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Review

Getting to the bottom of anal evolution

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ABSTRACT

The digestive tract is an essential organ system that allows animals to efficiently digest food and take up nutrients to maintain growth and sustain the body. While some animal groups possess a sack-like gut, others possess a tube shaped alimentary canal with an opening for food uptake, the mouth, and an opening for defecation, the anus. The evolutionary origin of the hindgut with the anal opening remains unclear. Bilaterally symmetric animals (Bilateria) have a great diversity of gut architectures and also show a great variety in the development of this important organ system. In this review, we summarize current knowledge about gut morphology, its development, and the underlying molecular mechanisms of the alimentary canal of Bilateria. We focus on gastrulation, the fate of the blastopore and its connection to hindgut and anus development in different animal taxa. We evaluate scenarios about bilaterian evolution on the basis of recent insights of animal phylogeny and widening knowledge about animal developmental diversity.

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1. Introduction

The digestive tract of animals is an essential organ system. It allows animals to efficiently digest food and take up nutrients to maintain growth and sustain the body. The animal gut can have different shapes and different degrees of specializations, and it is often connected to other organ systems, such as excretory organs (nephridia) and gonadal structures (Schmidt-Rhaesa, 2007; Yonge, 1937). While some animal lineages show the absence of a digestive tract (e.g., Porifera, Placozoa, Cestoda, Acanthocephala), others possess a sack-like gut with only one opening to the environment functioning both for food uptake and excretion (e.g., Ctenophora, Cnidaria, Acoelomorpha, Platyhelminthes, Gnathostomulida). The sack-like digestive tract can be elaborated by the evolution of additional branches of the digestive tract (e.g., triclads, polyclads), the evolution of additional holes, and subdivisions by lamellae-like structures.

Many animals possess an alimentary canal (through-gut), with an opening for food uptake, the mouth, and another opening for defecation, the anus. Such unidirectional gut is found in many bilaterian lineages, and is often subdivided in highly specialized regions (such as stomachs), since the food in general passes only once through each area. Specializations in the ectodermal mouth region (i.e., foregut) include muscular (pharynx) or cuticular (teeth) structures that help to grasp, suck and process food. The endodermal midgut is mainly specialized on the secretion of digestive enzymes by gland cells and absorption of nutrients through phagocytotic and pinocytic cells that can be aligned along the epithelia of different regions (stomachs, intestines) (Marianes and Spradling, 2013; Yonge, 1937). The midgut cells thus provide the nutrients to the rest of the body by using a blood-vascular system, coelomic cavities, active transportation, or diffusion. The hindgut and the anal opening are often of ectodermal origin, and correspond to the region where the remnants of food are discharged. A hindgut region is sometimes lacking, as the anal opening can get fused with the openings of the excretory system and/or reproductive organs (cloaca). A one-way gut enables the processing of food much more efficiently and allows the uptake of food while the animal is still digesting. The evolution of such alimentary canal was in some lineages likely connected to the evolution of larger or at least longer body sizes. However, despite this clear advantage for food digestion, some groups have secondarily reduced the alimentary canal to a sack-like gut (e.g., ophiuroid echinoderms, terebratellid brachiopods, nectonemaoid nematomorphs, asplanchnoid rotifers, urodasyoid gastrotrichs).

This review focuses on the morphology, development, and evolution of the opening that makes the one-way gut what it is – the anus. It is the anal opening that discriminates the sac-like gut from the unidirectional alimentary canal, since recent data suggest that the mouth openings of bilaterians seem to be homologous (see Arendt et al., 2001; Hejnol and Martindale, 2008a). Understanding the evolutionary origin(s) of the anus is key to understand the evolution and diversification of animal body plans, and thus, deserves special attention.

2. Gut morphology in the Bilateria

A tripartite digestive tract that comprises an ectodermal foregut or stomodaeum, an endodermal midgut, and an ectodermal hindgut or proctodaeum is widely distributed in the Bilateria (Schmidt-Rhaesa, 2007). However, the digestive tract architectures in this group of animals differ in its cell type composition and lineage specific specializations, which result in an astonishing variation that sometimes makes it difficult to distinguish the different gut parts in certain animals (Fig. 1).

2.1. The beneficial architecture of a unidirectional alimentary canal

The common structure of an alimentary canal is a tube that extends from the anterior mouth to the posterior anus (Schmidt-Rhaesa, 2007). Several sessile taxa, of which some of them live in tubes, possess a so-called U-shaped gut with a curved midgut and the mouth and anus are close to one another. Examples are found in bryozoans, entoprocts, brachiopods, phoronids, pterobranchs, and sipunculans. The close proximity of mouth and anus does not seem to be a problem, with ciliary structures and a concerted regulation preventing the interference between food uptake and defecation. The subdivision of the alimentary canal into foregut, midgut, and hindgut usually follows embryological criteria in which fore- and hindgut are invaginations of the ectoderm and solely the midgut is of endodermal origin. However, in different animal groups this separation is not as "clear cut". In arthropods, the foregut and hindgut are covered with cuticle of ectodermal origin and are molted. Here, cuticular specializations such as the chitinous ridges of the mastax indicate its ectodermal origin. In other animal groups, it is more difficult to draw a border between the ectodermal and endodermal parts of the digestive system (Zorn and Wells, 2009). The inclusion of molecular data makes the traditional discrimination between midgut and hindgut more difficult since the molecular boundaries do not always coincide with the morphological and developmental boundaries (see e.g., *Capitella*, Fröbius and Seaver, 2006; Meyer et al., 2010). Furthermore, it has been shown that in some cases a malfunction of a single gene can change the fate of midgut precursors to cells with hindgut properties (see e.g., Reuter, 1994).

Despite the fact that a through gut is of great advantage as it allows the unidirectional movement of the food throughout the body, several mostly-parasitic lineages and several species that live in anoxic environments have secondarily completely reduced the digestive tract. Major groups to name are acanthocephalans, cestodes, *Acholades asteris* (rhabdocoel platyhelminth) (Jennings, 1989), siboglinids (Katz et al., 2011), and also rare cases in other groups such as in oligochaetes (Giere, 1985), rhizocephalan crustaceans, and molluscs (Felbeck, 1983). Such complete loss of the digestive tract is usually connected with the ectodermal uptake of nutrients in parasites, and the hosting of symbiotic bacteria responsible for novel biochemical pathways for reducing sulfate in anoxic environments.

### 2.2. The blind gut – a common theme in the Bilateria

In other bilaterally symmetrical animals, the digestive tract is composed out of a sack-like gut that lacks the anal opening, similar to that in cnidarians and ctenophores. We find such a blind gut in xenoturbellids, acoelomorphs, gnathostomulids, platyhelminthes and micrognathozoans. It remains an open question whether the absence of an anal opening is plesiomorphic for the Bilateria (Hejnol and Martindale, 2008a,b, 2009; Schmidt-Rhaesa, 2007), since it largely depends on progress in resolving the phylogenetic framework of the major bilaterian groups (Dunn et al., 2014; Edgecombe et al., 2011).

