

Origin and evolution of alternative developmental strategies in amphibious sarcopterygian parasites (Platyhelminthes, Monogenea, Polystomatidae)

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Abstract

Most integrative studies involving phylogenetic, developmental and ecological trends showed that the diversity of developmental modifications among the Platyhelminthes was linked to transmission opportunity pressures. For parasitic flatworms with complex life cycles it was suggested that the evolutionary forces that constrained or enhanced developmental strategies implied heterochronic patterns. Similar patterns were also reported from the Monogenea with direct life cycles, especially for Polystomatidae, which infest amphibious Sarcopterygians. *Polystoma*, whose members are recovered almost exclusively from anuran hosts of the Neobatrachia, is capable of following two alternative developmental strategies depending on the physiological stage of its host. Processes by which parasites reach maturity are strikingly different, and lead to discrete adult phenotypes within the same parasite species. In the present study, we investigate the origin and evolution of developmental patterns of polystomatids in a phylogenetic framework, using an integrative approach of heterochrony and evolutionary ecology. The results suggest that both phenotypes have coexisted during the early stages of polystome evolution, and that neither of them can be considered as the ancestral one. The two developmental pathways, each associated with one life cycle, may have arisen independently prior to polystome diversification, when strictly aquatic sarcopterygians attempted colonization of temporary freshwater environments. The occurrence of these two patterns within species of the genus *Polystoma* is suggested to reflect the ancestral condition, and to have allowed both developmental strategies to be successful depending on shifts in transmission opportunities. Thus, host evolutionary ecology may be the main factor in shaping developmental strategies within polystomatids.

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Introduction

Since the beginning of evolutionary developmental biology, heterochrony – defined as changes in the timing and/or rates of processes underlying the ontogenic

formation of morpho-anatomical traits – has been recognized as an important recipe in the evolution of organisms (Gould 1977; Alberch et al. 1979). The diversity of developmental modifications resulting from heterochrony was classified in two main categories, peramorphosis and paedomorphosis, which correspond to extended or truncated development between ancestors and descendents, respectively (Alberch et al. 1979).

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Besides heterochrony, other possibilities of overlapping developmental modifications, e.g. heterotopy, can also be involved, considering that evolution of ontogenies is not strictly a matter of rhythm (Zelditch and Fink 1996; Smith 2003; Webster and Zelditch 2005). However, it has been emphasized that, apart from a strong phylogenetic framework to assess ancestral ontogenies, heterochrony did not warrant further investigation (Alberch and Blanco 1996; Reilly et al. 1997; McKinney 1999; Hall 2003; Smith 2003; Webster and Zelditch 2005). Because of the lack of conceptual tools in both heterochrony and phylogeny, the earliest reports of heterochronic patterns that were proposed to explain the evolution of phenotypic diversity probably need to be thoroughly re-examined.

Investigating the evolution of ontogenetic pathways and life cycles among parasitic flatworms has taken advantage of a strong phylogenetic background (see Olson and Tkach 2005; Park et al. 2007). So far, studies have been focused largely on parasitic lineages alternating between sexual and asexual reproductive stages through their complex life cycles, which allowed direct interpretation of developmental shortcuts or extensions (Cribb et al. 2002, 2003; Poulin and Cribb 2002; Lefebvre and Poulin 2005). Most integrative studies involving phylogenetic, developmental and ecological trends showed that the diversity of developmental modifications among platyhelminths, especially within the Digenea, were linked to transmission opportunity pressures (Poulin 1996; Combes 2001; Lefebvre and Poulin 2005). It was suggested that the evolutionary forces that have constrained or enhanced developmental strategies of digeneans could imply paedomorphic patterns such as progenesis (Lefebvre and Poulin 2005; Lagrue and Poulin 2007). Paedomorphosis was also proposed to explain developmental modifications among the Monogenea, which possess one single host species through their life cycle (Gallien 1935; Williams 1961; Williams and McKenzie 1995; Sinnappah et al. 2001).

Within the Monogenea, almost all species parasitize the gills or skin of actinopterygian and chondrichthyan fish (Rohde 1994; Cribb et al. 2002). With approximately 150 species, Polystomatidae is the most diverse family of the Monogenea whose members infest specifically sarcopterygian hosts, among them the Australian lungfish, amphibians, freshwater turtles and the African hippopotamus. Like all other monogeneans polystomes present a direct life cycle, but unlike fish monogeneans are found mostly in the urinary bladder of amphibians and in the bladder, palpebral and pharyngeal cavities of their chelonian hosts (Prudhoe and Bray 1982). It is well known that several species of the most diversified genus of the Polystomatidae, namely *Polystoma*, which infest almost exclusively anuran hosts of the Neobatrachia, complete their life cycle either in

the bladder of adult frogs or in the branchial chamber of tadpoles (Combes 1968; Maeder 1973; Murith 1979, 1981; Kok and du Preez 1989, 1998; Kok 1990). Depending on the life cycle, the developmental processes by which parasites reach maturity are strikingly different, leading to discrete adult phenotypes within the same parasite species (Gallien 1935; Williams 1961; Combes 1968). The bladder phenotype encountered across almost all amphibians was considered for a long time to be the result of normal adult development, whereas the branchial phenotype, which is less common, was considered to be paedomorphic, more precisely neotenic after Gallien (1935).

