These muscles control hydroskeleton tone, body shape, and feeding, which might be the original functions of muscle elements in animal ancestors.

Figure 10 illustrates cilia diversity in *Beroe abyssicola* with different types of cilia in the mouth (some serve as teeth for prey capture [101–103]) and body wall. At least six types of cilia [104] construct the aboral organ as a gravity center with dozens of living cells—**lithocytes** containing statolith [105–107]. Ciliated furrows are also efficient conductive pathways mediating various behaviors. There are multiple types of ciliated receptors formed by nonmotile cilia [102, 108–110].

The cilia are primarily used for locomotion with the unique ability to reverse cilia beating [111] and contain ctenophore-specific proteins CTENO64 and CTENO189, which are required for paddling of comb plates and locomotion of ctenophores [112] as well as reinforce the elastic connection among cilia to overcome the hydrodynamic drag of giant multiciliary plates [113].

A diverse spectrum of behaviors, ciliated and muscular locomotion, as well as feeding [30, 68, 93, 107], is controlled by quite complex neural systems, and, at least in part, it is coordinated by the aboral organ [107], an analog of the elementary brain.

The study of the neural organization of ctenophores was started in 1880s by R. Hertwig [114] as a logical expansion of

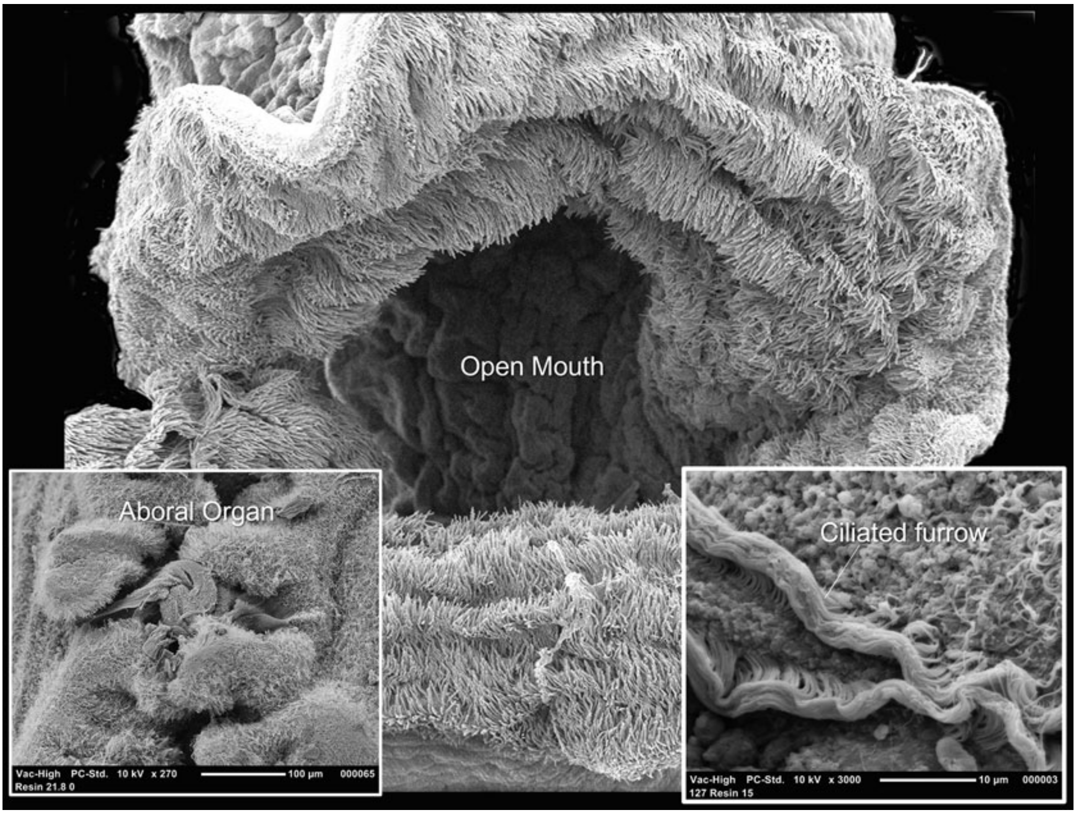

Fig. 10 Scanning electron microscopy of the mouth, aboral organ, and ciliated furrows of *Beroe abbysicola*. (See details in Refs. [102, 103])

similar studies on cnidarians by Hertwig's brothers [115–117]. This fundamental work led to the most well-known hypothesis of nervous system evolution [118, 119]. However, ctenophore neurons are elusive cells to stain with convenient histological dyes or bilaterian molecular markers due to the lack of pan-neuronal genes across Metazoa [120].