2.2.1. The case of Acoelomorpha
Acoelomorphs are simple, bilateral worms that lack coeloms and a through gut (Bourlat and Hejnol, 2009). The lack of an anal opening was one of the few morphological arguments that were used to group acoel flatworms into the Platyhelminthes (Ax, 1996; Ehlers, 1985; Hyman, 1951; von Graff, 1891). With the emergence of the alternative hypothesis of the phylogenetic position of acoels as sister group to all remaining Bilateria (Carranza et al., 1997; Ruiz-Trillo et al., 1999), the absence of an anus in this group of animals was interpreted as a reflection of the bilaterian ancestral state

(Hejnol and Martindale, 2008a). Given that the acoelomorphs with the single opening to their digestive tract are the sister group to the remaining Bilateria (Nephrozoa), how does their mouth relate to the mouth of other animals? The acoel mouth might correspond to the single opening of the Cnidaria, but it could be that it is the opening that in the last common ancestor of Bilateria gave rise to both the mouth and anus of some of the Nephrozoa by extension and separation along the anterior–posterior axis. The expression of the genes *brachyury* and *goosecoid* has been used to homologize the mouth between hemichordates and annelids: in the mouth, a combined expression of *brachyury* and *goosecoid* is present, while the anus is lacking the *goosecoid* expression and only expresses *brachyury* (Arendt et al., 2001). The closer inves-

tigation of these marker genes in acoels reveals that the single opening of the digestive tract of acoelomorphs likely corresponds to the mouth of cnidarians and the remaining Bilateria since it shows the combined expression of *goosecoid* and *brachyury* (Hejnol and Martindale, 2008a). Interestingly, marker genes such as *caudal/cdx* and *brachyury* are expressed in the male gonopore of the two acoel species investigated so far, which might correspond to the posterior *brachyury* domain that lacks *goosecoid* expression (Hejnol and Martindale, 2008a; Moreno et al., 2011) (Fig. 2). The authors stated the possibility that the posterior expression of these hindgut-markers in the male gonopore of acoels could indicate a close evolutionary relationship of the bialaterian anus and the acoel male gonopore (Hejnol and Martindale, 2008a). This is supported
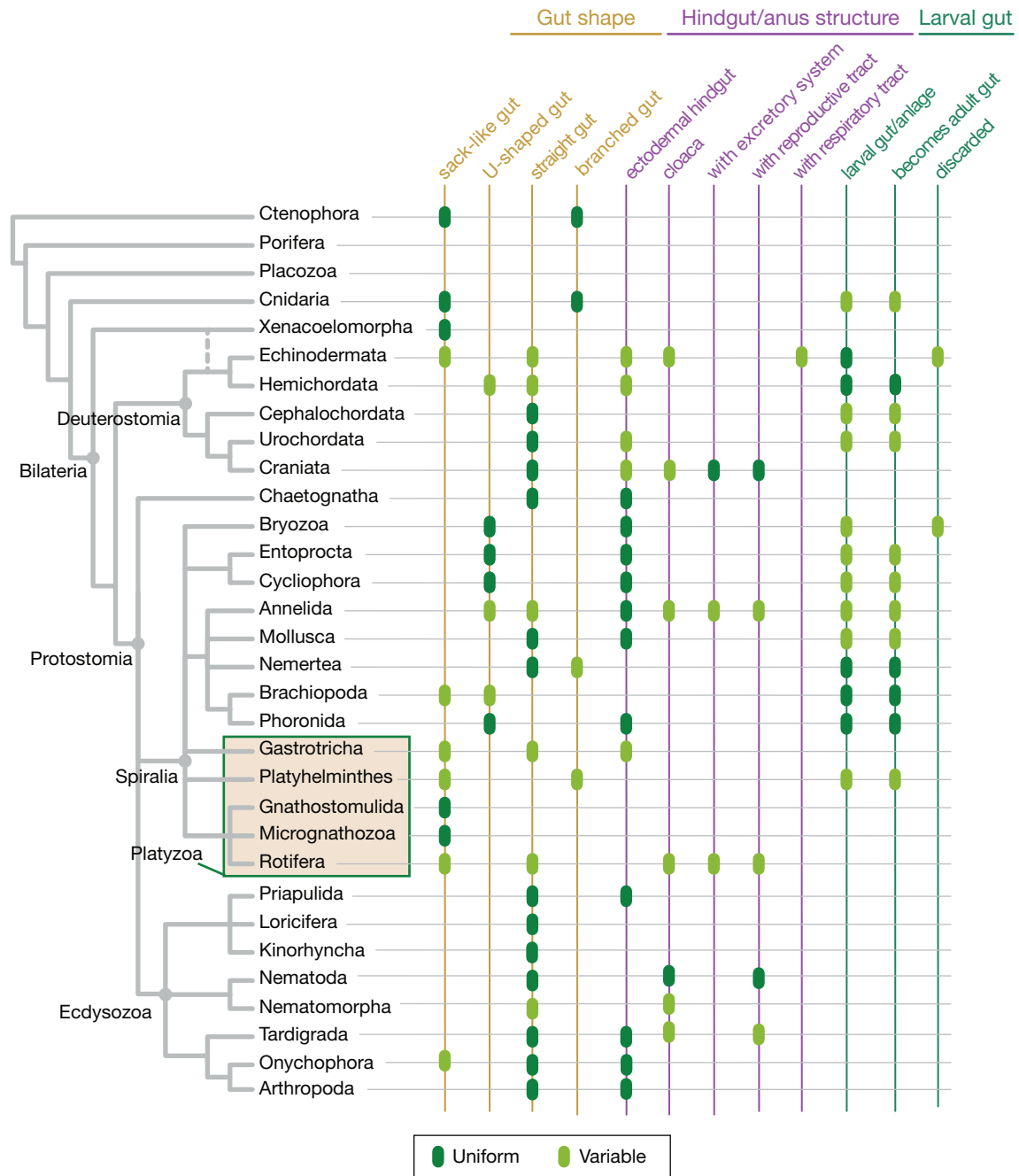
Fig. 1. Gut architecture and hindgut types across animal lineages.
Diversity of gut morphologies and types of hindguts observed in the different metazoan lineages, in relation to the most recent animal phylogeny (Dunn et al., 2014). The diagram is a simplification, and exceptions from the general coding are possible.
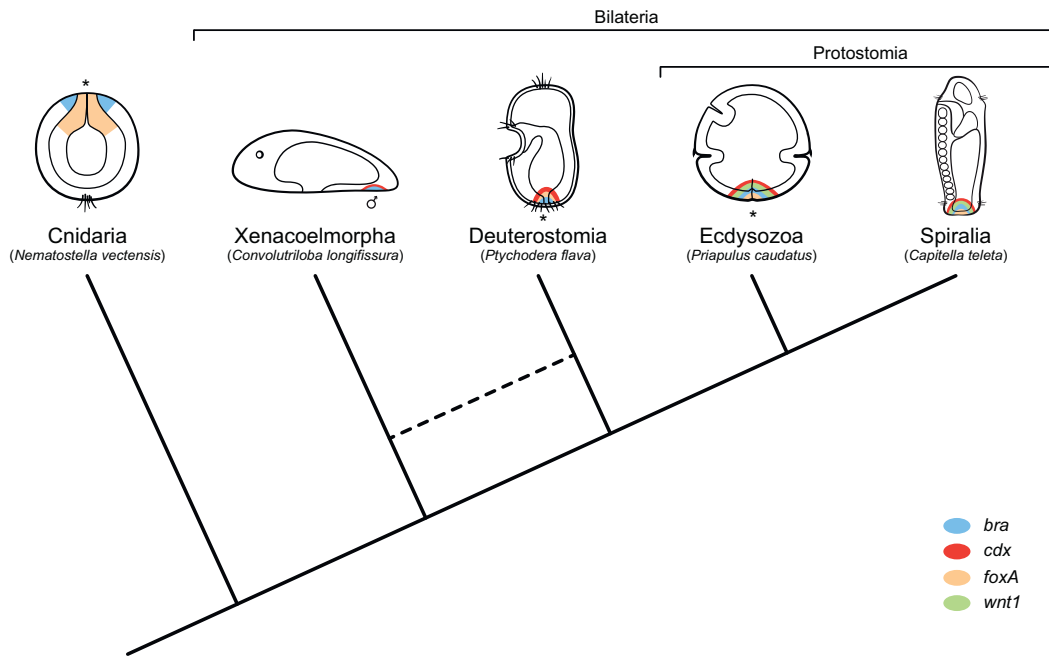
Fig. 2. Position of the anus relative to the expression of hindgut markers.
The genes *brachyury* (*bra*), *caudal* (*cdx*), *foxA* and *wnt1* are consistently expressed in the hindgut region of members of the Deuterostomia and Protostomia, as well as in the male copulatory opening of acoels (male symbol), suggesting the last common ancestor of bilateria had a sack-shaped gut and the anus evolved later. Only gene expression related to the hindgut is indicated in this diagram. Areas of color do not represent actual gene expression domains. For references on the expression data, see main text. The asterisk denotes the position of the blastopore, and the dashed line in the cladogram the alternative position of Xenacoelomorpha as members of the Deuterostomia.

