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# Chapter 14

## Xenacoelomorpha, a Key Group to Understand Bilaterian Evolution: Morphological and Molecular Perspectives



Ulf Jondelius, Olga I. Raikova and Pedro Martinez

**Abstract** The Xenacoelomorpha is a clade of mostly marine animals placed as the sister group of the remaining Bilateria (Nephrozoa) in most phylogenomic and morphological analyses, although alternative hypotheses placing them within deuterostomes have been proposed. This key phylogenetic position has raised recently a great interest in the study of their constitutive clades, since they can provide us with character states that illuminate different aspects of the origin of bilateral animals. Moreover, the recent availability of genomic and transcriptomic data from different species has been used in inferring the internal relationships among xenacoelomorph clades and the deciphering of molecular mechanisms that contribute to the evolution of metazoan genomes. Having access to molecular data paves the way to the systematic analysis of the genetic control of xenacoelomorph development and, additionally, to a better-informed study of bilaterian innovations. Here we revisit what has been learned over the last decades on the morphology, genomics and phylogenetic relationships of the Xenacoelomorpha.

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## 14.1 Xenacoelomorpha: Taxonomic Status

Superphylum Xenacoelomorpha (Philippe et al. 2011) currently includes two phyla: Xenoturbellida (Bourlat et al. 2006) and Acoelomorpha (Ehlers 1985) with two subgroups that are traditionally considered to be orders: Nemertodermatida (Karling 1940) and Acoela (Uljanin 1870). Xenacoelomorpha lack any type of nephridia, which distinguishes them from the rest of Bilateria (Nephrozoa).

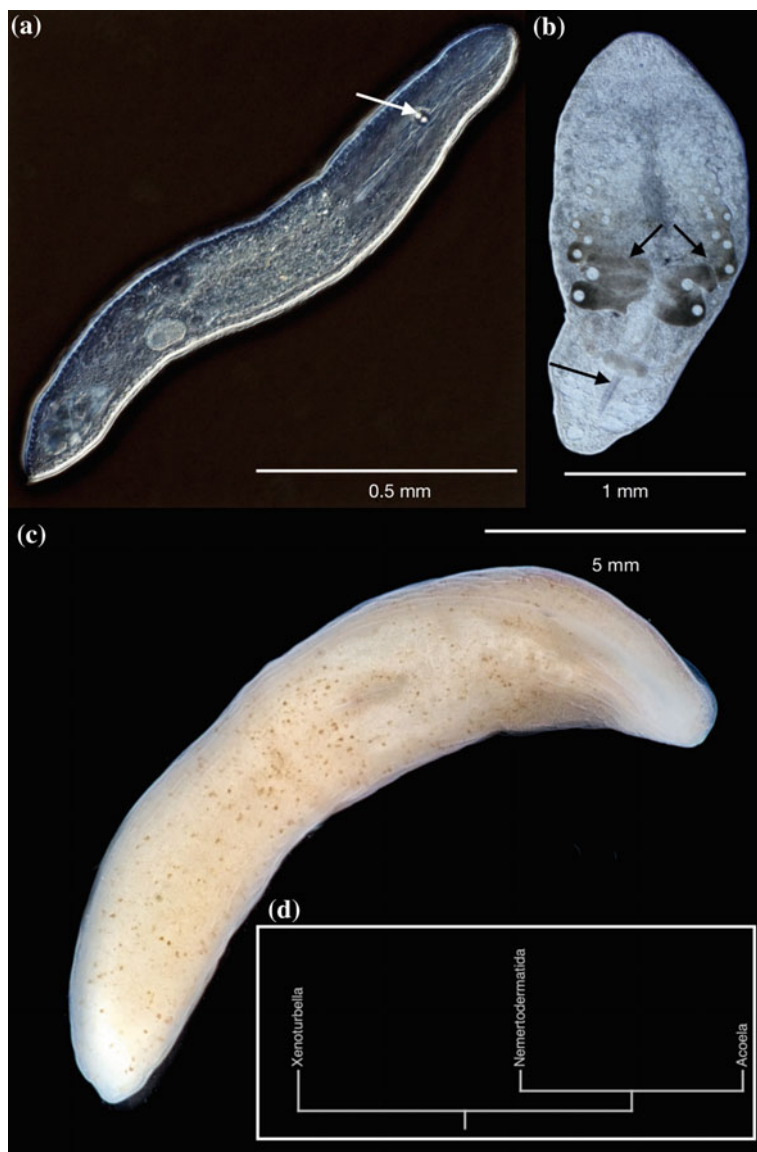
## 14.2 Xenacoelomorpha and Its Constitutive Clades: Phylogenetic Considerations

Xenacoelomorpha are soft-bodied marine hermaphroditic worms comprising three subgroups: Acoela, Nemertodermatida and *Xenoturbella* (see Fig. 14.1 for representatives). Acoela is by far the most diverse group with about 400 nominal species abundant in many marine habitats ranging from sandy beaches to deep-water mud and also with a few pelagic species. The 18 nominal species of Nemertodermatida are much rarer, many of them known from just a few specimens from marine sandy and muddy sediments below the intertidal zone (Sterrer 1998). Endosymbiotic species occur within both groups: *Meara stichopi* is an endosymbiotic nemertodermatid occurring in the intestine of the holothurian *Parastichopus tremulus* (Westblad 1949a), and within the acoel genus *Avagina*, there are three species that occur in echinoids (Dörjes 1972). Acoels and nemertodermatids are small animals with a maximum size of a few millimetres whereas the five species of *Xenoturbella* are much larger, attaining more than 20 cm in *X. monstrosa* (see below for detailed descriptions).

When they were first described, both Acoela and Nemertodermatida were classified as flatworms (“turbellarians”). The name Acoela (Uljanin 1870) refers to the lack of both intestinal cavity and body cavity in these animals. Nemertodermatida alludes to the thick gland-rich epidermis similar to that of nemertines. In contrast to acoels, nemertodermatids possess an epithelial gut, and they were classified in a separate order (Karling 1940). The first known *Xenoturbella*, *X. bocki*, was described in 1949 (Westblad 1949b) and, as the name indicates, it was considered to be a “strange turbellarian”, i.e. also a flatworm, but strange due to its large size and very simple anatomy.

Acoela and Nemertodermatida are considered sister groups making up the group Acoelomorpha (Ehlers 1984). This hypothesis was first based on ultrastructural similarities in their epidermal ciliary rootlets and later received support from phylogenomic analyses (Hejnol et al. 2009).

Acoelomorphs, based on similarities of morphological characters (lack body cavities (acoelomate structure), a hindgut or an anus), were originally classified within the Platyhelminthes (i.e. Conway-Morris et al. 1985). However, this original classification of Acoelomorpha within Platyhelminthes was difficult to reconcile with some of their morphological features, e.g. the absence of protonephridia, the divergent



**Fig. 14.1** **a.** Nemertodermatida. Live specimen of *Flagellophora apelti*. Charactersitsic double statoliths can be seen in anterior (arrow). **b.** Acoela. In this live specimen of *Childia macroposthium*, the male copulatory organ with proximal seminal vesicle can be seen at the posterior end (arrow) and the two ovaries are visible in the mid-third of the body (arrows). **c.** Swimming specimen of *Xenoturbella bocki* from the Gullmar fjord. **d.** Current phylogenetic hypothesis for Xenacoelomorpha where Xenoturbella is the sister taxon of Acoela and Nemertodermatida, which together form Acoelomorpha

sperm morphology and embryonic development. Even greater confusion pertained to *Xenoturbella*, which differs considerably from a flatworm in its morphology with larger size, rugby ball-like body shape, lack of defined gonads and epidermal nervous system. Its classification within Platyhelminthes was soon questioned; there has been an extraordinary array of different morphology-based phylogenetic hypotheses placing *Xenoturbella* close to Enteropneusta due to their epidermal histology and the morphology of the statocyst (Reisinger 1960), within Acoelomorpha on account of specialisations of its ciliary ultrastructure (Franzen and Afzelius 1987), as sister group to all other Bilateria based on epithelial ultrastructure (Ehlers 1985), and within Mollusca owing to similarities in oocyte morphology (Israelsson 1997).

The first molecular phylogenetic study that tested the position of Acoela in metazoan phylogeny and attempted to control for bias in phylogeny reconstruction potentially caused by among-lineage heterogeneity of substitution rates (so-called long branch attraction) was based on sequences from the 18S rRNA gene. A sister-group relationship between Acoela and Nephrozoa (Bilateria excluding Acoelomorpha and *Xenoturbella*) was supported in this study (Ruiz-Trillo et al. 1999). Subsequent analyses corroborated this result and added data on Nemertodermatida as well as sequences from other genes consistently demonstrating that the two taxa are not part of Platyhelminthes. In early studies, where much of the signal was derived from 18S and 28S rRNA genes, Acoela and Nemertodermatida did not form a monophyletic group but placed at the first and second split within Bilateria (Jondelius et al. 2002; Ruiz-Trillo et al. 2002; Telford et al. 2003), but subsequent phylogenomic studies supported a monophyletic Acoelomorpha, e.g. (Hejnol et al. 2009; Cannon et al. 2016, Rouse et al. 2016).

*Xenoturbella* initially proved difficult to place phylogenetically also when the first sequence data became available. In the first instance, a close relationship with molluscs was suggested (Noren and Jondelius 1997), but the first sequences from the cytochrome oxidase I mitochondrial gene (COI) were likely a food contamination from protobranch bivalves and analyses of new COI and 18S sequence data supported a position within deuterostomes as sister group to Ambulacraria (Bourlat et al. 2003).

A comprehensive phylogenomic study of metazoan groups supported a topology where Acoelomorpha and *Xenoturbella* formed the sister group to Nephrozoa (Hejnol et al. 2009). Re-analysis of data from Hejnol et al. by Philippe et al. (2011) using the CAT substitution model allowing for across sites rate heterogeneity under a Bayesian framework did not recover the Acoelomorpha and *Xenoturbella* as sister group to Nephrozoa, but instead placed the two former close to or within deuterostomes with varying degrees of statistical support depending on the taxonomic composition of the data set. Philippe et al. (2011) suggested that previous studies had been affected by long branch attraction, which, they claimed, was overcome by the use of the CAT model in their study. They also proposed the name Xenacoelomorpha for the group *Xenoturbella* + Acoelomorpha. Cannon et al. (2016) added more data from acoelomorphs and made efforts to control for long branch attraction through analysis of a number of data sets with differing taxonomic composition under the CAT substitution model. Cannon et al. found strong support for a sister-group relationship between Xenacoelomorpha and Nephrozoa and proposed that missing data

and misleading signal from ribosomal protein genes in Philippe et al. (2011) caused Xenacoelomorpha to associate with deuterostomes in that study (for a discussion about ribosomal protein genes in deep animal phylogeny see: (Bleidorn et al. 2009)). The sister-group relationship between Xenacoelomorpha and Nephrozoa provides a parsimonious explanation for the lack of excretory organs, through gut and body cavity in Xenacoelomorpha; these features evolved within the Nephrozoan stem line. As noted by Philippe et al. (2011), a placing of Xenacoelomorpha within deuterostomes requires numerous ad hoc hypotheses to explain the absence of these features as well as specific deuterostome characters such as gill slits and endostylar tissue.

An early classification of Acoela in the two families Proporidae (acoels with one gonopore) and Convolutidae (acoels with two gonopores) was proposed by von Graff (1905, 1911) who also elevated Acoela to the rank of subclass within Platyhelminthes. Einar Westblad studied the Swedish acoel fauna in a series of papers where he gave detailed accounts of their anatomy and proposed a comprehensive classification of Acoela (Westblad 1940, 1942, 1945, 1946, 1948). Westblad's system, which comprised four additional families, was based on the histology of the gonads, presence or absence of female copulatory organs and the position and anatomy of the male copulatory organ. A major transformation of acoel taxonomy was carried out by Dörjes (1968) who described a large number of new species and introducing six new families and numerous new genera. None of the above classifications were based on explicit phylogenetic hypotheses. In more recent years, Hooge (2001) emphasized the configuration of the body wall musculature as phylogenetically informative and introduced four new families based on features of the body wall musculature and morphology of the male copulatory organ.

Jondelius et al. (2011) used ribosomal and mitochondrial nucleotide sequences in combination with morphological characters to reconstruct phylogeny and character evolution of Acoela and provide a phylogenetic classification of the group down to "family" level. They found a basal split in the acoel tree between Diopisthoporidae with a single hermaphrodite gland and Bitesticulata with paired or follicular testes. The latter group comprises fifteen "family level" taxa. Acoela are relatively easily recognized from their general habitus (cylindrical or drop-shaped, sometimes flattened, ciliated, having no gut lumen and no anus, with obvious statocyst in the anterior and clear copulatory organs in the posterior; all visible in a simple dissecting microscope), and the group is monophyletic in all molecular analyses. In spite of this it is difficult to point to an easily discernible unique acoel feature. The presence of a digestive parenchyma in lieu of an intestinal lumen and a particular type of biflagellate spermatozoa are morphological synapomorphies at the histological and ultrastructural levels. Their position as part of the sister group of Nephrozoa paired with their relative morphological simplicity renders acoels important for understanding the evolution of bilaterian complexity. Of particular interest is the reconstruction of ancestral features of acoels and of Xenacoelomorpha. Among the most studied acoels are several species of *Convolutriloba*, large acoels that are able to reproduce asexually through budding and that harbour symbiotic algae. The morphology of the *Convolutriloba* species was shown by Jondelius et al. (2011) to be highly divergent

from the ancestral acoel. Hence, caution is advised when drawing conclusions about acoel ancestral features based on *Convolutriloba*.