The overall microanatomy of neural systems is now described for 11 ctenophore species [27, 102, 108, 109, 121–128] and summarized in Fig. 11 [129]. About 10,000 neurons were counted in *Pleurobrachia bachei*, representing five distinct components: (i) the aboral organ, (ii) polar fields, (iii) conductive pathways, and (iv) subepithelial and (v) mesogleal nerve nets.

Integrative comparative analyses, including genomics, metabolomics, molecular mapping, and physiology, suggest that ctenophore neurons are remarkably different from all other studied neurons in Cnidaria and Bilateria, meaning, together with the current phylogenetic reconstruction, their independent origins and ongoing parallel evolution (summarized in [35–37, 130–133]. Recent volume electron microscopy reconstruction of

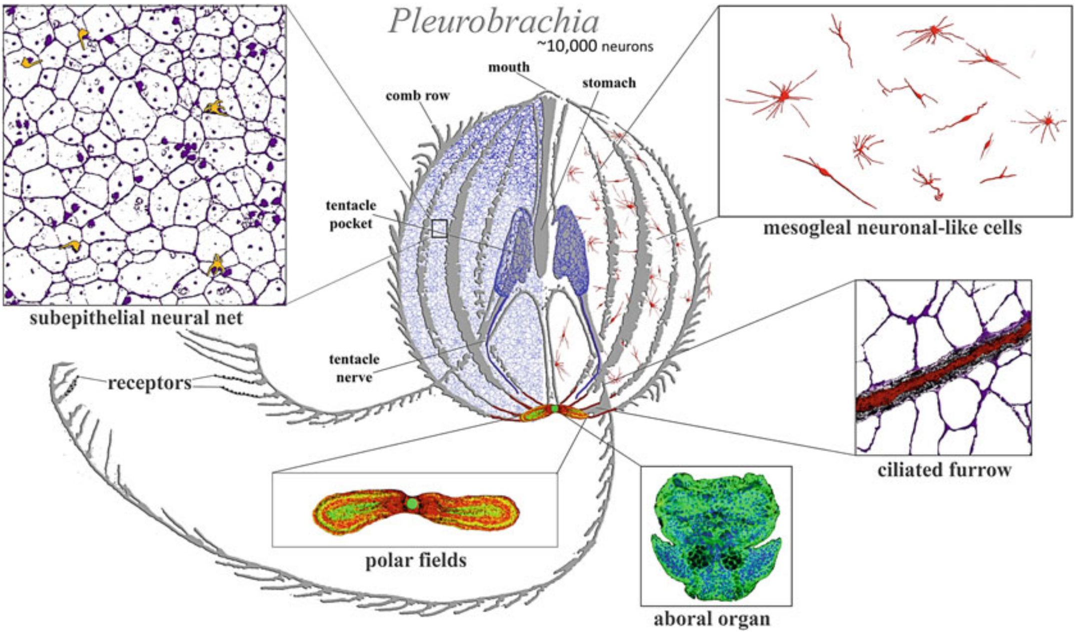

Fig. 11 Neural systems in ctenophores. The schematic diagram is based on the recent studies of several species [102, 108, 109, 127, 128, 196] with the cydippid *Pleurobrachia bachei* as a key reference model. Different colors indicate different cellular populations. Most neurons and receptors (yellow) are located within the subepithelial neural net in the skin (blue, magenta) and tentacle shields with two tentacular nerves (dark blue). There are two concentrations of neural elements: one in the aboral organ (green) with densely packed neurons and other cell types (the elementary brain?) and the second in the polar fields putative chemosensory structures (yellow/green, red marks phalloidin-labeled elements). The mesoglea has a diffuse population of neuron-like cells (red). Eight ciliated furrows (conductive ciliated cells—red lines) connect the aboral organ with comb plates. The ciliated furrows are closely associated with neural net elements (insert) and are possible under neuronal control. (Adapted from Ref. [129])

juvenile *Mnemiopsis* found that five neurons in the subepithelial network form the syncytium [134], which is likely a secondary adaptation for some neural elements. Still, most neurons and neuro-effector communications are chemical [197] with the distinct tripartite organization of ctenophore synapses, also known as "presynaptic triad." Each presumed presynaptic zone contains a three-layer complex of organelles: a single layer of synaptic vesicles lining the presynaptic membrane, a cistern of agranular endoplasmic reticulum just above the row of vesicles, followed by one or several mitochondria [27, 122, 125, 134–136].