by the fact that all acoelomorphs possess a male gonopore through which the male gametes are released. An alternative interpretation of the data would be that the marker genes *cdx* and *brachyury* are not useful for homologizing structures such as the hindgut/male gonopore, and are just related to the process of ectoderm invagination. However, these genes are not expressed in other openings, as for example the female gonopore of acoels, despite the fact that male and female gonopore share similar cell arrangements (Hejnol and Martindale, 2008a). Furthermore, within the acoels, the position of the mouth can vary between the very anterior (*Hofstenia*) and the posterior region (*Diopisthoporus*) of the body (Hooge, 2001; Rieger et al., 1991). Interestingly, the position of the male gonopore in *Hofstenia* is just posterior to the anterior mouth and not at the posterior tip of the animal. A more thorough investigation of these species would provide a good test of the hypothesis of an evolutionary relationship between the acoel male gonopore and the bilaterian hindgut (Hejnol and Martindale, 2009). It remains unclear if the same orthologous genes are expressed in the male gonopore of the nemertodermatids, the sister group of Acoela. Nemertodermatids have more plesiomorphic characters than acoels regarding their digestive tract, which is epithelial – just as in other animals – in contrast to the digestive syncytium of acoels (Smith and Tyler, 1985).

While large-scale phylogenomic analyses support the position of the Acoelomorpha + *Xenoturbella* (Xenacoelomorpha) as sister group to all remaining Bilateria (Hejnol et al., 2009; Srivastava et al., 2014), a previous phylogenomic study concludes an affiliation of the Acoelomorpha as sister-group to the Ambulacraria, albeit with low support (Philippe et al., 2011). Although a plesiomorphic absence of the anal opening cannot be excluded, the secondary reduction of the connection with the male gonoduct would seem more likely. In case the acoels are indeed the sister group to all remaining Bilateria, the last common ancestor of Bilateria would likely have possessed a sack-shaped gut and the

anus would have been evolved later (Hejnol and Martindale, 2008a) (Fig. 2).

2.2.2. The blind gut in "Platyzoa"

Many other bilaterian taxa with a sack-like gut belong to the group Platyzoa (Cavalier-Smith, 1998; Dunn et al., 2014; Giribet et al., 2000, 2009; Hejnol, 2010): Gnathostomulida, Micrognathozoa and Platyhelminthes. Gnathostomulids and Micrognathozoa group together with Rotifera to from the Gnathifera (Ahlrichs, 1995; Dunn et al., 2014; Laumer et al., 2015; Struck et al., 2014; Wey-Fabrizius et al., 2014; Witek et al., 2009, 2008). Most recent molecular analyses support Micrognathozoa – with its single species *Limnognathia maerski* – as sister group to all remaining Gnathifera (Laumer et al., 2015). Gnathostomulida with its sack-like gut would thus form the sistergroup to the Rotifera, of which most species possess a through gut with a cloaca that connects the gut and excretory organs, and sometimes the female opening (Fig. 1). In this arrangement, it is most parsimonious to assume the lack of the anus to be plesiomorphic for Gnathifera but this also depends on the sistergroup of the Gnathifera. If Platyzoa is monophyletic, this would be the Platyhelminthes and/or Gastrotricha. Platyhelminthes lack an anus, while gastrotrichs primarily have an anus (except *Urodasys*). The variation of gut morphologies in Platyzoa hinders the reconstruction of the ancestral state for the whole taxon. Furthermore, the most recent molecular phylogenies give less support for a monophyletic Platyzoa (Laumer et al., 2015; Struck et al., 2014). Instead "Platyzoa" renders to be polyphyletic, with Lophotrochozoa (sensu Halanych et al., 1995) nested within the Platyzoa (Laumer et al., 2015; Struck et al., 2014). In case paraphyletic Platyzoa receives further support in the future, it will change our view of the evolution of many body plan features within the Spiralia as a whole. Platyzoa are mainly small, acoelomate taxa and possess rather simply organized organ systems. Lophotrochozoa (Halanych et al., 1995) would have originated from such

ancestral microinvertebrate and the rather complex organ systems of annelids and molluscs would be based on convergent evolution (Struck et al., 2014). This result would also imply evidence for the multiple evolution of an anus within the Spiralia.

2.3. Transient anuses, new anal openings and multiple losses

The evolutionary history of digestive tracts is characterized by multiple intraphyletic changes from a unidirectional gut to a sack-like gut and vice versa. There are several cases in which additional openings, connections between openings of different organ systems, and reductions of a through gut to a sack-like gut can be identified. Ultrastructural investigations of some species led to the statements about the presence of a so-called temporary or transient anus in the gnathostomulid *Haplognathia* (Knauss, 1979) and the micrognathozoan *Limnognathia* (Kristensen and Funch, 2000). There are no direct observations of defecation through this possible opening and it is the absence of the basement membrane and the intercalation of the cells that led the authors to hypothesize the presence of a transient opening. A recent detailed study of the musculature of *Limnognathia* suggests that two longitudinal muscles might be involved in the defecation process (Bekkouche et al., 2014). Interestingly, in both species the potential temporal opening is on the dorsal side and not on the ventral or posterior tip of the animal (Knauss, 1979; Kristensen and Funch, 2000), which can indicate that it is not related to a proctodaeum of other animals.

While most Platyhelminthes lack an anus, there are several described cases in which anal openings have evolved independently in this group. Karling (1965) describes for the macrostomid *Haplopharynx* the presence of an anal pore, and several polyclad species possess multiple anal pores on their dorsal side (Kato, 1943; Lang, 1884). This secondary evolution of anal openings seems to be connected with the evolution of the extension of body length (*Haplopharynx*) or overall size of the body (polyclads) (Ehlers, 1985).

In addition to multiple observations of the independent gain of anal openings, there are also multiple cases of losses that resulted in the evolution of a blind gut. In the deuterostomes, several cases are known for echinoderms. The brittle stars (Ophiuroidea) lack an anus (Schechter and Lucero, 1968) and asteroid members of the large taxon Paxillosida possess a blind gut (Jangoux, 1982). Another deuterostome example for the reduction of an anus is found in the fast evolving appendicularians, which usually possess a through gut, but *Appendicularia sicula* lacks the anal opening (Brena et al., 2003). In the Ecdysozoa some shell-less cirripedes (*Trypetesa*) and mites (Acari) lack an anus (Mitchell, 1970), marine nematomorphs (*Nectonema*) possess a gut with blind end (Skaling and MacKinnon, 1988), and male nematodes lack an anus. In addition to the well-known example of the Platyhelminthes, several lineages of the Spiralia have taxa that have reduced an anus: this opening is lacking in rotifers of the group *Asplanchna*, but here the cloacal opening persists and forms only the opening for excretion and the release of the eggs (Clément and Wurdak, 1991). The gastrotrich *Urodasys* lacks an anus (Schoepfer-Sterrer, 1974), and in the Brachiopoda the terebratellid brachiopods (such as for example *Terebratalia transversa*) the short gut of the adult has lost its anal opening, in contrast to the linguliform and craniiform brachiopods (James, 1997).