Nemertodermatida comprise 18 nominal species, far fewer than Acoela, and most of them are known from a small number of specimens (Sterrer 1998; Meyer-Wachsmuth et al. 2014). Nemertodermatids are easily recognized by the statocyst that contains two statoliths. Phylogenetic reconstruction based on the ribosomal 18S and 28S genes and the nuclear Histone H3 demonstrated a basal split between Ascopariidae and Nemertodermatidae with the symbiotic *Meara stichopi* as sister to *Sterreria* within Nemertodermatidae (Meyer-Wachsmuth and Jondelius 2016). Species delimitation studies using nucleotide sequence data revealed a high incidence of cryptic species in the nemertodermatid genera *Sterreria* and *Nemertinoides* (Meyer-Wachsmuth et al. 2014). This suggests that global diversity of nemertodermatids may be much higher than previously understood. There is no study analysing a large number of specimens of a nominal acoel species to detect cryptic species, but K  nneby et al. (2015) found three new species of *Archaphanostoma* with limited distribution ranges when sampling on the Swedish west coast where the acoelomorph fauna is comparatively well studied. Data mining of metabarcoding sequences from pelagial and deep benthic habitats demonstrated the presence of new species and even potentially early branching groups of acoels (Arroyo et al. 2016). Thus, there are strong indications of extensive unknown acoel diversity in the oceans.

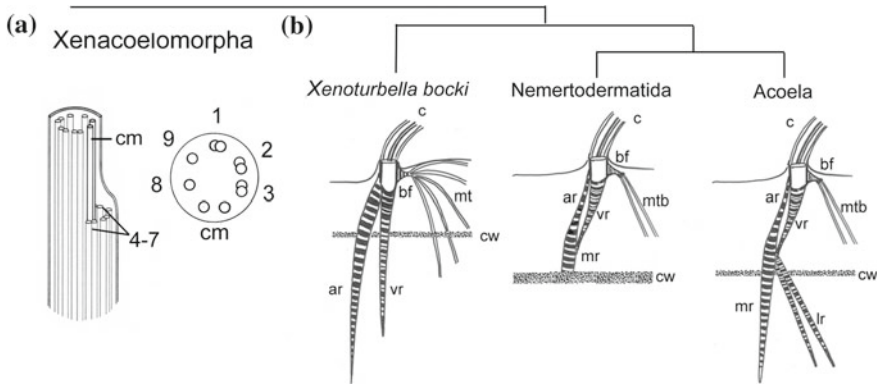
In summary, knowledge of acoelomorph fauna is fragmentary with a strong bias towards the Scandinavian coasts, the North Sea area, western Mediterranean, Parts of the North American east coast and the Sao Paulo area. The actual number of acoelomorph species is likely to be orders of magnitude larger than the current number of nominal species.

## 14.3 Morphological Characters of the Xenacoelomorpha

Herein we give a brief review of the main features of xenacoelomorph morphology and discuss the evolution of the various character states. As starting points, we have taken two recent excellent reviews of the current data published by Achatz et al. (2013) and Hazsprunar (2015), which has provided tables of references to phenotypic studies within the Xenacoelomorpha, on gene expression methodologies and molecular data.

### 14.3.1 Epidermis

Xenacoelomorpha share a multiciliary epidermis without accessory centrioles (Ehlers 1985). Cilia in all three groups of Xenacoelomorpha have unique thinned ciliary tips considered a synapomorphy of the group (for details see Fig. 14.2a and

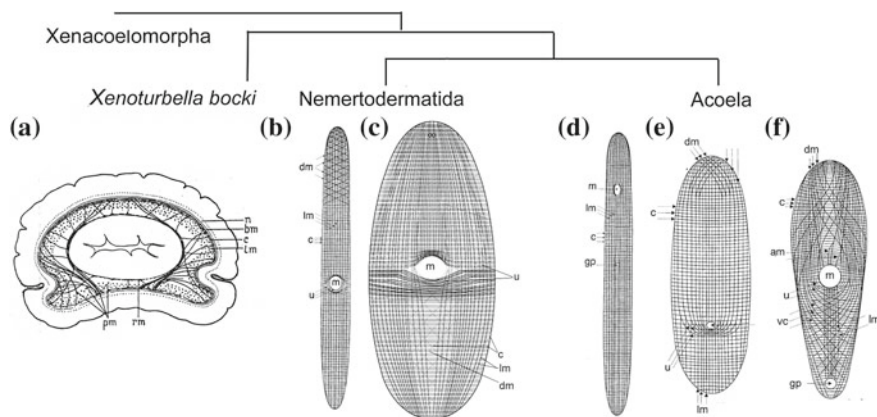


**Fig. 14.2** **a** Schematic representation of synapomorphic for Xenacoelomorpha thinned ciliary tips with a distinctive shelf where the doublets 4–7 terminate and central microtubules (cm) continue in a peripheral position (Tyler 1979; Ehlers 1985; Smith and Tyler 1985a, b; Franzen and Afzelius 1987; Lundin 1997, 1998). **b** Hypothesis of rootlet system evolution within Xenacoelomorpha, from Raikova (1991) with modifications. In *Xenoturbella bocki* (Franzen and Afzelius 1987) cilia (c) have two rootlets, anterior (ar) and vertical (vr) both running almost vertically down. The basal body has a well-developed basal foot (bf) with microtubules (mt) fanning from it. The rootlets are interconnected on a single level by a horizontal fibrous cell web (cw). In *Nemertodermatida* (Tyler and Rieger 1977) each cilium has an anteriorly directed main rootlet (mr) that forms a knee-like bend and continues vertically down. The main rootlet is bipartite in its upper part, supposedly formed by two fused rootlets: anterior and vertical. The basal foot is linked by microtubule bundles (mtb) with the main rootlets of the two posteriorly adjacent cilia, thus forming an upper interconnection of the rootlet grid. The rootlets are also interconnected by cell web. In *Acoela* (Dorey 1965; Hendelberg and Hedlund 1974; Tyler 1979; Ehlers 1985; Rohde et al. 1988), in addition to the described pattern, there are paired lateral rootlets (lr), linked to the main rootlets of two posteriorly adjacent cilia, forming the lower interconnection of the grid

ref. in the figure caption). Epidermal cilia are anchored within the cytoplasm of epidermal cells by ciliary rootlets (for details see Fig. 14.2b and ref. therein). Cilia in *Xenoturbella bocki* have two almost vertical rootlets interconnected only by fibrous cell web. Acoelomorphs have a single main rootlet, bipartite in its proximal part, likely formed by fusion of the two rootlets found in *X. bocki* (Raikova 1991). In Acoelomorpha, rootlet system reaches great complexity with rootlets interconnected at two (in Nemertodermatida) or at three levels (in Acoela).

### 14.3.2 Musculature of the Body Wall and Pharynges

Changes in the muscular system pattern were shown to be phylogenetically informative especially in Acoelomorpha (Hooze 2001; Tyler and Hooze 2004; Jondelius et al. 2011; Meyer-Wachsmuth et al. 2013). *Xenoturbella bocki* has only two muscle layers in the body wall: outer circular and inner longitudinal (for details see Fig. 14.3 and ref. in the figure caption). Such orthogonal muscle grid is presumably



**Fig. 14.3** Patterns of body wall musculature in Xenacoelomorpha. **a** *Xenoturbella bocki*, transverse section, from Westblad 1949b. The body wall is composed of an orthogonal grid of outer circular muscles and inner longitudinal muscles (Westblad 1949b; Sopott-Ehlers and Ehlers 1997; Raikova et al. 2000a). **b–f**—Schemes of arrangement of ventral body wall musculature from Hooge 2001 (**b**; **d–f**) and Meyer-Wachsmuth et al. 2013 **c**. Within Nemertodermatida, *Sterreria psammicola* **b** has mostly orthogonal body wall with few anterior diagonal muscles (Hooge 2001), while *Meara stichopi* **c** has three-layered body wall musculature with diagonal muscles and additional U-shaped muscles around the mouth. Among lower acoels, representatives of early emerging clades Solenofilomorphidae and *Proporus* cf. *lonchitus* **d** have only an orthogonal muscle grid (Hooge 2001); Paratomellidae **e** have few anterior diagonal muscles and U-shaped around the mouth (Hooge 2001). The more derived groups of Acoela (Crucimusculata) have very complex musculature with cross-over muscles, especially in the most derived Mecynostomidae **f** (Hooge 2001). **Abbreviations:** am—accessory muscles; bm—basal lamina; c—circular muscles; dm—diagonal muscles; gp—gonopore; lm—longitudinal muscles; m—mouth; n—nerve net; pm—parenchymal muscles; rm—reticular muscles of the gut; u—U-shaped muscles; vc—ventral cross-over muscles

plesiomorphic for Bilateria (Hooge 2001; Schmidt-Rhaesa 2007). Development of more complex muscle patterns likely took place independently within Acoela and Nemertodermatida, as in each of these taxa cases of plesiomorphic orthogonal grid occur in early emerging clades, while the more derived ones have quite complex muscular patterns (for details see Fig. 14.3 and references therein). There is no evidence of secondary simplification of muscles in some lineages and it seems highly improbable in free-living predators. Parasitic species (like *Meara stichopi*) show even more complex muscle patterns than their free-living relatives (Raikova et al. 2016). Pharynges are absent in *Xenoturbella* and nemertodermatids, but present in many early and mid-level acoel groups (Diopisthoporidae, Proporidae, Hofsteniidae, Solenofilomorphidae, Isodiametridae), where their presence compensates for the absence of cross-over muscles (Hooge 2001; Jondelius et al. 2011). Todt (2009) has demonstrated that different versions of pharynx simplex differ considerably in muscle patterns and speculated that they likely had developed independently.

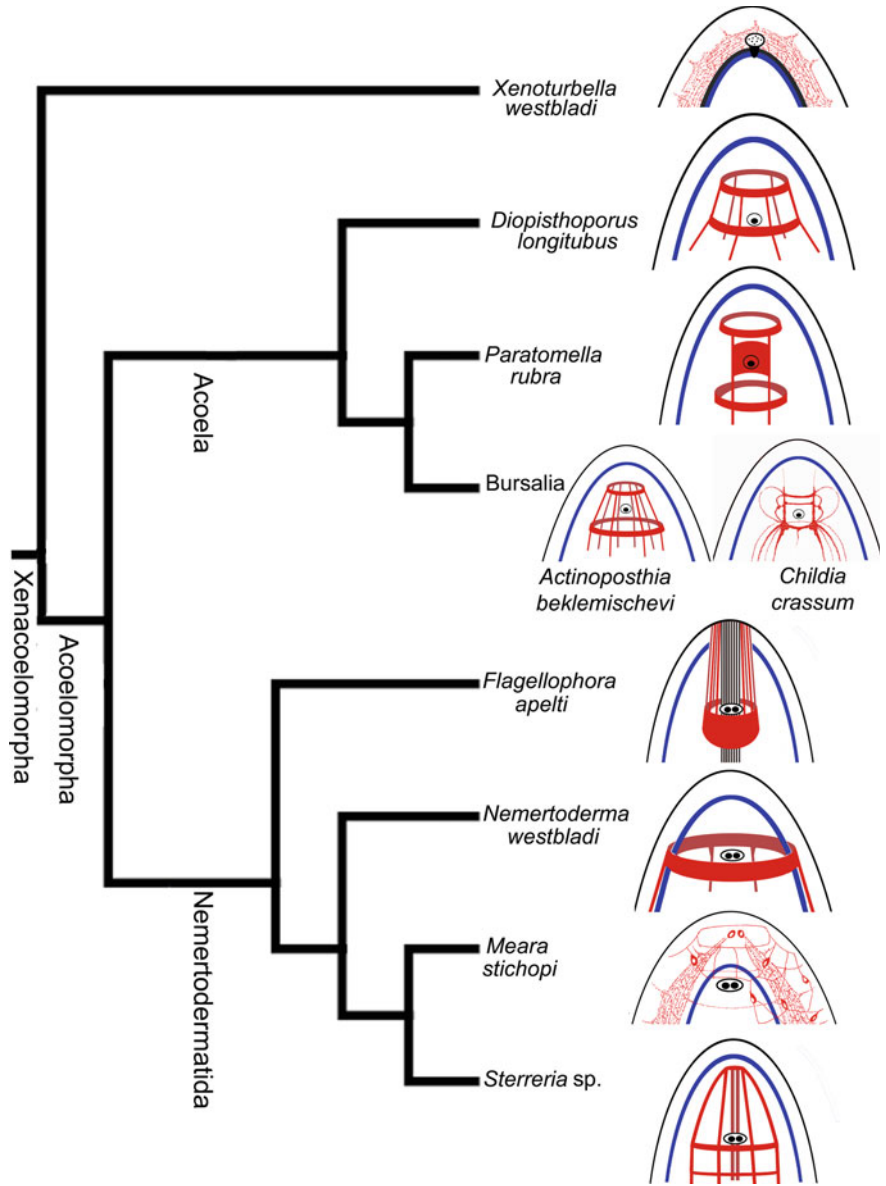
### 14.3.3 Glands

Glands in Xenacoelomorpha are diverse and abundant, especially those producing mucus for mucociliary gliding. Mucous glands form the newly described ventral glandular network in *Xenoturbella* species (Rouse et al. 2016; Nakano et al. 2017). In Nemertodermatida numerous mucous glands are concentrated on the frontal pole of the animal opening separately, which is interpreted as a plesiomorphic condition (Smith and Tyler 1988; Ehlers 1992c). The same condition occurs in the early emerging taxa of Acoela: *Diopisthoporus*, (Smith and Tyler 1985a, b), *Paratomella* (Ehlers 1992a, b, c) and *Afronta* (Proporidae) (Rieger et al. 1991). In the rest of acoels, the glandular ducts of the frontal glands discharge together through a common pore at the anterior pole of the body forming the “frontal organ” (Smith and Tyler 1985a, b; Smith et al. 1986; Klauser et al. 1986; Ehlers 1992b, c).