The diversity of synaptic vesicles implies the variety of signal molecules and neurotransmitters—most of them are currently unknown. Gaseous nitric oxide (NO) was also implicated in intercellular signaling. However, nitric oxide synthase (NOS) was not detected in ctenophore neurons [137, 138]. Initial analysis of the *Pleurobrachia* genome and transcriptomes for dozen of related species, complemented by metabolomic and functional studies, indicated that the canonical bilaterian neurotransmitters such as serotonin, dopamine, octopamine, noradrenaline, adrenaline, histamine, and acetylcholine are absent in the ctenophores, and likely bilaterian innovations [37, 120, 139].

Glutamate was proposed as a candidate for neuromuscular transmission [5, 140] and small secretory peptides are major transmitters with about 100 of ctenophore-specific neuropeptides [5, 37]. The diversity and role of neuropeptides were subsequently validated in two other species *Mnemiopsis* [141] and *Bolinopsis* [142], confirming the hypothesis that the earliest transmitters can be secretory peptides [119] and neurons evolved from genealogically different secretory cell types [132]. Of note, none of the ctenophore neuropeptides had recognized homologs outside of this phylum, further supporting the hypothesis about the unique organization of ctenophore neural systems, their independent origins, and extensive parallel evolution.

5 Unique Ctenophore Development

Most ctenophores are direct developing, self-fertile hermaphrodites with a few exceptions, such as the presence of both sexes in *Ocyropsis* [143]. Gonads derive from the endoderm of meridional canals; one part represents the female and the second male gonads. Gametes are released through pores in the epidermis or through meridional canals and anal pores (personal observation in *Pleurobrachia bachei - see Fig 6, next Chapter*). Unlike other metazoans, **polyspermy** occurs in ctenophores such as *Beroe*. As many as 20 spermatozoa enter the egg, and the female pronucleus moves and "selects" a male pronucleus, and the position of the selection determines the position of the blastoporal pore [144–146]. Patterns of early development seemed to be shared across ctenophores and were observed for several decades of research, starting with classical pioneering work at the end of the nineteenth century [31, 147–161]. The latest progress is summarized in [162] using *Mnemiopsis leidyi* as a model. All available data indicate that ctenophore development distinctly differs from other basal metazoans (e.g., see Fig. 12 for *Pleurobrachia bachei*).

The early [147, 148] and controversial history of ctenophore embryology started with the pioneering work on biodiversity and the earliest developmental specification discovered in 1880s by C. Chun [149]. When C. Chun separated blastomers in two-cell embryos, he found that each half-embryo developed half of the adult structures in ctenophores, suggesting highly deterministic mechanisms even after the first division during the cleavage. G. Freeman showed that the oral-aboral axis is established at the time of the first cleavage that cleavage plays a causal role in

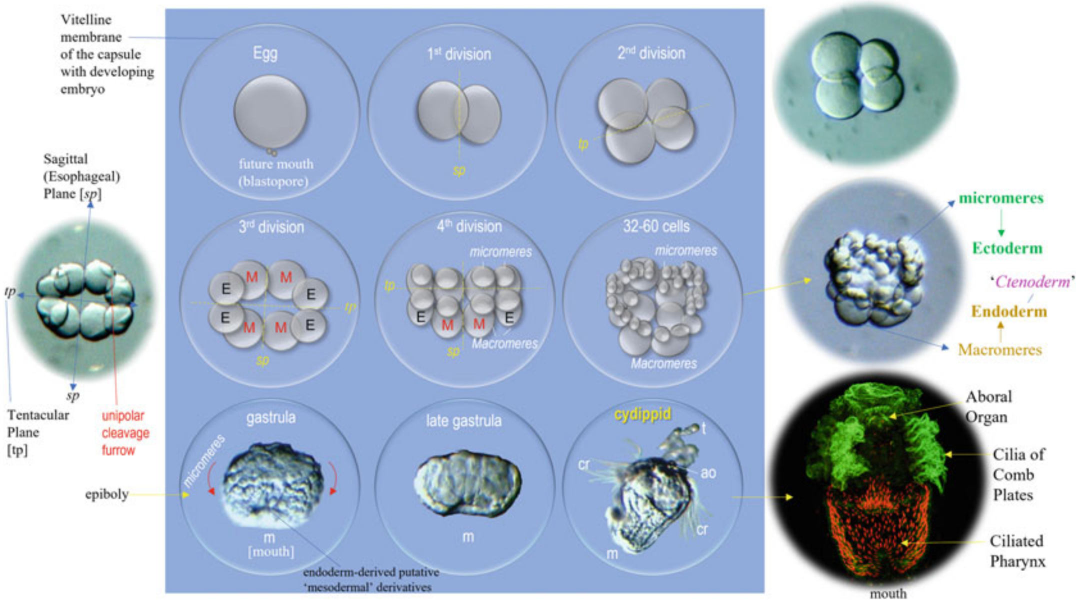

Fig. 12 Development in *Pleurobrachia bachei*. (Modified from Ref. [127]; see text for details)