This incomplete list of evolutionary transitions of anal aperture morphology indicates that making assumptions about ease of gain and loss events is problematic. The phylogenetic framework is the basis for the identification of the direction of the evolution of a through gut in each clade, and thus across all Bilateria. In the following sections, we will explore how developmental and molecular data might help clarify these evolutionary transitions.

3. Hindgut development in Bilateria

3.1. Anus development in different animal taxa: timing and relation to the blastopore

One key event in the development of the gut of bilaterians is gastrulation. During this process, the endodermal and mesodermal precursors that will form the future internal organ systems get internalized into the embryo. Gastrulation varies between animals, and a high number of molecular processes and diversity of cellular mechanisms coordinate this process (Stern, 2004). Bilaterians differ from non-bilaterian groups in that they gastrulate at the vegetal pole and not at the animal pole (Martindale and Hejnol, 2009). The site of endomesoderm internalization is called the blastopore, an opening that in some embryos gets coopted either for mouth formation or anus formation (Lankester, 1877).

The development of the digestive tract begins with the separation of the endodermal cells from the mesodermal cells followed by a progressing determination and differentiation of the cells that will form the major portions of the digestive tract. The separation of the mesoderm can already be completed at the beginning of gastrulation, or cells can immigrate as endomesoderm with a later separation inside the embryo, for example by a process called enterocoely (Salvini-Plawen and Splechtna, 1979; Technau and Scholz, 2003). In many cases, the site at which the cells were internalized does not remain open but is closed by the outer, ectodermal cells (Hejnol and Martindale, 2009) – a process that is called blastopore closure. How the anal opening relates to the blastopore and the future digestive tract openings can vary between animals. The formation of the hindgut and the anal opening differs in timing and location in relation to the other embryonic events of digestive tract formation between animal groups (Table 1). In some animals the blastopore gives rise to the later anal opening, which is the case for non-chordate deuterostomes, priapulids, nematomorphs, and some arthropods (Hejnol and Martindale, 2009; Martín-Durán et al., 2012). However, often the blastopore closes as for example in chaetognaths and chordate deuterostomes, or embryonic events such as elongation and axis formation cause a translocation of the blastopore to the future anterior part of the animal. In the two latter cases, the anus is usually formed later during development (Hejnol and Martindale, 2009). The earliest formation of an anal opening occurs in all non-chordate deuterostomic embryos in which the blastopore corresponds to the anus (Table 1). In chordate deuterostomes, the blastopore-anus relationships is modified by the growth of the post-anal tail, and the blastopore closes in all cases and the anus forms elsewhere (Lacalli, 2010). In *Branchiostoma*, the blastopore – often called 'primary anus' – is overgrown during neurulation and opens into the neurenteric canal. Later, the anus is formed newly on the right side and migrates later in development to the left side of the body (Stokes and Holland, 1995). In most vertebrate embryos the post-anal tail growth impacts the positioning of the cells that were at the edge of the closed blastopore (Bijtel, 1931; Davis and Kirschner, 2000; Kanki and Ho, 1997).

Most embryos form the anal opening rather late in development – usually after the mouth has been formed (Table 1). In embryos that form the anus late in development, the ectoderm forms an indentation that connects to the neighboring endoderm of the gut. In hindguts that are formed by many cells, the different cellular components undergo the usual sequence of organ system formation: the specification of the cells to form the hindgut primordium, followed by cell differentiation that impacts shape and physiological properties (Annunziata and Arnone, 2014; Wu and Lengyel, 1998). Mesodermal musculature and its innervation develop in those cases in which the anal opening has a sphincter (Andrikou et al., 2013; Kondo et al., 1996).

Table 1
Referenced table with timing/position of anus development in relation to gastrulation and blastopore.

Taxon	Embryonic position	Embryonic timing	References
Acoelomorpha*	(Posterior end)	(After hatching)	Børve and Hejnol (2014) and Ladurner and Rieger (2000)
Hemichordata	Blastopore	Gastrulation	Heider (1909)
Echinodermata	Blastopore	Gastrulation	Annunziata et al. (2014)
Craniata	Blastopore	Gastrulation	Gilbert and Raunio (1997)
Urochordata	Posterior end	After mouth	Conklin (1905)
Cephalochordata	Blastopore	Gastrulation	Conklin (1933)
Chaetognatha	Posterior end	After mouth	Hertwig (1880)
Nematoda	Posterior end	After mouth	Schulze and Schierenberg (2011)
Nematomorpha	Blastopore	Gastrulation	Montgomery (1904)
Tardigrada	Posterior end	With mouth	Hejnol and Schnabel (2005)
Onychophora	Posterior end	After mouth	Eriksson and Tait (2012)
Arthropoda	Posterior end	Variable	Anderson (1973) and Gerberding and Patel (2004)
Priapulida	Blastopore	Gastrulation	Martín-Durán and Hejnol (2015)
Loricifera	?	?	–
Kinorhyncha	Posterior end	With mouth	Kozloff (2007)
Bryozoa	Posterior	After mouth	Stricker (1989)
Entoprocta	Posterior	After mouth	Marcus (1939)
Cycliophora	?	?	–
Annelida	Posterior end	After mouth	Anderson (1973)
Mollusca	Posterior	After mouth	Conklin (1897); Lyons et al. (2015)
Nemertea	Posterior	After mouth	Iwata (1985)
Brachiopoda	Posterior-dorsal	After mouth	Nielsen (1991)
Phoronida	Posterior end	After mouth	Rattenbury (1954)
Gastrotricha	Posterior end	After mouth	Sacks (1955) and Teuchert (1968)
Platyhelminthes	–	–	–
Gnathostomulida	–	–	–
Micrognathozoa	–	–	–
Rotifera	Posterior end	After mouth	Zelinka (1891)