### 14.3.4 Receptors

Sensillae are bipolar or multipolar nerve cells with dendrites bearing one or rarely 2 and more cilia and sometimes, in “collar receptors”, a collar of thickened microvilli, called “stereocilia”. Sensillae were studied by electron microscopy mostly in acoels and classified on morphological basis (Bedini et al. 1973; Todt and Tyler 2006; Bery et al. 2010; Zabotin 2019). In acoels up to five different types of sensillae are usually present in various combinations. The simplest receptor type, likely plesiomorphic, is that with a long and slender ciliary rootlet—and only this type was so far described in *Xenoturbella* (Raikova et al. 2000a). It is also present in Nemertodermatida (Smith and Tyler 1988; Ehlers 1992c). Collar receptors are completely lacking in *Xenoturbellida*, Nemertodermatida or in lower acoels, suggesting an independent origin of collar receptors within the acoels and in other invertebrates (Todt and Tyler 2006). Eyes. *Xenoturbellida* and Nemertodermatida are devoid of eyes. Among acoels, epidermal eyespots occur in some Isodiametridae (Lanfranchi 1990), and very simple eyes are described in Convolutidae (Popova and Mamkaev 1985; Yamasu 1991). These structures show quite different organization and likely evolved independently in originally eyeless Acoela.

Statocysts. Representatives of all three groups of Xenacoelomorpha have anteriorly situated statocysts thoroughly described and discussed by Ehlers (1991). In *Xenoturbella bocki*, the statocyst is intraepidermal; it lies at the anterior pole anchored in the basal lamina and surrounded by thick basiepidermal nerve net. Acoelomorph statocysts are submuscular and lie within the brain, more specifically surrounded by statocyst ganglion. All xenacoelomorph statocysts have a capsule composed of extracellular matrix and inlaid on the inside by parietal cells. Inside the capsule, there are freely movable lithocyte cells with intracellular statoliths. In *X. bocki*, the lithocytes are numerous and have flagella, as do the parietal cells (Ehlers 1991). In Nemertodermatida, there are always two lithocytes (Fig. 14.1a), and a few parietal



◀**Fig. 14.4** Schematic drawings of the organization of the nervous system (NS) and its position with respect to the body wall musculature (BWM) in Xenacoelomorpha. For all species only the anterior part is shown, statocysts and broom organ (*Flagellophora*) are shown for morphological context. The NS is shown in red, the layers of BWM in blue. *Xenoturbella* possesses the hypothesized ancestral basiepidermal nerve net without anterior centralization. In Nemertodermatida, the NS is diverse and does not show a trend from an epidermal nerve net towards an insunk centralized lobed brain. It lies outside the BWM in *N. westbladi* and *M. stichopi* and inside the BWM in *F. cf. apelti* and *Sterreria* sp. *Meara stichopi* has no brain, but only a pair of basiepidermal lateral neurite bundles. *Nemertoderma westbladi* has a basiepidermal brain ring and a pair of ventrolateral neurite bundles. *Sterreria* sp. shows a commissural-like brain similar to that observed in Acoela and several neurite bundles going in frontal and caudal directions. *Flagellophora apelti* has a neuropile, located at the level of the statocyst, providing innervation for the broom organ. In Acoela, the NS is mostly submuscular with anterior centralisations of different degrees ranging from two rings in basal taxa to more complex structures with several commissures and paired peptidergic marker neurons. Figure adapted from Achatz and Martinez (2012). Nemertodermatid phylogeny is based on Meyer-Wachsmuth and Jondelius (2016), that of Acoela on Jondelius et al. (2011). Morphological information based on the following studies: *Xenoturbella westbladi* (Westblad 1949b; Raikova et al. 2000a); *Diopisthoporus longitubus* (Raikova 2004); *Paratomella rubra* (Crezée 1978); *Actinoposthia beklemischevi*: (Raikova et al. 1998; Raikova 2004); *Childia crassum* (Reuter et al. 2001a, b; Raikova et al. 2004b); *Nemertoderma westbladi* (Raikova et al. 2004a and Raikova et al. 2016); *Meara stichopi* (Raikova et al. 2000b; Børve and Hejnol 2014), *Flagellophora cf. apelti* and *Sterreria* spp. (Raikova et al. 2016)

cells with ventral nuclei; while in Acoela there is always one single lithocyte and two parietal cells with dorsal nuclei. No flagella are encountered within the statocysts of Acoelomorpha (Ferrero 1973; Ehlers 1985, 1991). Ehlers (1991) proves that statocysts of Xenacoelomorpha are non-homologous neither between each other, nor with those found in Platyhelminthes.

### 14.3.5 Nervous System

The nervous system (NS) patterns in xenacoelomorphs are extensively studied (see list of references in Hazsprunar (2015), or Martinez et al. (2017) and in the more recent of Dittmann et al. (2018). Here we give a brief summary of the currently available data (for details see Fig. 14.4 and ref. therein).

*Xenoturbella bocki* has a very simple NS, consisting solely of a basiepidermal nerve net lying above the thick basal lamina with no brain detected. Nemertodermatids have a very plastic NS, either basiepidermal or submuscular with or without a brain (for details see Fig. 14.4). Acoels show a considerable variety in the details of NS patterns (for details see Fig. 14.4), but some common features can be found. According to Raikova (2004), acoel brain has two parts: (1) a 5-HT- or RF-amide immunoreactive “commissural brain” (Raikova et al. 1998) more often composed of one to several rings, with paired longitudinal cords (or neurite bundles according to terminology proposed by Richter et al. 2010) starting caudal from it and (2) a

ganglion surrounding the statocyst. Unlike the commissural brain, the statocyst ganglion shows no 5-HT- or RF-amide like immunoreactivity; therefore, it is considered non-homologous with “true” cerebral ganglia of Nephrozoa and a synapomorphy of Acoelomorpha. Martinez et al (2017) disputed the use of “commissural brain” terminology, at least for the most recent clades of Acoela, due to the presence of a compact brain in convolutids, with a periphery occupied by the cell bodies and a dense internal neuropile. The acoel NS is currently being extensively studied in species of Convolutidae (Gaerber et al. 2007; Semmler et al. 2010; Bery et al. 2010; Bery and Martínez 2011; Gavilán et al. 2016; Martinez et al. 2017) or Isodiametridae (Achatz and Martinez 2012; Dittmann et al. 2018) as model animals. Moreover, the acoel *S. roscoffensis* is nowadays used to test the functionality of the NS given the fact that adult worms are able to regenerate a whole head from scratch (Sprecher et al. 2015; Arboleda et al. 2018).

The earliest bilaterian NS is thought to consist of a diffuse subepidermal nerve net similar to that of cnidarians (i.e. Gröger and Schmid 2001). Reisinger (1925) proposed that in the course of evolution nerve elements become more concentrated and the NS sinks below the muscle layers. According to this hypothesis, the basiepidermal NS of *Xenoturbella*, *Meara* and *Nemertoderma* would represent the ancestral state, while the submuscular brains of nemertodermatids and acoels would be apomorphic (Westblad 1948; Rieger et al. 1991). Under this hypothesis, a submuscular nervous system evolved multiple times independently in Acoelomorpha (Raikova et al. 2016).

A common for most animals stomatogastric nervous system is absent in all xenacoelomorphs (Westblad 1937, 1949b; Raikova et al. 2000a, b; Raikova 2004). It seems to be a symplesiomorphy of these groups, as a loss of stomatogastric system in primitively predatory organisms is unlikely (Haszprunar 2015; Gavilán et al. 2019).

### 14.3.6 Digestive System

Mouth is situated mid-ventrally in *Xenoturbella* and nemertodermatids while in acoels mouth position shows great variability. Most commonly it is situated mid-ventrally, sometimes subfrontally, but in the basalmost acoel taxon Diopisthoporidae the mouth opens at the posterior end. Beklemishev (1963) pointed out that the mouth in acoels was homologous to blastopore which makes its posterior position primitive (plesiomorphic). *Xenoturbella* and nemertodermatids have a saccular blind gut with amoeboid gastrodermis (Westblad 1949b, 1949a) that seems to be plesiomorphic for Xenacoelomorpha. Some acoels have cellular amoeboid digestive parenchyma (Smith and Tyler 1985a, b; Ehlers 1992b), but most often there is a central digestive syncytium (Rieger et al. 1991). For a recent review on the digestive system of xenacoelomorphs, check Gavilán and collaborators recent review (Gavilán et al. 2019).

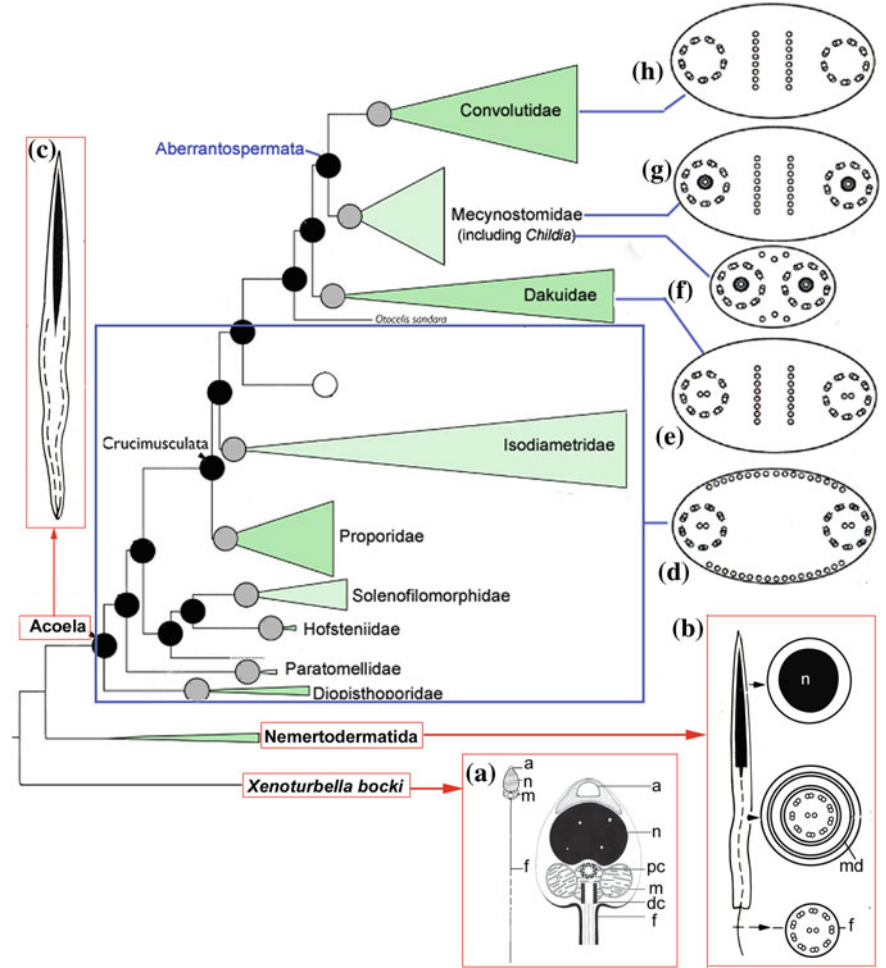
### 14.3.7 Reproductive Organs

The Acoelomorpha are simultaneous or protandric hermaphrodites suggesting hermaphroditic Urbilateria as likely (Haszprunar 2015). *Xenoturbella* lacks copulatory organs entirely (Westblad 1949b). Nemertodermatids have only a seminal vesicle opening subterminally. Acoels display a striking variety of copulatory organs. Most are not yet sufficiently investigated to be properly classified, especially the copulatory stylets (Tekle et al. 2007a, b; Achatz et al. 2013).

The agonadal condition with free eggs and sperm found in *Xenoturbella* and in several taxa of Acoelomorpha is presumably plesiomorphic (Rieger et al. 1991). The oocytes in *Xenoturbella* and Acoelomorpha are entolecithal with non-sclerotized egg envelope which seem to be plesiomorphic features (Falleni et al. 1995; Raikova et al. 1995 and ref. therein).