setting up the axis and that comb plate-forming potential begins to be localized in the aboral region of the embryo at this time [163].

The first division starts with a characteristic unipolar cleavage furrow. Most cell fates are determined at the first cleavage stages and continue through 60-cell stages and gastrulation, as carefully characterized by microinjection and dye-tracing techniques [162, 164, 165]. Macromere lineages give rise to the endoderm and its derivatives (including endothelium of meridional canals, the mineral-containing lithocytes generated in the floor of the aboral organ). In contrast, aboral micromeres give rise to the ectoderm and its components (skin, comb rows, most of the aboral organ, tentacle epidermis with colloblasts, some neurons, and pharyngeal epithelium). Furthermore, in ctenophores, the epithelial might also be regulated differently than in bilaterians and cnidarians. Specifically, Par protein localization during the early development of *Mnemiopsis leidyi* suggests other modes of epithelial organization [166].

The most fascinating is the "mesoderm" development. According to the carefull work of E. Metschnikoff [150] in 1885, "ctenophores have a 'true' mesoderm of entodermal origin" [31] derived from small cells at their oral poles. These cells carried inward during the gastrulation process proliferate and "become the cells of the collenchyme, including muscle cells" [31].

Recent studies of Martindale and Henry on *Mnemiopsis* convincingly identified a distinct subset of macromer-derived "oral" micromeres, which subsequently move inside the embryo and differentiate into mesenchymal cells [162, 165]. The muscle cells are supposedly derived from a type of mesenchyme cell in the mesoglea; they are segregated early in embryonic development and, therefore, can be considered as "true" mesodermal derivatives (separate from epidermis and gastrodermis [167, 168]). Separate comparative analyses of *Pleurobrachia* [5] and *Mnemiopsis* [33] genomes revealed that ctenophores do not possess many canonical developmental regulatory genes required for bilaterian mesoderm specification. Moreover, these data and the ctenophore-sister phylogeny imply that muscles and mesoderm evolved independently in ctenophores. Thus, the ctenophore "mesoderm" might not be homologous to the bilaterian mesoderm as we know it today. As a result, the term "ctenoderm" was proposed to refer to cells residing in this layer [169].

Later, post-hatching development varies more than embryonic development, creating enormous diversity of ctenophore forms across the phylum. Lobate ctenophores are generally flattened in the tentacles plane, while Platyctenida are flattened in the aboral-oral direction.

For example, after hatching as a classical cydippid larva/or juvenile, tentacles are dramatically reduced in Lobata representatives and can even be lost in adult *Ocyropsis.* Representatives of the order Beroida lost their tentacles at all developmental stages and in adults.

In some benthic ctenophores Platyctenids, adults can also lose comb plates from their cydippid larvae. A fascinating case was discovered in the Greenland sessile *Tjalfiella tristoma*, which is viviparous; the young ctenophores grow in a womb [31, 170]. Finally, one species *Lampetia* has an undifferential larval stage that parasitizes salps [170]. This larval stage was initially not recognized as the same species and was called *Gastrodes.*

Does dissogeny exist in ctenophores? In *Mnemiopsis* (and possibly *Beroe*), *continuous* reproduction was reported from early juvenile animals to large mature adults [171]. These observations challenge the concept of **dissogeny** or the presence of *separate phases* of larval and adult reproduction (see also [172]). Edgar and colleagues suggested that "spawning at small body size should be considered the default, on-time developmental trajectory rather than precocious, stress-induced, or otherwise unusual for ctenophores. The ancestral ctenophore was likely a direct developer, consistent with the hypothesis that multiphasic life cycles were introduced after the divergence of the ctenophore lineage" [171]. Whether such an exceptional situation would be applied to other ctenophore species would be the subject of future research [172].