3.2. The larval gut and its anus

Relevant for the discussion of the evolution of the alimentary canal, is the question of how the larval gut of animals with biphasic life cycle is related to the adult gut. In planktotrophic larva, the larval gut is the first alimentary canal that is formed during gastrulation and this raises the question of the comparability of the larval gut with the gut of the adult. Larval planktotrophy has played an important role in discussions about the evolution of the biphasic life cycle (Strathmann, 1993), and some scenarios that suggest a 'larva'-like ancestor of the Bilateria (Nielsen, 2009). Can the larva be seen as a 'second body' plan and thus a different entity to the adult reproductive stage? How different is the formation of the larval gut in comparison to the formation of the gut of direct developers? We face a fascinating diversity of different life cycle strategies in many animals and on the first view it seems difficult to find a common pattern. However, when we focus only on the digestive tract, and more specifically on the hindgut, a comparison between taxa might be more feasible. Fig. 1 summarizes taxa that possess a larval gut or anlage, and indicates taxa in which the larval gut is at least to a large part transferred to the adult. Only in very rare cases is the larval gut a specific larval organ system such as in the bryozoan cyphonautes (Stricker, 1989). Echinoderms transfer only the endodermal stomach to the adult, while ectodermal portions are discarded during metamorphosis (Chia and Burke, 1978). Descriptions of the 'larva' as separate body plan might be justified for some taxa for example echinoderms (Raff, 2008), but not for most of the animals. Echinoderms also seem to be the outliers in mouth development, while the larva still has the larval mouth, a second mouth can be formed in the rudimentary adult which suggests that a mouth-specific developmental program can be active in the same individual at two different places (Lacalli, 2014). Despite its different development and role in lecithotrophic and planktotrophic larva, the gut is patterned by the same genes as the gut of direct developing species. However, it seems the case at least for some of the spiralian larvae, such as the nemertean pillidium and various polyclad larvae, that they are new 'intercalated' dispersal stages not related to other larvae (Maslakova, 2010; Rawlinson, 2010, 2014). Interestingly, in the nemertean feeding larva, the pillidium, the gut is only sack-like and lacks the anal opening which is only formed after metamorphosis (Norenburg and Stricker, 2002). However, what defines a larva is for most cases blurred by the fact that the amount of larval material that is transferred to the adult differs even between closely related species. The knowledge about the transition of 'larval' organs to the adult is fundamental for the classic discussion about the 'larva-first'-hypothesis (Page, 2009). Pure 'larval' organs are rare and are limited to apical organs, ciliary bands and specialized portions of the nervous system (Strathmann, 1993). Other 'larval' organs can be often seen as heterochronic ('paedomorphic') adult organs, which develop already in the dispersal stage before they get modified to the adult morphology during morphogenesis. In other cases, larval organs can be formed by the recruitment of adult patterning systems to the larval stages (Love et al., 2008; Raff, 2008). In most cases, the larval formation of the gut corresponds to the formation of the adult digestive tract since it will get transferred to the adult. This means that most cases allow a direct comparison of the molecular digestive tract patterning of the larval gut with the adult gut since both are identical. Recent studies indeed show that similarities in the molecular system between direct and indirect developers are high (Annunziata et al., 2014). However, care must be taken when comparing anlagen of the adult gut present in larvae, since they are often non-functional, rudimentary anlagen. We can summarize, however, that the larval gut including its anal opening – when present – is transferred to the adult gut in most cases (Fig. 1). The exception from this general observation is the bryozoan cyphonautes in which the whole gut including the anus is discarded during metamorphosis. Studies of gene expression of hindgut markers in different cases of planktotrophic and lecithotrophic larvae are still scarce so that a general picture cannot be drawn. However, echinoderms that discard nearly the whole digestive tract still show the hindgut patterning genes expressed in an expected pattern (Annunziata et al., 2014). The lecitotrophic coronate larva of the bryozoan *Bugula neritina* that lacks an anlage of the gut, shows *cdx* expression in the blastemic cells that give rise to the adult gut (Fuchs et al., 2011). These data show that the expression patterns of hindgut genes can vary according to the future fate

of the larval digestive tract and each case needs to be examined closely.

3.3. Conserved molecular program for gut development

The molecular background of the development of the digestive tract in bilaterians has been mainly studied in the classical model systems (Annunziata et al., 2014; Marianes and Spradling, 2013; Roberts, 2000; Sheaffer and Kaestner, 2012; Stainier, 2002, 2005) and to some extent also in other species. Despite the morphological, developmental and lineage specific differences in these species, a set of conserved transcription factors and signaling molecules seem to play a common role in gut formation across bilaterians (Heath, 2010; Lengyel and Iwaki, 2002). However, the evolutionary distance between the classical model systems and their usually common fast rate of molecular evolution, which is often combined with excessive gene loss, limits the amount of genes that are available as candidate markers for comparisons with non-model species. In addition, there are a number of lineage specific genes that play an important role in hindgut formation in *Drosophila*, but these genes are absent in non-arthropods (Domazet-Loso and Tautz, 2003). In *Drosophila* for example *torso*, *huckebein*, and *bowl* are lineage specific genes of arthropods or ecdysozoans and have been recruited for the digestive tract later during evolution (Iwaki et al., 2001). We can expect that future investigations of non-model species will provide a more extensive list of candidate genes that have been lost in the species *Caenorhabditis elegans* and *Drosophila melanogaster* (Ruvkun and Hobert, 1998). Conserved transcription factors and signaling molecules in the posterior region of the alimentary canal are *caudal/cdx*, *brachyury*, *Wnt* ligands, *even-skipped/evx*, *foxA/HNF3* and posterior *Hox* genes (Fig. 3). The expression of these 'hindgut' markers is not germ layer specific: in vertebrates, these markers are expressed in the posterior endoderm, while in arthropods in the posterior ectodermal part of the gut, and in *Caenorhabditis* in the endo- or ectoderm. It is thus often mainly the combination of the expression patterns of these genes in comparable morphological structures that can hint to homology assumptions. One has to keep in mind that these genes are not only playing a role in digestive tract formation, but are additionally involved in multiple developmental processes and essential for the formation of other germ layers and organ systems. *Brachyury*, for example has a well-known additional role in mesoderm specification and when embryonic cells undergo epithelial–mesenchymal transition (Technau, 2001; Turner et al., 2014). In the following sections, we will discuss the role of these genes in hindgut development in more detail and we include descriptions of expression patterns in bilaterian outgroups.

3.3.1. Caudal/cdx

The ParaHox gene *caudal/cdx* encodes a homeodomain transcription factor that plays an important role in hindgut patterning in vertebrates and insects (Duprey et al., 1988; Wu and Lengyel, 1998). The three ParaHox genes *gsx*, *xlox* and *cdx* have been affiliated with the evolution of anterior to posterior gut patterning across the Bilateria (Holland, 2001, 2015), although several bilaterian lineages have lost one or more of the ParaHox genes (Fröbius and Seaver, 2006; Martín-Durán and Romero, 2011). As shown in Fig. 3, the gene *cdx* is present in most bilaterian genomes sequenced so far, and its hindgut expression is largely consistent in all investigated species (Annunziata et al., 2013; Brooke et al., 1998; Cole et al., 2009; Edgar et al., 2001; Fröbius and Seaver, 2006; Hryniuk et al., 2012; Hui et al., 2009; Ikuta et al., 2013; Joly et al., 1992; Katsuyama et al., 1999; Kulakova et al., 2008; Martín-Durán et al., 2012; Samadi and Steiner, 2010; Schulz et al., 1998; Wu and Lengyel, 1998). In mouse, the *cdx2* paralog is expressed in the early posterior endoderm, and functions as the upstream regulatory gene that promotes intestinal cell fates (Heath, 2010). In *Drosophila*, *cdx* is expressed as a

gradient with its highest levels at the posterior pole of the embryo, and is essential for the invagination of the hindgut primordium (Wu and Lengyel, 1998). In *C. elegans*, the *cdx* ortholog *pal-1* is involved in rectum development, among other functions (Edgar et al., 2001). Altogether, expression and functional data strongly support a conserved role of *caudal/cdx* genes in developing posterior structures, and in particular the hindgut region. Outside the Bilateria reports about the presence of a clear *cdx* ortholog are ambiguous. It is absent from the placozoan genome, and in the genome of the anthozoan cnidarian, *Nematostella vectensis*, a gene *NVHD065* which shares features with the bilaterian parahox genes *Xlox* and *Cdx* has been identified (Ryan et al., 2007). *NVHD065* is expressed asymmetrically along two mesenteries and its expression is difficult to relate to any pattern in bilaterians (Ryan et al., 2007). More recently, a possible *cdx* ortholog has been detected in calcisponges where it is uniformly expressed in the whole choanoderm (Fortunato et al., 2014). However, its relation to the bilaterian *cdx* remains only weakly supported and further investigations are required to confirm its orthology.