The three taxa of Xenacoelomorpha radically differ by their sperm structure. *Xenoturbella bocki* (Fig. 14.5a) has a primitive monoflagellate sperm with a roundish head and a free  $9 + 2$  flagellum that reflects ectaquatic fertilization (Obst et al. 2011). Nemertodermatida (Fig. 14.5b) have sperm with a single  $9 + 2$  flagellum starting from the nucleus, a condition clearly plesiomorphic for Acoelomorpha (Tyler and Rieger 1975). However, nemertodermatid sperm is filiform and highly modified in order to penetrate tissues (introsperm). Acoels (Fig. 14.5c) have aberrant filiform biflagellate sperm with incorporated flagella in inverted position and supporting microtubules (cortical or axial) (Hendelberg 1969, 1977, 1986). In Acoela, three configurations of sperm axonemes are described:  $9 + 2$ ;  $9 + 0$  and  $9 + 1$  (for details see Fig. 14.5d–h and ref. therein). It should be emphasized that the aberrant  $9 + 1$  configuration where only one central microtubule remains surrounded by a halo of electron dense material (Tekle et al. 2007a, b), found in acoel family Mecynostomidae, is non-homologous with  $9 + 1$  configuration in flatworm taxon Trepaxonemata, as the central cylinder (“1”) in Trepaxonemata does not contain tubulin (Iomini et al. 1995). Sperm characters in Acoela proved to be those most concordant with molecular phylogeny (Petrov et al. 2004; Jondelius et al. 2011); thus, they were extensively studied (see references in Haszprunar (2015)).

Recently data on the sperm of Xenacoelomorpha have been re-evaluated by Buckland-Nicks et al. (2018). The authors propose a very interesting hypothesis that, assuming Xenacoelomorph monophyly and ancestral introsperm in this taxon, the re-expression of the aquasperm form could have happened in *Xenoturbella* as a secondarily derived state through “progenetic spermiogenesis” with the precocious development of round spermatids to maturity.



◀**Fig. 14.5** Schematic drawings of the sperm morphology in Xenacoelomorpha. **a** *Xenoturbella bocki*. Primitive monoflagellate sperm in with a roundish head and a free 9 + 2 flagellum after Westblad (1949a) and Obst et al. (2011). **b** Nemertodermatida modified filiform monoflagellate sperm with long proximal nucleus, mitochondrial derivatives and supporting microtubules, after Hendelberg (1986), Lundin and Hendelberg (1998), Boone et al. (2011), Buckland-Nicks et al. (2018). **c**. *Acoela*. Aberrant filiform biflagellate sperm with incorporated flagella in inverted position (with basal bodies at the distal end of the cell), after Hendelberg (1986). **d–h** Cross sections of the sperm shaft in different acoel groups showing axoneme configuration and the arrangement of cytoplasmic microtubules. **d** Plesiomorphic axoneme configuration with nine peripheral microtubule doublets and two central microtubules (9 + 2), characteristic for all basal and mid-level taxa of the Acoela (Petrov et al. 2004; Jondelius et al. 2011). Cortical (positioned under the plasma membrane) cytoplasmic microtubules are also plesiomorphic. **e**. Sperm with 9 + 2 axonemes and axial (running along the main axis of the sperm) cytoplasmic microtubules, described in *Philactinoposthia saliens* (Raikova and Justine 1999), *Philocelis brueggemanni* and *Daku woorimensis* (Petrov et al. 2004). **f**. Sperm in *Childia* spp. (Mecynostomidae) with 9 + 1 axonemes and distal microtubules found only in the distal region of the sperm (Tekle et al. 2007a, b). **g**. Sperm in other Mecynostomidae with 9 + 1 axonemes and axial microtubules (Petrov et al. 2004; Tekle et al. 2007a, b). **h**. Sperm in Convolutidae with 9 + 0 axonemes and axial microtubules. Note that Mecynostomidae and Convolutidae due to their aberrant sperm axonemes are united into AberrantospERMata group. The phylogenetic tree used is based on Jondelius et al. (2011) and Hejnol et al. (2009), but simplified. Abbreviations: a—acrosome; a—flagellum; dc—distal centriole; m—mitochondrion; md—mitochondrial derivative; n—nucleus; pc—proximal centriole

## 14.4 Molecular Regulation in Xenacoelomorphs

The characterization of xenacoelomorph genomes is still in its infancy. Our current knowledge derives, fundamentally, from the isolation of single genes using classical PCR screens. However, and mostly owing to the needs of having large data sets for phylogenomic analysis, several transcriptomes have been obtained over the last number of years. This provides a very rich source of data for downstream characterization of specific genes/gene families, though at present, the data is, with this purpose, very seldom used. Moreover, we have generated data that represents a wide array of species, most of which are unsuitable for developmental studies given the paucity with which they are found and the undeveloped systems for their culture. Most developmental studies have been focused on just a few species, for which we are starting to gather some critical data on the use of regulatory genes and certain families of differentiation markers.

### 14.4.1 *Xenacolomorph Genomes and Transcriptomes*

Before proceeding further, let us recapitulate what has been learned in terms of the molecular composition of different xenacoelomorph genomes and transcriptomes. Table 14.1 provides a comprehensive list of our current knowledge.

**Table 14.1** Summary of the current genomic and transcriptomic resources available for different xenacoelomorph species

| Species                               | Family           | Data source                            | References                                          |
|---------------------------------------|------------------|----------------------------------------|-----------------------------------------------------|
| <i>Acoela</i>                         |                  |                                        |                                                     |
| <i>Childia submaculatum</i>           | Mecynostomidae   | Transcriptome                          | Cannon et al. (2016)                                |
| <i>Convolutiloba macropyga</i>        | Convolutidae     | Transcriptome                          | Cannon et al. (2016)                                |
| <i>Diopisthoporus gymnopharyngeus</i> | Diopisthoporidae | Transcriptome                          | Cannon et al. (2016)                                |
| <i>Diopisthoporus longitubus</i>      | Diopisthoporidae | Transcriptome                          | Cannon et al. (2016)                                |
| <i>Eumecynostomum macrobursarium</i>  | Mecynostomidae   | Transcriptome                          | Cannon et al. (2016)                                |
| <i>Hofstenia miamia</i>               | Hofsteniidae     | Transcriptome                          | Cannon et al. (2016)                                |
| <i>Isodiametra pulchra</i>            | Isodiametridae   | Transcriptome                          | Cannon et al. (2016)                                |
| <i>Symsagittifera roscoffensis</i>    | Convolutidae     | Mitochondrial genome                   | Mwinyi et al. (2010)                                |
| <i>Isodiametra pulchra</i>            | Isodiametridae   | Mitochondrial genome                   | Robertson et al. (2017)                             |
| <i>Paratomella rubra</i>              | Paratomellidae   | Mitochondrial genome (partial)         | Ruiz-Trillo et al. (2004)                           |
| <i>Paratomella rubra</i>              | Paratomellidae   | Mitochondrial genome                   | Robertson et al. (2017)                             |
| <i>Archaphanostoma ylvae</i>          | Isodiametridae   | Mitochondrial genome                   | Robertson et al. (2017)                             |
| <i>Symsagittifera roscoffensis</i>    | Convolutidae     | Transcriptome (mix stage embryos)      | Philippe et al. (2019), Perea-Atienza et al. (2015) |
| <i>Symsagittifera roscoffensis</i>    | Convolutidae     | Draft genome                           | Philippe et al. (2019), Perea-Atienza et al. (2015) |
| <i>Hofstenia miamia</i>               | Hofsteniidae     | Transcriptome (embryos + reg. animals) | Srivastava et al. (2014)                            |
| <i>Isodiametra pulchra</i>            | Isodiametridae   | Transcriptome (adults)                 | Brauchle et al. (2018)                              |
| <i>Symsagittifera roscoffensis</i>    | Convolutidae     | Transcriptome (juveniles)              | Brauchle et al. (2018)                              |
| <i>Hofstenia miamia</i>               | Hofsteniidae     | Genome (complete)                      | Gehrke et al. (2019)                                |
| <i>Hofstenia miamia</i>               | Hofsteniidae     | Transcriptome (adults)                 | Gehrke et al. (2019)                                |
| <i>Praesagittifera naikaiensis</i>    | Convolutidae     | Genome (complete)                      | Arimoto et al. (2019)                               |

(continued)

**Table 14.1** (continued)

| Species                  | Family            | Data source                    | References                                          |
|--------------------------|-------------------|--------------------------------|-----------------------------------------------------|
| <i>Nemertodermatida</i>  |                   |                                |                                                     |
| Ascoparia sp.            | Nemertodermatidae | Transcriptome                  | Cannon et al. (2016)                                |
| Meara stichopi           | Nemertodermatidae | Transcriptome                  | Cannon et al. (2016)                                |
| Nemertoderma westbladi   | Nemertodermatidae | Transcriptome                  | Cannon et al. (2016)                                |
| Sterria sp.              | Nemertodermatidae | Transcriptome                  | Cannon et al. (2016)                                |
| Nemertoderma westbladi   | Nemertodermatidae | Mitochondrial genome (partial) | Ruiz-Trillo et al. (2004)                           |
| <i>Xenoturbellida</i>    |                   |                                |                                                     |
| Xenoturbella bocki       | Xenoturbellidae   | Transcriptome (adults)         | Cannon et al. (2016)                                |
| Xenoturbella profunda    | Xenoturbellidae   | Transcriptome (adults)         | Rouse et al. (2016)                                 |
| Xenoturbella monstrosa   | Xenoturbellidae   | Mitochondrial genome           | Rouse et al. (2016)                                 |
| Xenoturbella hollandorum | Xenoturbellidae   | Mitochondrial genome           | Rouse et al. (2016)                                 |
| Xenoturbella churro      | Xenoturbellidae   | Mitochondrial genome           | Rouse et al. (2016)                                 |
| Xenoturbella bocki       | Xenoturbellidae   | Draft genome                   | Philippe et al. (2019); Perea-Atienza et al. (2015) |
| Xenoturbella bocki       | Xenoturbellidae   | Transcriptome (adults)         | Brauchle et al. (2018)                              |

As mentioned before, the first characterization of xenacoelomorph genes was derived from the need for obtaining molecular markers to infer phylogenetic relationships, whether they were for understanding the position of different Xenacoelomorpha clades within the Metazoa or inferring the internal phylogeny of those clades. In this context, it was, historically, natural that cloning ribosomal (i.e. 18S) and mitochondrial genes were taken as a priority (see the earlier sections for details). The advent of powerful phylogenomic methodologies prompted the use of transcriptomic and genomic data for the inference of clade relationships. With this in mind, the studies of Hejnol et al. (2009), Philippe et al. (2011) and Cannon et al. (2016) were pioneers (see Sect. 2.1 for more details). They used, mostly Cannon and collaborators, large sets of xenacoelomorph transcriptomes, including a wide range of species from various xenacoelomorph families. The species used in these studies are all found in Table 14.1, which summarizes all the species for which there is genomic or transcriptomic data.

Very little is known about the structure and composition of xenacoelomorph genomes. Only two complete genomes, with long scaffolds, have been produced,

that of the early divergent acoel *Hofstenia miamia* (Gehrke et al. 2019) and that of the more recently diverged species *Praesagittifera naikaiensis* (Arimoto et al. 2019). *H. miamia* has a genome with a size of 950 Mb, encoding for (predicted and supported by transcriptome data) 22,632 genes in an environment of 53% of repetitive sequences, mostly derived from transposons. *P. naikaiensis* genome is a bit smaller, approx. 656 Mb, with 70% of the sequence being repetitive elements, though encoding for 22,143 genes (a number supported also by transcriptome data). Other genomic data, in relatively small scaffolds has been obtained for the genomes of the xenoturbellid, *Xenoturbella bocki*, and the acoel, *Symsagittifera roscoffensis* (Philippe et al. 2019). The complete descriptions of those last genomes are, however, still missing. Of the general characteristics, only the sizes (~150 Mb for *X. bocki* and ~1.4 Gb for *S. roscoffensis*) are known, plus the fact that those genomes are full of repetitive elements, accounting for the large size of the *S. roscoffensis* genome, which is nearly half the size of the human genome. Several features of the genome organization of acoels were known, through the use of technologies that include the development of BAC libraries and chromosomal mapping. These were instrumental in understanding that the Hox genes in acoels were dispersed, located on different chromosomes (Moreno et al. 2009). Unfortunately, the potential of these technologies was not explored any further.

In the absence of xenacoelomorph genomes, large-scale analysis of long fragments has been conducted in a few acoelomorphs targeting mitochondrial genomes, shorter gene complexes easier to sequence. Complete or substantial fractions of mitochondrial genomes are known for one nemertodermatid and four acoels (Table 14.1 and: (Ruiz-Trillo et al. 2004; Mwinyi et al. 2010; Robertson et al. 2017). Strikingly, all these reports indicate that the arrangement of genes within these mitochondrial genomes is very different from what has been observed in other metazoans (and with each other), pointing towards a clade-specific set of rearrangements.