6 Ctenophores Are Kings of Regeneration

In contrast to highly deterministic "mosaic" development, many ctenophore species are capable of fast and efficient regeneration [173–181], the most characteristic for very fragile lobate ctenophores (Fig. 13), but also observed for tentacles and additional body parts (e.g., tentacles) in other lineages within Cydippida [182] and Platynectida. The creeping Platyctenida even can reproduce asexually from their fragments that could regenerate the whole animal with all organs [173, 176, 183, 184]. In contrast, Beroids have a minimal regeneration capability.

In *Bolinopsis* and *Mnemiopsis,* we noted the remarkable regeneration of the aboral organ, which takes 2.5–3.5 days at ambient temperatures, and restoration of observable behaviors within 5–6 days (n=45, author's observations). For example, I observed the regeneration of the aboral organ four times from the very same animal. After the first regeneration event, I fed animals following the recovery of their behaviors and repeated the procedure four times! Cellular, molecular, and genomic bases of such unique

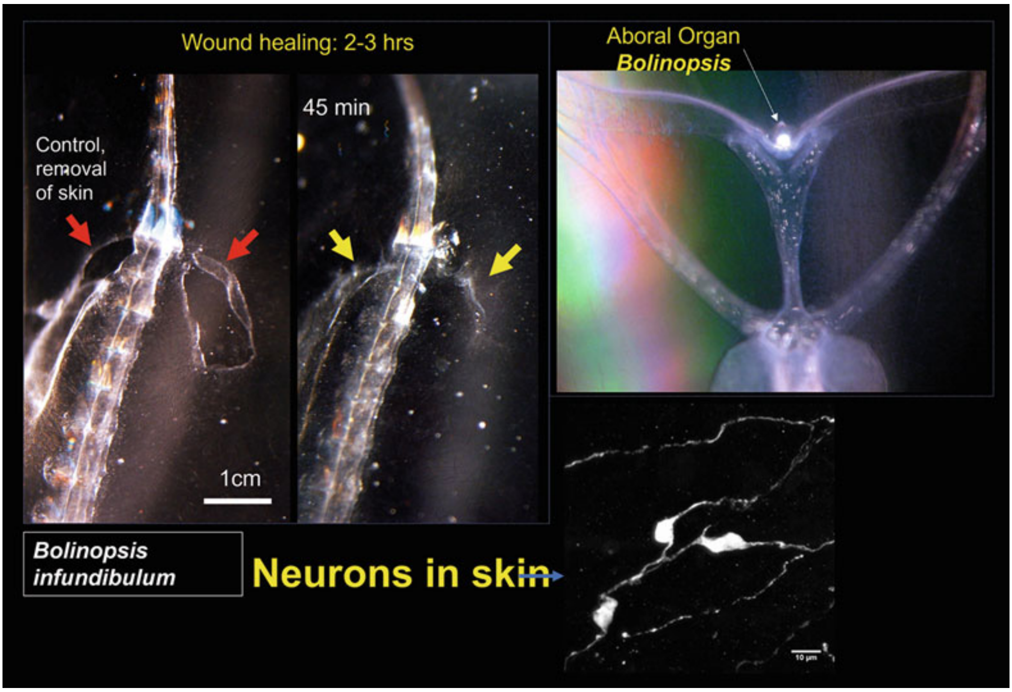

Fig. 13 Ctenophore regeneration. An illustrative example of wound healing in *Bolinopsis microptera*, where an experimental cut of the skin area induced its rapid closing within 1 h after the injury. The aboral organ in this species (shown on the right) can efficiently regenerate within 3 days (see text for details). The aboral organ's wound healing and regeneration are accompanied by notable reorganization of the subepithelial neural net (lower right)

regeneration capabilities are under intensive investigation [182, 185, 186] and can provide deep insights into the synthetic biology of the future.

7 Future Directions: Ctenophores as Key Reference Species: Culturing, Genomics, and Gene Editing

Systematic interdisciplinary studies of ctenophores are in their infancy, and deciphering the remarkable morphological and functional diversity is one of the hot topics in biological research over the following decades, with many anticipated surprises. Many of these surprises would be from examples of convergent evolution, including deciphering lineage-specific diversification across integrative systems and signaling in ctenophores (Fig. 14).

Several reasonably straightforward directions in the field are outlined below.