3.3.2. Brachyury

Brachyury is related to gastrulation and digestive tract development in most metazoans (Technau, 2001). The expression of *brachyury* around the blastopore in cnidarians (Scholz and Technau, 2003) has been interpreted as a support for a common origin of the bilaterian mouth and anus from the single cnidarian opening to the digestive tract (Arendt, 2004). Indeed, in some animals, such as echinoderms, hemichordates, annelids, and molluscs, *brachyury* is expressed in both the mouth and the anus (Arendt et al., 2001; Boyle et al., 2014; Gross and McClay, 2001; Lartillot et al., 2002b; Röttinger and Martindale, 2011; Tagawa et al., 1998), with the mouth domain lost in Ecdysozoa (Kispert et al., 1994; Martín-Durán et al., 2012; Singer et al., 1996) and Chordata (Holland et al., 1995), except urochordates (Yasuo and Satoh, 1994) (Fig. 3). On the other hand, chaetognaths seem to have lost the hindgut expression (Takada et al., 2002). The variability of *brachyury* expression may indicate a diminishing role for this gene in foregut and/or hindgut formation in several bilaterian lineages (Arenas-Mena, 2013). In this regard, *brachyury* was lost in the lineage to the nematode *Caenorhabditis* (Pocock et al., 2004; Woollard and Hodgkin, 2000) and also in the lineage to planarians (Martín-Durán and Romero, 2011). While in Platyhelminthes this loss can be correlated with the possible loss of an anus, nematodes develop a hindgut despite the absence of *brachyury*. However, the variability of expression domains and developmental functions reported for *brachyury* likely indicates that this gene is not a specific marker for mouth and/or anus. Instead, *brachyury* seems to have a conserved role in early stages of mesoderm determination and internalization (Marcellini, 2006; Technau, 2001; Technau and Scholz, 2003), and it is thus, the diversity of modes of mesoderm development that accounts for the variability in the expression domains of this gene across metazoan lineages.

3.3.3. FoxA/HNF3

The forkhead-box containing protein *foxA* (also known as *hepatocyte nuclear factor 3*, *HNF3*) has a broadly conserved expression in the endoderm and digestive system of metazoan embryos (Boyle et al., 2014; Hejnol and Martindale, 2008a; Martindale et al., 2004), and it is considered a core regulator of the endodermal gene regulatory network (Davidson et al., 2002). It is thus expressed in the hindgut region in some protostome embryos (Azzaria et al., 1996; Boyle and Seaver, 2008, 2010; Lartillot et al., 2002a; Martín-Durán et al., 2012; Weigel et al., 1989), echinoderms (Annunziata et al., 2014), cephalochordates (Shimeld, 1997), and urochordates (Di Gregorio et al., 2001). Moreover, *foxA* is also expressed in the single gut opening of Platyhelminthes (Adler et al., 2014; Martín-
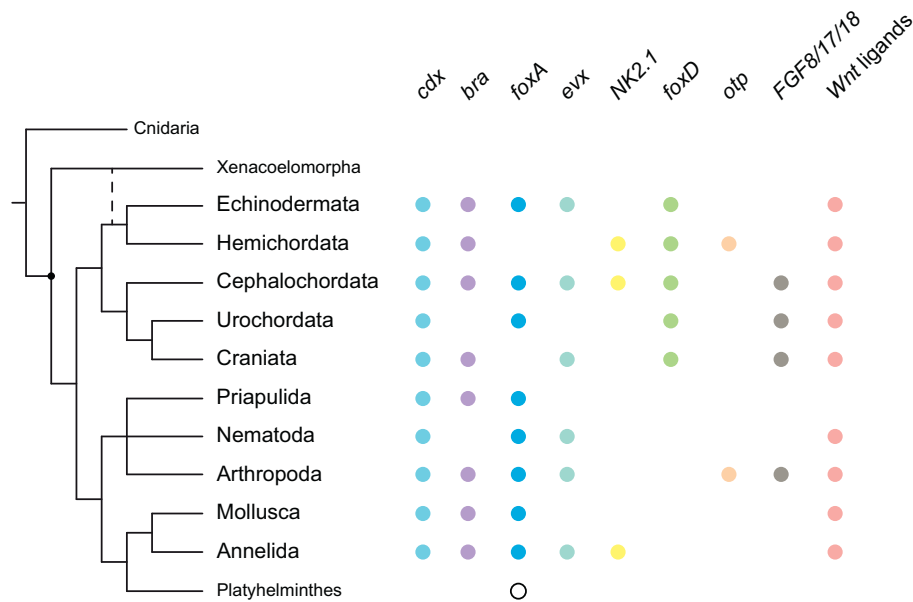
Fig. 3. Expression of hindgut genes in bilaterian lineages.
Presence/absence of expression of developmental genes associated with hindgut formation in different lineages of the bilateria. Full dots indicate presence of expression, absence of dot indicates lack of data and/or absence of expression in the studied species. For the Platyhelminthes (taxon with a sack-like gut), the circle in *foxA* indicates expression in the single gut opening. See main text for references.

Durán et al., 2010) and cnidarians (Martindale et al., 2004). It is difficult to assign a specific role in hindgut patterning for *foxA*, since the transcription factor seems to play a general role in digestive tract and endoderm patterning.

3.3.4. Other transcription factors

Apart from the transcription factors *caudal/cdx*, *brachyury*, and *foxA*, a variety of developmental genes are related to the formation of the hindgut (Fig. 3). For instance, the homeobox gene *even-skipped/evx* is consistently required for posterior and hindgut development in bilaterian lineages (Ahringer, 1996; Brown et al., 1997; de Rosa et al., 2005; Ferrier et al., 2001; Pyati et al., 2006; Ransick et al., 2002; Seaver and Kaneshige, 2006), and the NK gene *NK2.1* is expressed in the hindgut regions of hemichordates, cephalochordates, and annelids (Boyle et al., 2014; Takacs et al., 2002; Venkatesh et al., 1999). The paired gene *orthopedia* (*otp*) is required for hindgut specification in *Drosophila* (Iwaki et al., 2001), and it is also expressed in this region in hemichordates (Lowe et al., 2003). The forkhead domain containing protein *foxD* seems to be expressed in the hindgut region of the Deuterostomia (Fritzenwanker et al., 2014; Imai et al., 2002; Odenthal and Nüsslein-Volhard, 1998; Pohl and Knöchel, 2002; Tu et al., 2006; Yu et al., 2002), but seems to be absent from this region in the lineages of the Protostomia studied so far. The FGF ligand *FGF8/17/18* is also expressed in and associated with the development of the posterior gut in *D. melanogaster* (Stathopoulos et al., 2004), and chordates (Bertrand et al., 2011; Imai et al., 2004; Maruoka et al., 1998). In vertebrates, Hedgehog signaling is involved in cloaca development (Parkin et al., 2009; Seifert et al., 2009). Finally, evidence from multiple bilaterian lineages supports the role of *Wnt* ligands in posterior embryonic development, placing the canonical Wnt pathway as an upstream regulator of *caudal/cdx* (Martin and Kimelman, 2009; Petersen and Reddien, 2009). Altogether, the expression of these developmental genes in the hindgut region, which can be extremely evolutionarily conserved in some cases and not so much in many others, demonstrate how developmental pathways are prone to evolutionary modifications, integrating novel genes into existing networks, but also losing and/or replacing key elements. Higher taxon sampling across metazoans will reveal a better understand-

ing of these processes and how these integration and disintegration happens on the molecular level and finally how this is connected to the morphological outcome. The use of marker genes as a support for homology statements has to be made with care. Since so far only a handful of markers are known, only the combination of their expression in a comparable morphological structure can hint for common ancestry. Future tissue and single-cell sequencing efforts might reveal additional marker genes that can support homology hypotheses, that can be tested with the use of a solid phylogenetic framework.