Returning to draft genomes, we need to stress that independent of the completeness they represent, in the current state, they have facilitated the characterization of the size and diversity of certain gene families in these two species, for instance, those of the transcriptional regulators, bHLH or Hox, the signalling Wnt family of ligands and the complete set of GPCR receptors (Perea-Atienza et al. 2015; Gavilán et al. 2016). Other families have been characterized through the deep analysis of transcriptomes. In addition to the previous ones (for which we have genomic and transcriptomic data), the whole complement of homeobox-containing families (11 in total) have been identified in *X. bocki* and the two acoels, *S. roscoffensis* and *Isodiametra pulchra* (Brauchle et al. 2018), or the GPCR complements of different xenacoelomorphs (Thiel et al. 2018). In addition, the latter study of transcriptomes carried out by Thiel and colleagues has permitted a comprehensive description of neuropeptide evolution in this phylum.

Before ending, this section is important to emphasize that the phylogenetic position of Xenacoelomorpha allows us to study with detail those genomic novelties associated to the origin and diversification of bilaterian animals and how the activities of those new or lost genes affect the evolutionary transformations of body plans. The important paper of Paps and Holland (2018) describes the particularities of the

birth and loss of gene groups in the metazoans (see their Fig. 14.1 for a graphical view). These authors have shown that the emergence of multicellular animals was accompanied by the incorporation of many genomic novelties (1189 gene homology groups; HG). Other, more recent phylogenetic nodes, such as that representing the last common ancestor of Cnidaria + Bilateria (Planulozoa) and the Bilateria, are also characterized by the incorporation of 1201 and 1580 new HG, respectively. In fact, these nodes are also characterized by some specific HG losses, here 72 and 520. Many of these genes encode for regulatory functions (transcription factors or signalling molecules). Since no comprehensive expression analysis has been done for those specific genes in most metazoan clades, it becomes quite obvious that the genomic underpinnings of the emergence of bilaterian animals (including the early diversification of xenacoelomorphs) are, mostly, a matter of speculation. The study of those regulatory functions should become a major future focus of analysis in the Xenacoelomorpha because they will illuminate many aspects of the origin of bilaterians.

#### 14.4.2 Gene Activity in Xenacoelomorphs

Transcriptomes are ideal sources for the identification of particular genes, avoiding the cumbersome procedure of PCR screens. They can provide sequence information necessary to generate probes for in situ hybridization, thus providing us with entry to viewing particular developmental or physiological processes. The exploitation of transcriptomes for this purpose has been instrumental in identifying genes involved in dorsoventral patterning (Srivastava et al. 2014), mesoderm specification (Chiodin et al. 2013) and stem cell biology (De Mulder et al. 2009) in different acoels or the study of excretion processes in acoelomorphs (Andrikou et al. 2019). All these studies have provided the necessary tools to understand the molecular underpinnings of those processes at a level that was impossible previously using more conventional, gene-by-gene cloning methods (i.e. Ramachandra et al. 2002; Cook et al. 2004; Moreno et al. 2009).

The identification of all those genes plus the development of colorimetric or fluorescent in situ methodologies has supplied a basic understanding of developmental processes. It is true that, in comparison with well-established model systems, our knowledge of xenacoelomorph development, from the molecular point of view, is rather limited. The paucity of functional tools (see the following) and a detailed description of embryogenesis have hampered progress in this area. Nevertheless, it is fair to stress here that in some areas, our knowledge is more detailed than that we have for many other phyla. A clear case would be our characterization of the patterning mechanisms employed along the two major body axes (AP and DV). The genes involved in both processes were of primary interest as it was revealed that acoelomorphs were the first offshoot of Bilateria. It was clear that in order to determine how bilateral animals acquired their specific symmetry, we needed to know

how acoelomorphs specified their orthogonal axis. Hox genes were the primary candidates as they were used as a vectorial system to specify the position of structures along the AP axis across all bilaterians. The study of different acoels uncovered early on that the complement of Hox genes was quite reduced, numbering at only three, one orthologous to each of the so-called Anterior, Central and Posterior classes. Studies of *Convolutriloba longifissura* (Hejnowicz and Martindale 2009) and *Symsagittifera roscoffensis* (Moreno et al. 2009) exhibited a pattern of staggered domains for the three genes along the major body axis, in agreement with what is observed in other bilaterians. The expression of those genes was initiated during embryogenesis, just after gastrulation (Hejnowicz and Martindale 2009) and resolved in the staggered pattern seen in hatchlings and juveniles. In budding specimens of *Convolutriloba retrogemma*, it has also been shown that during the budding process (that involves a reversal of polarity in new animals), expression along the bud also conforms to the expected AP Hox pattern (Sikes and Bely 2010). Interestingly, the staggered pattern is not the result of chromosomal clustering (Moreno et al. 2009), just as in other bilaterian animals. However, it seems clear clustering is not necessary for the establishment of a staggered expression pattern (i.e. Seo et al. 2004). Other regulators of the AP axis are the Wnt ligands, and in the acoel *Hofstenia miamia*, they are used in the same process (Srivastava et al. 2014). In regenerating *Convolutriloba retrogemma*, the chemical inhibition of the Wnt pathway also affects the polarity of the regenerates (Sikes and Bely 2010). The role of Hox and Wnt ligands in the specification of posterior structures has been further explored through the use of RNAi technologies in the acoels, *Isodiametra pulchra* (Moreno et al. 2010) and *Hofstenia miamia* (Srivastava et al. 2014). These represent two of the very few functional studies carried out for any xenacoelomorph; in fact, we only have data obtained for acoels. The DV axis of bilaterian animals is specified through the BMP pathway. In the case of *H. miamia*, the interplay between the ligands, BMP and ADMP, seems to specify the DV axis, as probed through the use of RNAi technology (Srivastava et al. 2014). Additional data on the expression of BMP-ADMP genes has been obtained from the study of the nemertodermatid, *Meara stichopi*, and the acoel, *Isodiametra pulchra* (Martín-Durán et al. 2018). These ligands are also present in other xenacoelomorphs, such as *S. roscoffensis* or in *X. bocki* (Chang et al. 2015), though their expression or function has not been evaluated.

A cautionary note before proceeding all the functional experiments using RNAi carried out in acoels have been conducted in regenerating adults. No embryo RNAi methods have been developed to date.

During animal development, the establishment of the major body axis is followed by a regionalization process followed (or in concert) with the specification of the major tissues. With acoels, for the most part, much effort has been devoted to characterizing the molecular players involved in the specification of tissues, with a major focus placed on understanding mesodermal and neural developmental mechanisms. The mesodermal derivatives in acoels have been studied, fundamentally, in two species—*Isodiametra pulchra* and, to a lesser degree, *Symsagittifera roscoffensis*. While in the latter, the study of the musculature, and its structural components, has been assessed (Chiodin et al. 2011), in the case of *I. pulchra*, there is a relatively

complete understanding of how different mesodermal derivatives are molecularly regulated (Chiodin et al. 2013). The study of Chiodin and collaborators demonstrated that the mesoderm in acoels is composed of a few different cell types and that the gonads and stem cells are all derived, most probably, from the endomesoderm. The specific role of the stem cell system has been explored in the past (Gschwentner et al. 2001), but it is through the more detailed studies of De Mulder et al. (2009) that we came to understand various molecular players. In particular, we learned that the piwi orthologues (there are two in *I. pulchra*) are involved in specifying/maintaining the stem cell state. This has been proven through the use of RNAi methods in regenerating animals.

The development of the nervous system in acoels has been evaluated through the identification and characterization of candidate genes belonging to different transcription factor families. A major focus has been allocated to the identification of the so-called neurogenic genes of the bHLH family. The characterization of their expression patterns has been carried out in embryos and hatchlings of the species, *Symsagittifera roscoffensis* (Perea-Atienza et al. 2018). Interestingly, the different members of this family seem to have quite different spatio-temporal patterns of expression, with some of them being specifically restricted to neurogenic areas (from early on in embryogenesis). Their sometimes overlapping domains hint at the possibility of a combinatorial code of genes responsible for the specification of different neural domains or phenotypes. In addition to this family, other genes involved in neurogenesis have been characterized, though sometimes in the context of exploring other processes. This is the case of SoxB relatives in *Convolutiloba longifissura* (Hejnlol and Martindale 2008) or in *S. roscoffensis* (Semmler et al. 2010). In both cases, the domain of expression of SoxB is compatible with their use in the specification of the neurogenic domain. Other regulatory genes expressed in presumptive neural tissue have been described in *C. longifissura* (Cdx, Otp and NK2.1; Hejnlol and Martindale 2008) or *Neochildia fusca* (Brn1 and Brn3; Ramachandra et al. 2002). Very little is known of the regulation of nemertodermatid nervous system formation. However, the expression domains of certain key regulatory genes have been recently reported by Martín-Durán and colleagues utilizing *Meara stichopi* (Martín-Durán et al. 2018).

In spite of the knowledge gathered over the last number of years on the molecular components that regulate certain basic processes in xenacoelomorphs, it is clear that what needs to be done in order to “understand” both the development and physiology of this group of animals represent still an enormous task. This would be one that should involve the generation of more, and better, sequence data and the incorporation of novel technologies to assess gene function.

## 14.5 Future Perspectives

The realization that Xenacoelomorpha represent a clade of early divergent bilaterian has prompted a renewed interest in its study. While the collection and initial morphological characterization of different species within this clade date to more than

a century ago, it has been the introduction of molecular technologies that have revolutionized their modern study. Large gene data sets have allowed, on the one side, to reanalyse the phylogenetic affinities of Xenacoelomorpha, and its constitutive clades, and on the other, to initiate the characterization of the regulatory functions of many genes. While the functional analysis of these genes are still in its infancy, the promise of new technologies such as single-cell sequencing, CRISPR, high throughput genomic and proteomic analysis, should bring the study of these animals to a new level. However, it is important to note here that in absence of a careful analysis of the morphology and developmental processes, as well as a detailed understanding of the diversity of this group, molecular data per se will be of little value. Understanding how xenacoelomorphs have diversified over time and how the patterns of divergence are reflected in changes in developmental processes will need of a continuous investigation of the interplay between morphology changes, development and evolutionary diversification (Martinez 2018). If we follow this recommendation, we anticipate that the study of Xenacoelomorpha will become a key research program geared at a better understanding of the origin and diversification of Bilateria.

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## References

- Achatz JG, Martinez P (2012) The nervous system of *Isodiametra pulchra* (Acoela) with a discussion on the neuroanatomy of the Xenacoelomorpha and its evolutionary implications. *Frontiers Zool* 9. <https://doi.org/10.1186/1742-9994-9-27>
- Achatz JG, Chiodin M, Salvenmoser W, Tyler S, Martinez P (2013) The Acoela: On Their Kind and Kinships, Especially with Nemertodermatids and Xenoturbellids (Bilateria Incertae Sedis). *Org Diversity Evol* 13(2):267–286. <https://doi.org/10.1007/s13127-012-0112-4>
- Andrikou C, Thiel D, Ruiz-Santisteban JA, Hejnol A. (2019) Active mode of excretion across digestive tissues predates the origin of excretory organs. *PLoS Biol* 17(7):e3000408. <https://doi.org/10.1371/journal.pbio.3000408>
- Arboleda E, Hartenstein V, Martinez P, Reichert H, Sen S, Sprecher SG, Bailly X (2018) An emerging system to study photosymbiosis, brain regeneration, chronobiology, and behavior: the marine acoel symagittifera roscoffensis. *BioEssays* 40(10). <https://doi.org/10.1002/bies.201800107>
- Arimoto A, Hikosaka-Katayama T, Hikosaka A, Tagawa K, Inoue T, Ueki T, Yoshida M et al (2019) A draft nuclear-genome assembly of the acoel flatworm *Praesagittifera naikaiensis*. *GigaScience* 8(4):1–8. <https://doi.org/10.1093/gigascience/giz023>
- Arroyo A, López-Escardó D, de Vargas C, Ruiz-Trillo I (2016) Hidden diversity of Acoelomorpha revealed through metabarcoding. *Biol Lett* 12(9). <https://doi.org/10.1098/rsbl.2016.0674>
- Bedini C, Ferrero E, Lanfranchi A (1973) The ultrastructure of ciliary sensory cells in two Turbellaria Acoela. *Tissue Cell* 5(3):359–372. [https://doi.org/10.1016/S0040-8166\(73\)80030-8](https://doi.org/10.1016/S0040-8166(73)80030-8)