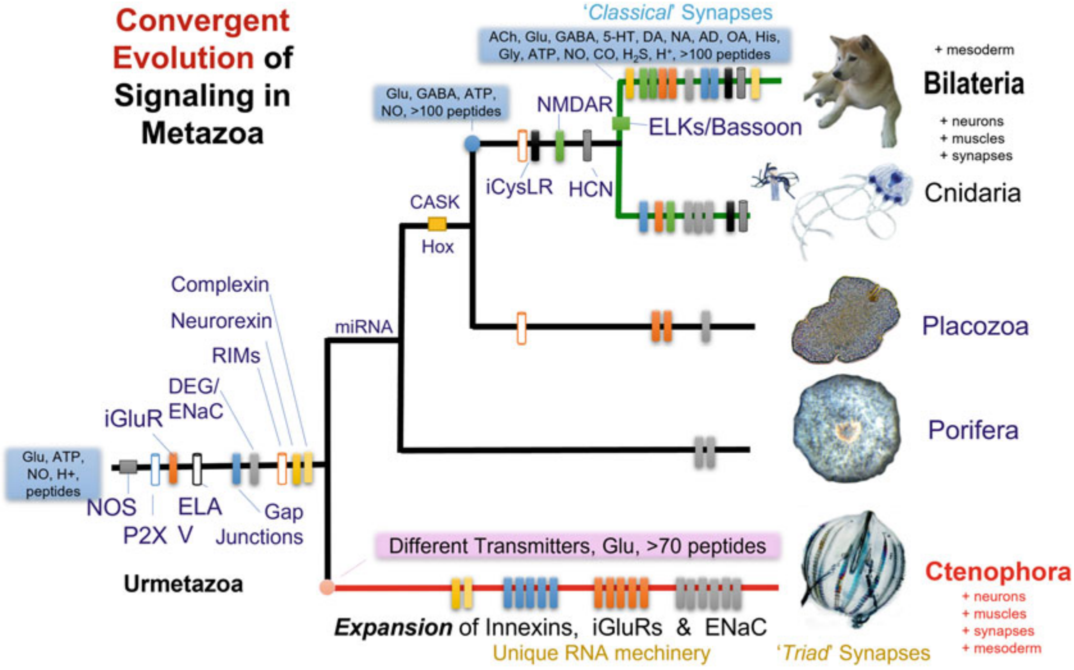

Fig. 14 Molecular innovations underlying the parallel evolution of neuromuscular organization and respective (neuro)transmitter systems in ctenophores vs. other basal metazoan lineages (Modified from Refs. [5, 37]). Bars indicate the presence or independent radiation of selected gene families (e.g., ionotropic glutamate receptors [iGluR], innexins [5, 199], acid-sensitive channels (ENA) in ctenophores and Cnidaria+Bilateria clades. Our model suggests that sponges and placozoans never developed "true" neural and muscular systems. However, both neurons and muscles independently evolved in common ancestors of the ctenophore vs. Cnidaria and Bilateria lineages with a distinct complement of signaling molecules and secretory peptides

1. Although most ctenophores cannot be routinely maintained in laboratory culture, we already see remarkable progress in this direction for some species [187–192], primarily using facilities of marine stations.
2. Ctenophore cells can be efficiently maintained in cell culture, enabling a diversity of experimental manipulations [95, 193, 194].
3. The remarkable breakthrough was a success in gene editing using CRISPR-cas9 technology in *Mnemiopsis* [188] and morpholinos in *Bolinopsis* [142].
4. Sequencing, chromosome-level, and functional annotation of genomes from dozens of diverse ctenophore species representing all families of the phylum is needed and will be achievable soon. This research will decipher ctenophore innovations and be a critical platform for virtually all directions in the field.
5. Nevertheless, most surprises are anticipated in the sea, from investigations of animals in their native habitats toward little explored functional biodiversity for these enigmatic species. This strategy would expand work from standard model organisms such as specialized and abundant *Mnemiopsis* to dozens of other ctenophore species. Here, the progress relies on the infrastructure of already established marine laboratories as the first step.
6. However, we expect the most discoveries by direct access to ctenophores in their native living habitats using remote operation vehicles (ROV) and even full-scale interdisciplinary floating laboratories at sea, such as the Ship-seq approach [195] introduced earlier and leading to the first systematic molecular access to more than 30 species [8].
7. Finally, we expect a shift from more traditional genomic or embryological/developmental approaches to a deeper experimental analysis of ctenophore cellular and system physiology, neuroscience, and deciphering cellular bases of behaviors and use this knowledge for future synthetic biology to make new cell types, tissues, organs, organisms, and behaviors.
8. Finally, we anticipate discoveries in (micro)paleontology using novel techniques and approaches to expand our understanding of basal metazoan lineages' origins and early radiation.

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