4. The evolutionary origin of the through gut

The origin of the through gut is mainly a question about the evolution of the anus, since morphology and gene expression data suggest that the mouth opening of the major groups of bilaterians is homologous (Arendt et al., 2001; Hejnol and Martindale, 2008a; Martindale and Hejnol, 2009) [for chordates see discussion in Lacalli (2010)]. Although a specific discussion of this subject has not been specifically addressed yet, it has been an important element of several hypotheses about the evolution of bilaterian organ systems and body plans.

4.1. The 'bursa' and 'ductus intestinalis' in flatworms – a debate between zoologists

The origin of the anus has been subject to a historical debate between mainly german zoologists in the 1950s and early 1960s. During this pre-cladistic era, zoologists tried to explain evolution by assembling stages of evolutionary transformations of observed morphologies of different species ("evolutionsreihen", see Remane, 1971). This misleading approach also provoked the formulation of 'evolutionary trends', which usually lead from simple to complex. In addition, these hypotheses about assumed transformations were used to assemble the phylogenetic relationships of animals. With Willi Hennig's, revolutionary introduction of phylogenetic systematics (cladistics) (Hennig, 1950, 1966), evolutionary biology received its scientific ground, and 'tree-thinking' replaced the thinking of evolutionary series and trends (Gee, 1999). The progress

in molecular phylogenetics allows for the first time the assembly of the animal tree of life, which is independent from morphological characters, and thus, avoids the danger of circulatory reasoning (Dunn et al., 2014). Despite the progress, we repeat key-arguments from this debate, since they still play a role when discussing the evolution of an anus in the light of new data and the better knowledge of animal phylogenetic relationships.

In this debate, Platyhelminthes and Acoela were taken as the example to discuss the likeliness of the gain or loss of an anus (Reisinger, 1961). The phylogenetic position of Platyhelminthes, including the possible exclusion of the Acoela from the Platyhelminthes (Remane, 1951), played a major role in the discussion. Some flatworms (e.g., *Coelogynopora* [Proseriata], and Tricladida) possess a connection – Ductus genito-intestinalis – between the gut and the complex genital system (bursal organ) with female and male openings (Steinböck, 1924) (Fig. 4). The connection was thought to function as a pathway by which the fertilized eggs are transported to the gut to get extruded through the mouth opening of the adult. The female opening is in many cases functional for taking up sperm to fertilize the eggs, which later get transferred to the digestive tract. In some cases, however, the female opening is also used for laying the eggs. Important is that the blind gut is connected to the exterior at the posterior end over this ductus intestinalis. The evolutionary interpretation of the ductus intestinalis depends on the general view of the phylogeny and evolution of animals: Remane (1951), as a proponent of the 'Enterocoely-Hypothesis' which implies a rather complex coelomate ancestor, saw this architecture as remnant of the loss of an anus and thus as remnant of a hindgut. Steinböck (1966) and Karling (1940), who interpreted platyhelminthes and acoels as evolutionary ancestral groups with simple body plans, saw the structure as an evolutionary intermediate stage to gain a connection of the genital system with the digestive tract (Reisinger, 1961; Steinböck, 1966). The arguments raised for the different hypotheses were mainly on the morphological level, but also included ontogenetic arguments mainly referring to the 'biogenetic law' of recapitulation of Ernst Haeckel (1866). For example, Remane (1951) argued against Steinböck's view (Fig. 4) that the bursa of platyhelminthes never develops from the digestive tract, and thus, can not have its evolutionary origin in the endoderm since it is not 'recapitulated' in the ontogeny (Reisinger, 1961). Steinböck (1966) rejected this argument by pointing out that the lack of an ontogenetic recapitulation is not a valid argument and brought the example that the syncytial gut of acoels also does not undergo an epithelial developmental stage, despite the common view that the syncytial gut of acoels is derived from an epithelial gut of an ancestor (Reisinger, 1961). The validity of ontogeny for establishing homology hypotheses has been questioned by developmental biologists from the beginning, and it is now clear that there is not an ontogenetic 'criterion' for the homology of adult structures (Scholtz, 2005; Wilson, 1894).

The dispute can teach us that the lack of a solid phylogenetic framework makes it impossible to solve the debate and reconstruct the direction of evolution. All opponents struggled in their argumentation because the phylogenetic position of the many flatworm species that have been carefully examined regarding the bursa and hindgut morphology was unclear. Because the theoretical toolkit of cladistics, which makes the development of testable hypotheses possible, was not fully established yet, the debate stagnated in different opinions standing against each other. However, the dispute teaches us that the broad knowledge of animal diversity can reveal insights into evolution that cannot be gained by studying a handful of representative model systems in greater detail. With the current decay of taxonomical knowledge and expertise in academia, future researchers will depend more and more on reassessing the old literature. The value of the debate between Remane, Marcus, Steinböck, Karling and Reisinger for today's open

questions about the evolution of anal openings lies in the list of examples for different hindgut and gonadal architectures within and outside the platyhelminths and acoels (Fig. 4). This variation in animals indicates that ectodermal-, gonadal-connections with the endoderm can arise relatively 'easy' in evolution and point to a common cellular mechanism of the fusion of epithelia. Using the different examples, we can now phrase testable hypotheses, investigate these examples with molecular tools, and test the hypotheses on the background of an improving phylogenetic framework.