- Beklemishev VN (1963) On the relationship of the Turbellaria to the other groups of the animal kingdom. In: Dougherty EC (ed) The lower Metazoa. University California Press, Berkeley, pp 234–244
- Bery A, Martínez P (2011) Acetylcholinesterase activity in the developing and regenerating nervous system of the acoel symagittifera roscoffensis. *Acta Zoologica* 92(4):383–392. <https://doi.org/10.1111/j.1463-6395.2010.00472.x>
- Bery A, Cardona A, Martínez P, Hartenstein V (2010) Structure of the central nervous system of a juvenile acoel, *Symagittifera roscoffensis*. *Dev Genes Evol* 220(3–4):61–76. <https://doi.org/10.1007/s00427-010-0328-2>
- Bleidorn C, Podsiadlowski L, Zhong M, Eeckhaut I, Hartmann S, Halanych KM, Tiedemann R (2009) On the phylogenetic position of Myzostomida: can 77 genes get it wrong? *BMC Evol Biol* 9(1):150
- Boone M, Bert W, Claeys M, Houthoofd W, Artois T (2011) Spermatogenesis and the structure of the testes in Nemertodermatida. *Zoomorphology* 130:273–282
- Børve A, Hejnol A (2014) Development and juvenile anatomy of the nemertodermatid *Meara stichopi* (Bock) Westblad 1949 (Acoelomorpha). *Front Zool* 11(January):50. <https://doi.org/10.1186/1742-9994-11-50>
- Bourlat SJ, Nielsen C, Lockyer AE, Littlewood DT, Telford MJ (2003) *Xenoturbella* is a deuterostome that eats molluscs. *Nature* 530:925–928
- Bourlat SJ, Juliusdottir T, Lowe CJ, Freeman R, Aronowicz J, Kirschner M, Lander ES et al (2006) Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. *Nature* 444(November):85–88. <https://doi.org/10.1038/nature05241>
- Brauchle M, Bilican A, Eyer C, Bailly X, Martínez P, Ladurner P, Bruggmann R, Sprecher SG (2018) Xenacoelomorpha survey reveals that all 11 animal Homeobox gene classes were present in the first Bilaterians. *Genome Biol Evol* 10(9):2205–2217. <https://doi.org/10.1093/gbe/evy170>
- Buckland-Nicks J, Lundin K, Wallberg A (2018) The sperm of Xenacoelomorpha revisited: implications for the evolution of early Bilaterians. *Zoomorphology*. <https://doi.org/10.1007/s00435-018-0425-8>
- Cannon JT, Vellutini B, Smith J, Ronquist F, Jondelius U, Hejnol A (2016) Xenacoelomorpha is the sister group to Nephrozoa. *Nature* 530(7588):89–93. <https://doi.org/10.1038/nature16520>
- Chang YC, Pai CY, Chen YC, Ting HC, Martinez P, Telford MJ, Yu JK, Su YH (2015) Regulatory circuit rewiring and functional divergence of the duplicate admp genes in dorsoventral axial patterning. <https://doi.org/10.1016/j.ydbio.2015.12.015>
- Chiodin M, Achatz JG, Wanninger A, Martinez P (2011) Molecular architecture of muscles in an acoel and its evolutionary implications. *J Exp Zool B Mol Dev Evol* 316(6):427–439. <https://doi.org/10.1002/jez.b.21416>
- Chiodin M, Børve A, Berezikov E, Ladurner P, Martinez P, Hejnol A (2013) Mesodermal gene expression in the acoel *Isodiametra pulchra* indicates a low number of mesodermal cell types and the endomesodermal origin of the gonads. *PLoS ONE* 8(2):e55499. <https://doi.org/10.1371/journal.pone.0055499>
- Conway-Morris S, George JD, Gibson R, Platt HM (1985) The origins and relationships of lower invertebrates. Clarendon Press, Oxford
- Cook CE, Jimenez-Guri E, Akam M, Salo E (2004) The hox gene complement of acoel flatworms, a basal bilaterian clade. *Article Evol Dev* 6(3):154–163
- Crezée M (1978) *Paratomella rubra* Rieger and Ott, an amphiatlantic acoel turbellarian. *Cah Biol Mar* 19:1–9
- Dittmann IL, Zauchner T, Nevard LM, Telford MJ, Egger B (2018) SALMFamide2 and serotonin immunoreactivity in the nervous system of some acoels (Xenacoelomorpha). *J Morphol* 279(5):589–597
- Dorey AE (1965) The organization and replacement of the epidermis in acoelous turbellarians. *Q J Microsc Sci* 106:147–172
- Dörjes J (1968) Die Acoela (Turbellaria) Der Deutschen Nordseeküste Und Ein Neues System Der Ordnung. *Z. Zool Syst Evolutionforsch* 6:56–452

- Dörjes J (1972) *Faerlea echinocardii* Sp. N. Und Diskussion Der Gattungen *Avagina* Leiper Und *Faerlea* Westblad (Turbellaria Acoela). *Zoolog Scr* 1(3):185–189
- Ehlers U (1984) Phylogenetisches system der plathelminthes. *Verh Natwiss Ver Hamburg* 27:291–294
- Ehlers U (1985) Das Phylogenetische System Der Plathelminthes. Gustav Fischer, Stuttgart
- Ehlers U (1991) Comparative morphology of statocysts in the Plathelminthes and the Xenoturbellida. *Hydrobiologia* 227:263–271
- Ehlers U (1992a) Dermonephridia—modified epidermal cells with a probable excretory function in *Paratomella rubra* (Acoela, Plathelminthes). *Microfauna Mar* 7:253–64
- Ehlers U (1992b) On the fine structure of *Paratomella rubra* Rieger & Ott (Acoela) and the position of the taxon *Paratomella* Dörjes in a phylogenetic system of the Acoelomorpha (Plathelminthes). *Microfauna Mar* 7:265–293
- Ehlers U (1992c) Frontal glandular and sensory structures in *Nemertoderma* (Nemertodermatida) and *Paratomella* (Acoela): ultrastructure and phylogenetic implications for the monophyly of the Euplathelminthes (Plathelminthes). *Zoomorphology* 112(4):227–236. <https://doi.org/10.1007/BF01632820>
- Falleni A, Raikova O, Gremigni V (1995) Ultrastructural and cytochemical features of the ovary in *Paratomella rubra* (Platyhelminthes, Acoela). *J Submicroscopical Cytol Pathol* 27:511–523
- Ferrero E (1973) A fine structural analysis of the statocyst in Turbellaria Acoela. *Zoologica Scripta* 2(1):5–16. <https://doi.org/10.1111/j.1463-6409.1973.tb00793.x>
- Franzen A, Afzelius B (1987) The ciliated epidermis of *Xenoturbella bocki* (Platyhelminthes, Xenoturbellida) with some phylogenetic considerations. *Zoolog Scr* 16(1):9–17. <https://doi.org/10.1111/j.1463-6409.1987.tb00046.x>
- Gaerber CW, Salvenmoser W, Rieger RM, Gschwentner R (2007) The nervous system of *Convolutriloba* (Acoela) and its patterning during regeneration after asexual reproduction. *Zoomorphology* 126(2):73–87. <https://doi.org/10.1007/s00435-007-0039-z>
- Gavilán B, Sprecher SG, Hartenstein V, Martínez P (2019) The digestive system of xenacoelomorphs. *Cell Tissue Res* (in press). <https://doi.org/10.1007/s00441-019-03038-2>
- Gavilán B, Perea-Atienza E, Martínez P (2016) Xenacoelomorpha: a case of independent nervous system centralization? *Philos Trans R Soc Lond S B Biol Sci* 371(1685):20150039. <https://doi.org/10.1098/rstb.2015.0039>
- Gehrke AR, Neverett E, Luo Y-J, Brandt A, Ricci L, Hulett RE, Gompers A et al (2019) Acoel genome reveals the regulatory landscape of whole-body regeneration. *Science* 363(6432):eaau6173. <https://doi.org/10.1126/science.aau6173>
- Graff LV (1905) Turbellaria I. Acoela. In: F Schulze (ed) *Das Tierreich, Eine Zusammenstellung Und Kennzeichnung Der Rezenten Tierformen Heft 23*, pp 23–34. Königl. Preuss. Akademie der Wissenschaften zu Berlin
- Graff LV (1911) Acoela, Rhabdocoela Und Alloecoela Des Ostens Der Vereinigten Staaten von Amerika. *Z Wiss Zool*. 99:321–428
- Gröger H, Schmid V (2001) Larval development in Cnidaria: a connection to Bilateria? *Genesis* 29:110–114
- Gschwentner R, Ladurner P, Nimeth K, Rieger R (2001) Stem cells in a basal bilaterian. S-phase and mitotic cells in *Convolutriloba longifissura* (Acoela, Platyhelminthes). *Cell Tissue Res* 304(3):401–408
- Haszprunar G (2015) Review of data for a morphological look on Xenacoelomorpha (Bilateria Incertae Sedis). *Org Diversity Evol* 16(2):363–389. <https://doi.org/10.1007/s13127-015-0249-z>
- Hejnol A, Martindale MQ (2008) Acoel development indicates the independent evolution of the bilaterian mouth and anus. *Nature* 456(7220):382–386. <https://doi.org/10.1038/nature07309>
- Hejnol A, Martindale MQ (2009) Coordinated spatial and temporal expression of Hox genes during embryogenesis in the acoel *Convolutriloba Longifissura*. *BMC Biol* 1(7):65
- Hejnol A, Obst M, Stamatakis A, Ott M, Rouse GW, Edgecombe GD, Martínez P et al (2009) Assessing the root of bilaterian animals with scalable phylogenomic methods. <https://doi.org/10.1098/rspb.2009.0896>

- Hendelberg J (1969) On the development of different types of spermatozoa from spermatids with two flagella in the Turbellaria with remarks on the ultrastructure of the flagella. *Zoologiska Bidrag Uppsala* 38:1–52
- Hendelberg J (1977) Comparative morphology of turbellarian spermatozoa studied by electron microscopy. *Acta Zoologica Fennica* 154:149–162
- Hendelberg J (1986) The phylogenetic significance of sperm morphology in the Platyhelminthes. In: *Advances in the biology of turbellarians and related platyhelminthes*, pp 53–58. Springer, Dordrecht
- Hendelberg J, Hedlund K-O (1974) On the morphology of the epidermal ciliary rootlet system of the acoelous turbellarian *Childia groenlandica*. *Zoon* 2:13–24
- Hooge MD (2001) Evolution of the body-wall musculature in the Platyhelminthes (Acoelomorpha, Catenulida, Rhabditophora). *J Morph* 249:171–194
- Iomini C, Raikova OI, Noury-Sraïri N, Justine J-L (1995) Immunocytochemistry of tubulin in spermatozoa of Platyhelminthes. *Adv Spermatozoal Phylogeny Taxonomy* 166:97–110
- Israelsson O (1997) *Xenoturbella*'s Molluscan Relatives...] and Molluscan Embryogenesis. *Nature* 390:32
- Jondelius U, Ruiz-Trillo I, Bagaña J, Riutort M (2002) The Nemertodermatida are basal bilaterians and not members of the Platyhelminthes. *Zool Scr* 31(2):201–215. <https://doi.org/10.1046/j.1463-6409.2002.00090.x>
- Jondelius U, Wallberg A, Hooge M, Raikova OI (2011) How the worm got its pharynx: phylogeny, classification and Bayesian assessment of character evolution in Acoela. *Syst Biol* 60(6):845–871. <https://doi.org/10.1093/sysbio/syr073>
- Kännöby T, Bernvi DC, Jondelius U (2015) Distribution, delimitation and description of species of *Archaphanostoma* (Acoela). *Zool Scr* 44(2):218–231. <https://doi.org/10.1111/zsc.12092>
- Karling TG (1940) Zur Morphologie Und Systematik Der Alloeoceola Cumulata and Rhabditophora Lecithophora (Turbellaria). *Acta Zool Fennica* 26:1–160
- Klauser MD, Smith JPS, Tyler S (1986) Ultrastructure of the frontal organ in *Convoluta* and *Macrostomum* spp.: significance for models of the turbellarian archetype. *Hydrobiologia* 132(1):47–52. <https://doi.org/10.1007/BF00046227>
- Lanfranchi A (1990) Ultrastructure of the epidermal eyespots of an acoel platyhelminth. *Tissue Cell* 22(4):541–46. <http://www.ncbi.nlm.nih.gov/pubmed/18620320>
- Lundin K (1997) Comparative ultrastructure of the epidermal ciliary rootlets and associated structures in species of the Nemertodermatida and Acoela (Plathelminthes). *Zoomorphology* 117(2):81–92. <https://doi.org/10.1007/s004350050033>
- Lundin K (1998) The epidermal ciliary rootlets of *Xenoturbella bocki* (Xenoturbellida) revisited: new support for a possible kinship with the Acoelomorpha (Platyhelminthes). *Zool Scr* 27(3):263–270. <https://doi.org/10.1111/j.1463-6409.1998.tb00440.x>
- Lundin K, Hendelberg J (1998) Is the sperm type of the Nemertodermatida close to that of the ancestral Platyhelminthes? *Hydrobiologia* 383:197–205
- Martín-Durán JM, Pang K, Børve A, Lê HS, Furu A, Cannon JT, Jondelius U, Hejnol A (2018) Convergent evolution of Bilaterian nerve cords. *Nature* 553(7686):45–50. <https://doi.org/10.1038/nature25030>
- Martínez P (2018) The Comparative method in biology and the essentialist trap. *Frontiers in ecology and evolution* 6 (AUG). <https://doi.org/10.3389/fevo.2018.00130>
- Martínez P, Hartenstein V, Sprecher SG (2017) Xenacoelomorpha Nervous Systems. In: SM Sherman (ed) *Oxford encyclopaedia of neurosciences*. Oxford University Press
- Meyer-Wachsmuth I, Jondelius U (2016) Interrelationships of Nemertodermatida. *Org Div Evol*. <https://doi.org/10.1007/s13127-015-0240-8>
- Meyer-Wachsmuth I, Raikova OI, Jondelius U (2013) The muscular system of *Nemertoderma westbladi* and *Meara stichopi* (Nemertodermatida, Acoelomorpha). *Zoomorphology* 132(3):239–252. <https://doi.org/10.1007/s00435-013-0191-6>

- Meyer-Wachsmuth I, Curini-Galletti M, Jondelius U (2014) Hyper-cryptic marine meiofauna: species complexes in Nemertodermatida. PLoS ONE 9(9):e107688. <https://doi.org/10.1371/journal.pone.0107688>
- Moreno E, De Mulder K, Salvenmoser W, Ladurner P, Martínez P (2010) Inferring the ancestral function of the posterior hox gene within the *Bilateria*: controlling the maintenance of reproductive structures, the musculature and the nervous system in the acoel flatworm *Isodiametra pulchra*. *Evol Dev May*. <https://doi.org/10.1111/j.1525-142x.2010.00411.x>
- Moreno E, Nadal M, Baguña, J, Martínez P (2009) Tracking the origins of the *Bilateria* hox patterning system: insights from the acoel flatworm *Symsagittifera roscoffensis*. *Evol Dev* 11 (5). <https://doi.org/10.1111/j.1525-142X.2009.00363.x>
- Mulder K De, Kualess G, Pfister D, Willems M, Egger B, Salvenmoser W, Thaler M et al (2009) Characterization of the stem cell system of the acoel *Isodiametra pulchra*. *BMC Dev Biol* 9:69. <https://doi.org/10.1186/1471-213X-9-69>
- Mwinyi A, Bailly X, Bourlat SJ, Jondelius U, Littlewood TJ, Podsiadlowski L (2010) The phylogenetic position of Acoela as revealed by the complete mitochondrial genome of *Symsagittifera roscoffensis*. *BMC Evol Biol* 10:309. <https://doi.org/10.1186/1471-2148-10-309>
- Nakano H, Miyazawa H, Maeno A, Shiroishi T, Kakui K, Koyanagi R, Kanda M, Satoh N, Omori A, Kohtsuka H (2017) A new species of *Xenoturbella* from the western Pacific Ocean and the evolution of *Xenoturbella*. *BMC Evol Biol* 17(1):245
- Noren M, Jondelius U (1997) *Xenoturbella*'s molluscan relatives. *Nature* 390:31–32. <https://doi.org/10.1038/36242>
- Obst M, Nakano H, Bourlat SJ, Thorndyke MC, Telford MJ, Nyengaard JR, Funch P (2011) Spermatozoon ultrastructure of *Xenoturbella bocki* (Westblad 1949). *Acta Zool* 92(2):109–115. <https://doi.org/10.1111/j.1463-6395.2010.00496.x>
- Paps J, Holland PWH (2018) Reconstruction of the ancestral metazoan genome reveals an increase in genomic novelty. *Nat Commun* 9(1):1730. <https://doi.org/10.1038/s41467-018-04136-5>
- Perea-Atienza E, Gavilán B, Chiodin M, Abril J-F, Hoff KJ, Poustka AJ, Martínez P (2015) The nervous system of Xenacoelomorpha: a genomic perspective. *J Exp Biol* 218(Pt 4):618–628. <https://doi.org/10.1242/jeb.110379>
- Perea-Atienza E, Sprecher SG, Martínez P (2018) Characterization of the bHLH family of transcriptional regulators in the acoel *S. roscoffensis* and their putative role in neurogenesis. *Evodevo* 9:8
- Petrov AA, Hooge M, Tyler S (2004) Ultrastructure of sperms in Acoela (Acoelomorpha) and its concordance with molecular systematics. *Invertebr Biol* 123:183–197
- Philippe H, Brinkmann H, Copley RR, Moroz LL, Nakano H, Poustka AJ, Wallberg A, Peterson KJ, Telford MJ (2011) Acoelomorph flatworms are deuterostomes related to *Xenoturbella*. *Nature* 470(7333):255–258. <https://doi.org/10.1038/nature09676>
- Philippe H, Poustka AJ, Chiodin M, Hoff KJ, Dessimoz C, Tomiczek B, Schiffer PH, Müller S, Domman D, Horn M, Kuhl H, Timmermann B, Satoh N, Hikosaka-Katayama T, Nakano H, Rowe ML, Elphick MR, Thomas-Chollier M, Ha MJ (2019) Mitigating anticipated effects of systematic errors supports sister-group relationship between Xenacoelomorpha and Ambulacraria. *Curr Biol* (in press)
- Popova NV, Mamkaev YV (1985) Ultrastructure and primitive features of the eyes of *Convoluta convoluta* (Turbellaria Acoela). *Dokl Akad Nauk SSSR* 283:756–759
- Raikova OI (1991) On phylogenetic significance of ultrastructural characters in Turbellaria. In: *Proceedings of the Zoological Institute of the Academy of Sciences of the USSR*, pp 26–52
- Raikova OI (2004) Neuroanatomy of basal bilaterians (*Xenoturbellida*, *Nemertodermatida*, *Acoela*) and its phylogenetic implications (Ph.D. Thesis). Åbo Akademi University. Åbo, Finland
- Raikova OI, Justine J-L (1999) Microtubular system during spermiogenesis and in the spermatozoon of *Convoluta saliens* (Platyhelminthes, Acoela): tubulin immunocytochemistry and electron microscopy. *Mol Reprod Dev* 52:74–85
- Raikova OI, Falleni A, Gremigni V (1995) Oogenesis in *Actinoposthia beklemishevi* (Platyhelminthes, Acoela): an ultrastructural and cytochemical study. *Tissue Cell* 27:621–633

- Raikova OI, Reuter M, Kotikova EA, Gustafsson MKS (1998) A commissural brain! The pattern of 5-HT immunoreactivity in Acoela (Plathelminthes). *Zoomorphology* 118(2):69–77. <https://doi.org/10.1007/s004350050058>
- Raikova OI, Reuter M, Jondelius U, Gustafsson MKS (2000a) An immunocytochemical and ultrastructural study of the nervous and muscular systems of *Xenoturbella westbladi* (Bilateria Inc. Sed.). *Zoomorphology* 120(2):107–18. <https://doi.org/10.1007/s004350000028>
- Raikova OI, Reuter M, Jondelius U, Gustafsson MKS (2000b) The brain of the Nemertodermatida (Platyhelminthes) as revealed by anti-5HT and anti-FMRamide immunostainings. *Tissue Cell* 32(5):358–365. <https://doi.org/10.1054/tice.2000.0121>
- Raikova OI, Reuter M, Gustafsson MKS, Maule AG, Halton DW, Jondelius U (2004a) Basiepidermal nervous system in *Nemertoderma westbladi* (Nemertodermatida): GYIRamide immunoreactivity. *Zoology* 107(1):75–86. <https://doi.org/10.1016/j.zool.2003.12.002>
- Raikova OI, Reuter M, Gustafsson MKS, Maule AG, Halton DW, Jondelius U (2004b) Evolution of the nervous system in *Paraphanostoma* (Acoela). *Zool Scr* 33:71–88
- Raikova OI, Meyer-Wachsmuth I, Jondelius U (2016) The plastic nervous system of Nemertodermatida. *Org Div Evol* 16(1):85–104. <https://doi.org/10.1007/s13127-015-0248-0>
- Ramachandra NB, Gates RD, Ladurner P, Jacobs DK, Hartenstein V (2002) Embryonic development in the primitive bilaterian *Neochildia fusca*: normal morphogenesis and isolation of POU genes Brn-1 and Brn-3. *Dev Genes Evol* 212(2):55–69. <https://doi.org/10.1007/s00427-001-0207-y>
- Reisinger E (1925) Untersuchungen Am Nervensystem Der *Bothrioplana semperi* Braun. (Zugleich Ein Beitrag Zur Technik Der Vitalen Nervenfaerbung Und Zur Vergleichenden Anatomie Des Plathelminthennervensystem). *Z Morphol Okol Tiere* 5:119–149
- Reisinger E (1960) Was ist *Xenoturbella*. *Z Wiss Zool* 164:188–198
- Reuter M, Raikova OI, Jondelius U, Gustafsson MKS, Maule AG, Halton, DV (2001) Organisation of the nervous system in the Acoela: an immunocytochemical study. *Tissue Cell* 33(2):119–28. <http://www.ncbi.nlm.nih.gov/pubmed/11392663>
- Reuter M, Raikova OI, Gustafsson MKS (2001) Patterns in the nervous and muscle systems in lower flatworms. *Belgian J Zool* 131 (Suppl:47–53
- Richter S, Loesel R, Purschke G, Schmidt-Rhaesa A, Scholtz G, Stach T, Vogt L, Wanninger A, Brenneis G, Döring C, Faller S, Fritsch M, Grobe P, Heuer CM, Kaul S, Möller OS, Müller CH, Rieger V, Rothe BH, Stegner ME, Harzsch S (2010) Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. *Front Zool.* 7:29. <https://doi.org/10.1186/1742-9994-7-29>
- Rieger RM, Tyler S, Smith JPS III, Rieger GE (1991) Platyhelminthes: Turbellaria. In: Bogitsh BJ, Harrison FW (eds) *Microscopic anatomy of invertebrates*. Wiley-Liss, New York
- Robertson HE, Lapraz F, Egger B, Telford MJ, Schiffer PH (2017) The mitochondrial genomes of the acelomorph worms *Paratomella rubra*, *Isodiametra pulchra* and *Archaphanostoma ylvae*. *Sci R* 7(1):1–16. <https://doi.org/10.1038/s41598-017-01608-4>
- Rohde K, Watson NA, Cannon LRG (1988) Ultrastructure of epidermal cilia of *Pseudactinoposthia* sp. (Platyhelminthes, Acoela); implications for the phylogenetic status of the Xenoturbellida and Acoelomorpha. *J Submicroscopical Cytol Pathol* 20:759–767
- Rouse GW, Wilson NG, Carvajal JI, Vrijenhoek RC (2016) New deep-sea species of *Xenoturbella* and the position of Xenacoelomorpha. *Nature*. <https://doi.org/10.1038/nature16545>
- Ruiz-Trillo I, Riutort M, Littlewood DT, Herniou EA, Bagaña J (1999) Acoel flatworms: earliest extant bilaterian metazoans, not members of Platyhelminthes. *Science* 283 (5409):1919–23. <http://www.ncbi.nlm.nih.gov/pubmed/10082465>
- Ruiz-Trillo I, Paps J, Loukota M, Ribera C, Jondelius U, Bagaña J, Riutort M (2002) A phylogenetic analysis of myosin heavy chain type II sequences corroborates that Acoela and Nemertodermatida are basal bilaterians. *Proc Natl Acad Sci* 99(17):11246–11251. <https://doi.org/10.1073/pnas.172390199>
- Ruiz-Trillo I, Riutort M, Fourcade HM, Bagaña J, Boore JL (2004) Mitochondrial genome data support the basal position of Acoelomorpha and the polyphyly of the Platyhelminthes. *Mol Phylogenet Evol* 33(2):321–332. <https://doi.org/10.1016/j.ympev.2004.06.002>