4.2. The evolution of the anus in the last common bilaterian ancestor

Despite the fact that fate of the blastopore is highly variable between animals (Hejnol and Martindale, 2009), it plays a key role in some of the different scenarios about 'major' transitions in animal evolution that have been proposed for more than a century (Arendt and Nübler-Jung, 1997; Hyman, 1951; Jägersten, 1972; see for example Lankester, 1877; Malakhov, 1977; Martindale and Hejnol, 2009; Masterman, 1898; Nielsen and Nørrevang, 1985; Salvini-Plawen, 1978; Sedgwick, 1884; von Graff, 1891). The blastopore is an organizing 'control center' of the embryo and a site at which a number of essential decisions and processes occur that vastly influence the future outcome: internalization of precursor blastomeres, differentiation and separation of the germ layers, axis determination and elongation (Solnica-Krezel and Sepich, 2012; Stern, 2004). In addition, it sometimes gives rise to either mouth or anus – or just disappears. This set of events that impact body plan organization naturally provokes hypotheses about its impact for the evolutionary change of body plans (Wolpert, 1992). It has been shown that the different processes occurring during gastrulation are orchestrated by a complex molecular and cell biological machinery (Stern, 2004), but their mostly simultaneous occurrence has been taken as a recapitulation of a major evolutionary event that transforms a simple ancestor into a complex one – for example, in the enterocoely hypothesis and its derivatives (Remane, 1950). The multiple innovations such as bilateral symmetry, mesoderm, brains, alimentary canal etc., that are necessary to transform the cnidarian-bilaterian stem species into a complex last common ancestor of protostomes and deuterostomes have been incorporated in different scenarios (De Robertis and Sasai, 1996; Hejnol and Martindale, 2008b). An essential part of these scenarios is the evolution of the alimentary canal. How did the sack-like gut with one opening get transformed to the alimentary canal of most bilaterians? How did the other opening that forms an additional connection of the ectoderm and endoderm evolve? A common theme relates Sedgwick (1884) and Masterman (1898) ideas that the elongation of the blastopore along the future anterior–posterior axis followed by its lateral closure leaving the two ends open which will give rise to mouth and the anus, showing that both orifices share a common origin. This process has been coined 'amphistomy' (Arendt and Nübler-Jung, 1997) in reference to the embryonic processes protostomy and deuterostomy. Although no description of a bona fide amphistomy exists for any animal embryo (Hejnol and Martindale, 2009; van den Biggelaar et al., 2002), it provides one possible explanation of how to transform a cnidarian-like ancestor with a single gut opening into an ancestor that has a gut with two openings. These scenarios imply that the mouth and anus originated simultaneously from the single opening of the last common cnidarian–bilaterian ancestor and that this process is also connected to the evolution of bilateral symmetry (Arendt, 2004). Expression patterns of bilaterian marker genes do not give a clear picture: a clear *caudal/cdx* ortholog is missing from cnidarians and the closest related ortholog in *N. vectensis*, homeodomain gene *NVHD065*, is asymmetrically expressed in the endoderm (Ryan et al., 2007). Orthologs of *foxA* and *brachyury* are expressed in
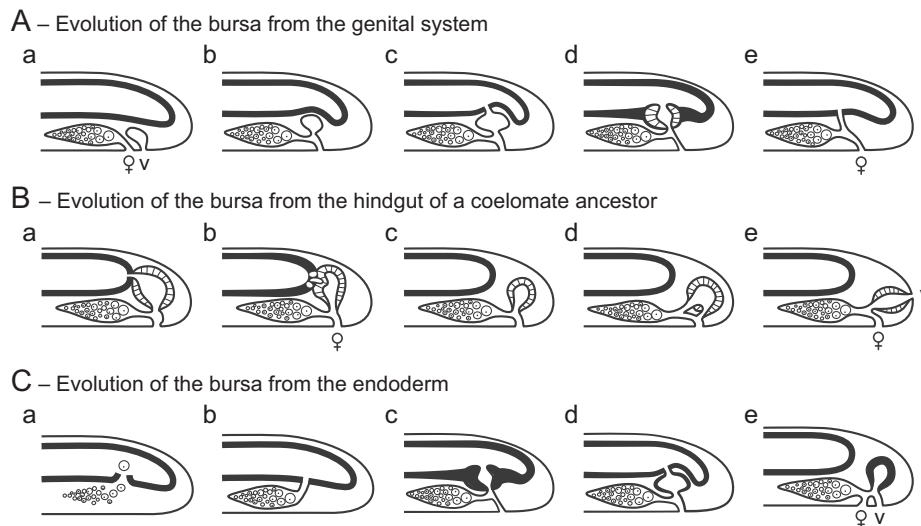
Fig. 4. Hypotheses about the evolution of the platyhelminth connection between gonads and digestive tract.
(A) According to Karling (1940), the bursa evolved from the genital system following this sequence of events: separate gonadal system for male (v) and female gonads (♀) (a) got connected with each other (b) and with the digestive tract (c) to resorb the sperm. The organ for the resorption of excessive sperm got elaborated (d) and after its digestive function disappeared, the ductus for the release of oocytes and connection with the gut remained (e). (B) According to Remane (1951), the bursa evolved from the hindgut of a coelomate ancestor as follows: at the beginning the hindgut and the female genital duct were connected in a cloaca (a). Then, the gut lost its function as transfer of digesting material (b) and the cloaca area that corresponds to the hindgut (bursa) acquired the function to digest material that accidentally entered there, such as remnants of sperm and yolk. This material could still be in some cases given to the gut by vacuoles. The bursa became the receptaculum seminis (c) and ducts from this cavity to the female gonad developed (d), until the receptaculum seminis formed its own channel (e). (C) According to Steinböck (1966), the bursa evolved from the endoderm: initially, the ancestor had a sack like gut through which oocytes were laid (a). After the female gonad gained complexity as an organ (b), a portion of the gut developed to a bulb-like structure (bursa, in c) and a genital tract and gonopore evolved (d). In some lineages, the endodermal bursa separates from the gut (e), resulting in the secondary evolution of a blind gut. Platyhelminth examples for different architectures: A(a) acoels, A(d), B(a), C(c): *Myozona*, *Promacrostomum* (Macrostomida), *Enterogonia* (Polycladida), *Coelogynopora* (Proseriata) A(c), B(a, b): *Kambanella*, *Pseudograffilla* (Rhabdocoela), B(c), C(e): some triclads and polyclads, B(d) and B(e) some rhabdocoels (see references in original literature).

Nematostella in portions of the endoderm in the region of the mouth (Martindale et al., 2004; Scholz and Technau, 2003). These data do not give clear evidence for the amphistomy scenario, nor do they speak against it.

With the possible placement of the Acoelomorpha as sister group to the remaining Bilateria the so-called 'acoeloid–planuloid' hypothesis from von Graff (1891) and Hyman (1951) was revisited (Baguñá and Riutort, 2004; Hejnol and Martindale, 2008b). This hypothesis implies a direct homology of the single mouth opening of the Cnidaria with the single opening of the Acoelomorpha. The anus would have evolved later, and this process is not connected with the transition from radial to bilateral symmetry, since acoelomorphs are bilaterally symmetric. However, this case would still include the possibility that the mouth of acoelomorphs would have extended afterwards and undergone the process of 'amphistomy' later. However, the gene expression of the acoel orthologs of *brachyury* and *cdx* in the posterior male gonopore suggests instead that there is an evolutionary relationship between the bilaterian hindgut and the acoel gonopore (Hejnol and Martindale, 2008a; Moreno et al., 2011). If acoelomorphs are indeed the sister group to the remaining Bilateria, the 'amphistomy'-based scenarios would be rejected. However, the case remains open since the phylogenetic position of Acoelomorpha remains uncertain (Dunn et al., 2014; Hejnol et al., 2009; Philippe et al., 2011; Srivastava et al., 2014). It is also vague how the acoel gonopore should be related to the bilaterian hindgut. A possible scenario could be the connection of the male reproductive organ system to the bilaterian digestive tract forming a cloaca (Hejnol and Martindale, 2008a, 2009) which is fundamentally different from the scenarios discussed in the context of the ductus intestinalis. This would thus provide an alternative hypothesis, which would connect first with the male reproductive system with the gut and later with the female reproductive system. The plausibility of the different ideas about the origin of the anus is clearly affected by the phylogenetic position of the Acoelomorpha,

but also the internal relationships of the remaining Bilateria impact the direction of evolution regarding gains and losses.

5. Conclusions and perspectives

Despite the progress in solving animal phylogenetic relationships, a broader taxon sampling in embryological studies and the inclusion of more molecular data is needed to solve the question about the (single or multiple) origin of the bilaterian hindgut. We can expect to come closer to a solution in the next decade, since the tools are now readily available. New developments in sequencing technologies, microscopy, sequencing technologies and gene manipulation, open the opportunity to study the development of animals in much greater detail. Advancing live imaging technologies in three dimensions allow to trace embryonic event is much greater detail (Hejnol and Schnabel, 2006; Reynaud et al., 2015). Single-cell and embryonic tissue sequencing are promising technologies to characterize better the molecular background of cell differentiation and will likely lead to the discovery of new marker genes (Crosetto et al., 2015; Shapiro et al., 2013). Such detailed studies of a larger diversity of animal taxa will ultimately lead to the necessary refinement of the vocabulary we use for describing the development of animals. The artificial subdivision into 'types' will likely be rendered useless, and previously assumed developmental constraints will disappear to the same degree the knowledge about developmental diversity grows. The results will lead to a better understanding how genes are contributing for shaping morphology and possibly to insights how this is connected to the evolution of organ systems such as the through gut. Fast progress in phylogenomic methods and genome sequencing will help to solve the animal relationships and will thus provide the framework for the interpretation of the developmental and morphological data from an evolutionary perspective. This will allow us not only to identify the time and sequence of the evolutionary changes in hindgut archi-

tecture, but also the identification of the molecular and cellular mechanisms.

Acknowledgements

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