- Schmidt-Rhaesa, A. (2007). The evolution of organ systems. Oxford University Press
- Semmler H, Chiodin M, Bailly X, Martinez P, Wanninger A (2010) Steps towards a centralized nervous system in basal bilaterians: insights from neurogenesis of the acoel *Symsagittifera roscoffensis*. Dev Growth Differ 52(8):701–713. <https://doi.org/10.1111/j.1440-169X.2010.01207.x>
- Seo HC, Edvardsen RB, Maeland AD, Bjordal M, Jensen MF, Hansen A, Flaot M, Weissenbach J, Lehrach H, Wincker P, Reinhardt R, Chourrout D (2004) Hox cluster disintegration with persistent anteroposterior order of expression in *Oikopleura dioica*. Nature 431(7004):67–71
- Sikes JM, Bely AE (2010) Making heads from tails: development of a reversed anterior–posterior axis during budding in an acoel. Dev Biol 338(1):86–97. <https://doi.org/10.1016/j.ydbio.2009.10.033>
- Smith III JPS, Tyler S (1988) Frontal organs in the Nemertodermatida (Turbellaria). Am Zool 28(4):140A, #747
- Smith III JPS, Tyler S, Rieger RM (1986) Is the Turbellaria Polyphyletic? Hydrobiologia 132:13–21
- Smith JPS, Tyler S (1985) Fine-structure and evolutionary implications of the frontal organ in Turbellaria Acoela. 1 *Diopisthoporus gymnopharyngeus* sp.n. Zool Scr 14(2):91–102. <https://doi.org/10.1111/j.1463-6409.1985.tb00180.x>
- Smith JPS, Tyler S (1985) The acoel turbellarians: kingpins of metazoan evolution or a specialized offshoot? In: Morris SC, George JD, Gibson R, Platt HM (eds) The origins and relationships of lower invertebrates, 123142. Oxford: Clarendon Press
- Sopott-Ehlers B, Ehlers U (1997) Ultrastructure of the subepidermal musculature of *Xenoturbella bocki*, the adelphotaxon of the Bilateria. Zoomorphology 117:71–79. <https://doi.org/10.1007/s004350050032>
- Sprecher SG, Bernardo-Garcia F-J, van Giesen L, Hartenstein V, Reichert H, Neves R, Bailly X et al. (2015) Functional brain regeneration in the acoel worm *Symsagittifera roscoffensis*. Biology Open 4 (12):1688–95. <https://doi.org/10.1242/bio.014266>
- Srivastava M, Mazza-Currl KL, van Wolfswinkel JC, Reddien PW (2014) Whole-body acoel regeneration is controlled by wnt and bmp-admp signaling. Curr Biol CB 24(10):1107–1113. <https://doi.org/10.1016/j.cub.2014.03.042>
- Sterrer W (1998) New and known Nemertodermatida (Platyhelminthes-Acoelomorpha): a revision. Belgian J Zool 128(1):55–92. [https://www.researchgate.net/publication/265923621\\_New\\_and\\_known\\_Nemertodermatida\\_Platyhelminthes-Acoelomorpha\\_A\\_revision](https://www.researchgate.net/publication/265923621_New_and_known_Nemertodermatida_Platyhelminthes-Acoelomorpha_A_revision)
- Tekle YI, Raikova OI, Justine J-L, Jondelius U (2007a) Ultrastructure and tubulin immunocytochemistry of the copulatory stylet-like structure in *Childia* species (Acoela). J Morphol 268:166–180
- Tekle YI, Raikova OI, Justine J-L, Hendelberg J, Jondelius U (2007b) Ultrastructural and immunocytochemical investigation of acoel sperms with 9+ 1 axoneme structure: new sperm characters for unraveling phylogeny in Acoela. Zoomorphology 126:1–16
- Telford MJ, Lockyer AE, Cartwright-Finch C, Littlewood TJ (2003) Combined large and small subunit ribosomal RNA phylogenies support a basal position of the acoelomorph flatworms. Proc Biol Sci 270 (1519):1077–83. <https://doi.org/10.1098/rspb.2003.2342>
- Thiel D, Franz-Wachtel M, Aguilera F, Hejnol A (2018) Xenacoelomorph neuropeptidomes reveal a major expansion of neuropeptide systems during early bilaterian evolution. Mol Biol Evol 35(10):2528–2543. <https://doi.org/10.1093/molbev/msy160>
- Todt C (2009) Structure and evolution of the pharynx simplex in acoel flatworms (Acoela). J Morphol 270(3):271–290. <https://doi.org/10.1002/jmor.10682>
- Todt C, Tyler S (2006) Ciliary receptors associated with the mouth and pharynx of Acoela (Acoelomorpha): a comparative ultrastructural study. Acta Zoologica 88(1):41–58. <https://doi.org/10.1111/j.1463-6395.2007.00246.x>
- Tyler S (1979) Distinctive features of cilia in metazoans and their significance for systematics. Tissue Cell 11:385–400
- Tyler S, Hooge MD (2004) Comparative morphology of the body wall in flatworms (Platyhelminthes). Can J Zool 82:194–210
- Tyler S, Rieger RM (1975) Uniflagellate spermatozoa in Nemertodermata (Turbellaria) and their phylogenetic significance. Science 188:730–732

- Tyler S, Rieger RM (1977) Ultrastructural evidence for the systematic position of the Nemertodermatida (Turbellaria). *Acta Zool Fennica* 54:193–207
- Uljanin WN (1870) Die Turbellarien Der Bucht von Sebastopol. *Arbeiten Der 2.Versammlung Russischer Naturforscher Zu Moskau*. 1869:1–96
- Westblad E (1937) Die Turbellarien-Gattung *Nemertoderma* Steinböck. *Acta Societatis pro Fauna et Flora Fennica* 60:45–89
- Westblad E (1940) Studien Über Skandinavische Turbellaria Acoela. I. *Arkiv För Zoologi* 32A(20):1–28
- Westblad E (1942) Studien Über Skandinavische Turbellaria Acoela. II. *Arkiv För Zoologi* 33A(14):1–48
- Westblad E (1945) Studien Über Skandinavische Turbellaria Acoela. III. *Ark Zool.* 36A(5):1–56
- Westblad E (1946) Studien Über Skandinavische Turbellaria Acoela. IV. *Ark Zool* 38A(1):1–56
- Westblad E (1948) Studien Über Skandinavische Turbellaria Acoela. V. *Ark Zool* 41:191–273
- Westblad E (1949a) On *Meara stichopi* (Bock) Westblad, a new representative of Turbellaria archophora. *Ark Zool Ser 2* 1(5):43–57
- Westblad E (1949b) *Xenoturbella bocki* N.g., N. Sp., a peculiar, primitive Turbellarian type. *Ark. Zool* 1:3–29
- Yamasu T (1991) Fine structure and function of ocelli and sagittocysts of acoel flatworms. *Hydrobiologia* 227(1):273–282. <https://doi.org/10.1007/BF00027612>
- Zabotin YI (2019) Ultrastructure of epidermal sensillae in three species of Acoela. *Invertebr Zool* 6(1):71–77

# Chapter 15

## Cooperation and Competition in Mammalian Evolution



### Gene Domestication from LTR Retrotransposons

Tomoko Kaneko-Ishino and Fumitoshi Ishino

**Abstract** Mammalian genomes have had to endure the integration of exogenous DNA sequences over the course of time. In most cases, such events have proven harmful to individuals thus afflicted, but in the long-term gene domestication of exogenous DNA sequences, such as LTR retrotransposons, has also served as a driving mechanism in biological evolution. This is especially the case in eutherian mammals, in which two lines of domesticated genes increased in number in a common eutherian ancestor, eleven sushi-ichi-related retrotransposon homologs (*SIRH*)/retrotransposon Gag-like (*RTL*) genes and more than fifteen paraneoplastic Ma antigen (*PNMA*) genes. It is clear that these *SIRH/RTL* and *PNMA* genes were positively selected due to the advantage conferred on eutherian reproductive success. Thus, the principle of “competition among individuals within the same species” in the Darwinian theory of evolution is effectively at work in the domestication process. However, when the number of domestication events is taken into account, how could the common eutherian ancestor have acquired that many domesticated genes? We suggest that sexual mating across multiple generations of individuals with one or a small number of such domesticated genes may have been critically important for accumulating all of them into a single line, thus leading to the common eutherian ancestor. Then, we would like to propose that “cooperation among individuals within the same species” in the form of interactive behaviors of the individuals within a tightly delimited species is also at work in this process.

## 15.1 Introduction

The acquisition of vivipary in therian mammals and subsequent divergence into viviparous marsupial and eutherian subgroups having a different placental system

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and reproductive strategy are critical events in the course of mammalian evolution. What underlies these events from the viewpoint of genomic function? It is probable that genomic imprinting and gene domestication from long terminal repeat (LTR) retrotransposons/retroviruses played important roles in these processes by promoting dramatic change in gene function of host organisms. This is because two paternally expressed imprinted genes, paternally expressed 10 (*PEG10*) and *PEG11*/retrotransposon Gag-like 1 (*RTL1*), are essential placental genes, even though they were actually derived from a *suchi-ichi*-related LTR retrotransposon (Ono et al. 2001, 2006; Charlier et al. 2001; Sekita et al. 2008). Moreover, at least some of the nine additional domesticated genes from the same retrotransposon, i.e., *sushi-ichi*-related retrotransposon homologs (*SIRH*)/*RTL* genes, have important roles not only in the placenta, but also in brain in the current developmental system (Naruse et al. 2014; Irie et al. 2015, 2016; Kaneko-Ishino and Ishino 2015). Interestingly, both the genomic imprinting and gene domestication mechanisms are related to the insertion of exogenous DNA. The former was apparently established as a defense mechanism against the insertion of exogenous cis-regulatory units (Barlow 1993; Kaneko-Ishino and Ishino 2019), while the latter is exaptation mechanism for creating genes of novel function from the inserted LTR retrotransposons/retroviruses (Gould and Vrba 1982; Brosius and Gould 1992).

In terms of genomic imprinting that regulates parent-of-origin-specific monoallelic expression of imprinted genes, a critical regulatory sequence in each imprinted region, the so-called differentially methylated region (DMR), is derived from exogenous DNA having cis-regulatory function, such as insulators, enhancers, and promoters of antisense RNA. The genomic imprinting mechanism then reorganized the gene expression profile around the insertion site so as to ensure the expression of all of the genes by regulating paternal- or maternal-specific monoallelic expression. It is probable that this mechanism is advantageous because additional imprinted regions repeatedly emerged independently in several different eutherian lineages (Kaneko-Ishino and Ishino 2019).

It is known that during biological evolution, certain essential genes were acquired by domestication (exaptation or cooption) of LTR retrotransposons/retroviruses and/or DNA transposons (Kaneko-Ishino and Ishino 2012), such as telomerase in eukaryotes (Nakamura and Cech 1998), recombination activating genes (*RAG1* and *RAG2*) for immunoglobulin class switching by somatic recombination in vertebrates (Agrawal et al. 1998; Hiomi et al. 1998), far-red elongated hypocotyls 3 (*FHY3*), and far-red-impaired response (*FAR1*) genes for photosynthesis in plants (Lisch et al. 2001; Hudson et al. 2003; Lin et al. 2007). Another famous example in mammals is syncytin gene that is essential for placental syncytiotrophoblast formation, but interestingly different syncytin genes were domesticated from different retroviruses in a lineage-specific manner (Blond et al. 2000; Mi et al. 2000; Dupressoir et al. 2005; Heidemann et al. 2009). Interestingly, comprehensive genome analysis in mammals revealed that the number of domesticated genes from another LTR retrotransposon, *paraneoplastic* *Ma* antigen (*PNMA*) genes, also increased in a common eutherian ancestor as well as *SIRH/RTL* genes (Brandt et al. 2005; Schüller et al. 2005; Youngson et al. 2005; Campillos et al. 2006; Ono et al. 2006). Accumulating evidence

has shown that these *PNMA* genes also play important roles in the current eutherian developmental systems (Cho et al. 2008a, b, 2011; Hanson et al. 2011a, b), indicating that *SIRH/RTL* and *PNMA* gene domestication was a driving force in evolution of marsupial and eutherians (Ono et al. 2006; Sekita et al. 2008; Cho et al. 2008a, b, 2011; Hanson et al. 2011a, b; Naruse et al. 2014; Irie et al. 2015, 2016).

These data suggest that the defense mechanism against the insertion of exogenous DNAs, such as DNA transposons and LTR retrotransposons/retroviruses, as well as the capacity for gene domestication from such inserted DNAs, is important for biological evolution of organisms. As we have already articulated the importance of genomic imprinting in mammalian evolution in a recent review (Kaneko-Ishino and Ishino 2019), in this chapter we focus on the gene domestication mechanism in mammalian evolution and consider how the expansion of the *SIRH/RTL* genes could have occurred in the common eutherian ancestor. We propose that both “cooperation” and “competition” among individuals within a species are important principles in mammalian evolution. The same idea may also apply to the domestication of other selfish genetic elements such as LINEs, SINEs, and retroviruses as well as non-retroviral RNA viruses such as bornavirus and ebola/marburgviruses (Horie et al. 2010; Belyi et al. 2010).

## 15.2 LTR Retrotransposon-Derived *SIRH/RTL* and *PNMA* Genes in Eutherians and Marsupials

*PEG10* and *PEG11/RTL1* were the first two domesticated genes derived from an LTR retrotransposon to be recognized in mammals (Ono et al. 2001; Charlier et al. 2001). They were first identified as paternally expressed imprinted genes in humans/mice and cows, respectively, exhibiting significant homology to a sushi-ichi retrotransposon isolated from fugu fish (Fig. 15.1). *PEG10* is a therian-specific gene, i.e., conserved in both the marsupials and eutherians (Suzuki et al. 2007), while *PEG11/RTL1* is a eutherian-specific gene (Edwards et al. 2008) (Fig. 15.2). The fact that both *PEG10* and *PEG11/RTL1* are essential placental genes in mice (Ono et al. 2006; Sekita et al. 2008) promoted the screening of related domesticated genes in humans and mice. Importantly, eutherians have nine additional genes domesticated from the same LTR retrotransposon family, which accordingly were named sushi-ichi-related retrotransposon homologs (*SIRH3–11*) [alternatively called *MART* or *SUSHI* genes and recently renamed retrotransposon Gag-like (*RTL*)] (Brandt et al. 2005; Youngson et al. 2005; Ono et al. 2006) (Figs. 15.1 and 15.3). Importantly, they are “eutherian-specific,” like *PEG11/RTL1*, because there are no orthologous genes present in marsupials (Ono et al. 2011). On the other hand, marsupials have another *SIRH12* that is absent from eutherians, suggesting that the expansion of the *SIRH/RTL* genes occurred independently in the marsupial and eutherian lineages except for *PEG10*. The tammar wallaby, an Australian marsupial species, has *SIRH12*, while opossum, a South American marsupial species, has *pseudoSIRH12* along with many