During the hosts' breeding period, the bladder parasites lay eggs which develop into free swimming larvae infesting the larval stage of the amphibian hosts. Depending on the physiological stage of the tadpoles, parasites trigger one of two alternative developments. When larvae enter the gill chamber of tadpoles older than 10–13 days, they develop very slowly. During the tadpoles' metamorphosis, they creep onto the skin, migrate to the bladder, and reach maturity when the hosts experience their first reproductive season about three years later (Gallien 1935; Williams 1961; Combes 1968). This developmental strategy is referred to as the 'bladder' type. On the other hand, when larvae attach to tadpoles less than 10–13 days old, they develop very rapidly. They mature and reproduce inside the branchial chamber and die during host metamorphosis (Gallien 1935; Williams 1961; Combes 1968). This developmental strategy is referred to as the 'branchial' type.

Early studies of polystome ontogenies showed that the branchial type reaches maturity with incomplete attachment organs and incomplete or undifferentiated genital apparatus compared to the bladder type (Gallien 1935; Williams 1961). Gallien (1935) then considered the branchial phenotype to be a neotenic parasite, which would mean that branchial development has arisen secondarily through paedomorphosis. On the other hand, Murith (1979, 1981) hypothesized that the branchial type could be ancestral within polystomatids on account of the high morphological, ecological and transmission-strategy similarities between the branchial types encountered among *Polystoma* and all other fish monogeneans. According to Murith (1979, 1981), the bladder type would derive from the branchial one, thus could have arisen through peramorphosis. Another hypothesis to explain the occurrence of two developmental strategies within the same species could be that neither life cycle type derives from the other (Malmberg 1981, 1990). The latter author argued like Murith (1979, 1981), that the branchial types have more in common with fish monogeneans than with bladder types of the same polystome species, and concluded from observations of the haptor ontogeny that the branchial phenotypes did not represent neotenic larvae. Instead,

he suggested that both life cycles could have arisen independently within polystomatids, which would mean that the bladder type was not derived by peramorphosis.

All of the proposed hypotheses were suggested outside of a phylogenetic framework, which makes them highly questionable. The recent advances in the systematics, phylogeny and evolution of the Polystomatidae (Sinnappah et al. 2001; Verneau et al. 2002) should now allow investigation of the origin and evolution of polystome life cycles. A global scheme of coevolution between polystomes and their amphibious hosts over the past 425 million years (Myr) since the origins of the first terrestrial vertebrates has been presented (Verneau et al. 2002), suggesting that the ecological transition from strictly marine vertebrates to amphibious tetrapods could be a key evolutionary event that constrained or enhanced the developmental strategies of polystomes.

The aim of the present study was to evaluate confidence in the conflicting hypotheses about the origins of the two life cycles within *Polystoma*. We used complete 18S and partial 28S rDNA sequences to assess the phylogeny of twelve of the main polystomatid genera. Ancestral developmental types were then deduced in a phylogenetic framework, using an integrative approach of heterochrony and evolutionary ecology, to investigate origin and evolution of polystomatid life cycles.

Material and methods

Parasite sampling

Sampling included 20 species covering 12 genera of the Polystomatidae and two fish monogeneans as an outgroup. Most sequences had been used in previous studies on the historical biogeography of anuran polystomes (Bentz et al. 2006; Badets et al. unpublished data) and retrieved from GenBank (Table 1). The six chelonian polystomes were taken from the bladder or from palpebral or pharyngeal cavities of their natural host species, whereas the two urodelan polystomes were collected from the gills or bladder of their hosts (Table 1). Only polystome species infesting chelonians and urodeleans were investigated on the molecular level.

Molecular work

All methods used for DNA extraction, amplification and sequencing followed those described in Sinnappah et al. (2001). The complete 18S rRNA gene was amplified in one round with the primers Forward F18, 5'-ACCTGGTTGATCCTGCCAGTAG-3' and Reverse IR5, 5'-TACGGAACCTTGTTACGAC-3', yielding a PCR product of about 2 kb. It was subsequently

sequenced with the same primers in addition to the internal primers Forward 18F3, 5'-GGACGGCATGTTACTTTGA-3'; S1, 5'-ATTCCGATAACGAACGAGACT-3'; and Reverse 18RC, 5'-TACGAGCTTTTAACTGCAG-3'; 18RG, 5'-CTCTCTTAACCATTACTTCGG-3'.

The partial 28S rRNA gene corresponding to the 5' terminal end was amplified with primers Forward LSU5', 5'-TAGGTGACCCGCTGAAYTTAAGCA-3' and Reverse LSU3', 5'-TAGAAGCTTCCTGAGGAAACTTCGG-3' (Snyder and Loker 2000), yielding a PCR product of about 1.4 kb. It was subsequently sequenced with the same primers in addition to the internal primers Forward: IF13, 5'-AGCAAACAAGTACCGTGAGGG-3'; IF15, 5'-GTCTGTGGCGTAGTGGTAGAC-3'; and Reverse IR13, 5'-GTCGTGGCTTACACCCTGAGG-3'; IR14, 5'-CATGTTAACTCCTTGGTCCG-3'. Internal primers were designed to ensure overlapping between sequenced fragments.

18S and 28S rDNA sequences have been deposited in GenBank under accession numbers FM992696–FM992708.

Phylogenetic reconstructions

18S and 28S sequences were edited and assembled using Sequencher 4.5TM software (Gene Codes Corp., Ann Arbor, MI) and manually aligned using the MUST package (Philippe 1993). Gaps as well as ambiguous regions were excluded from subsequent analyses. Phylogenetic reconstructions were performed with PAUP* 4.0b9 (Swofford 2002) from a combined dataset including characters from both rRNA genes. The Maximum Parsimony (MP) analysis was assessed following a branch-and-bound search on all equally-weighted informative characters. The best-fitting model of sequence evolution for Maximum Likelihood (ML) was chosen using the program Modeltest 3.06 (Posada and Crandall 1998), applying Hierarchical Likelihood Ratio Tests (hLRTs) and the Akaike Information Criterion (AIC). The ML analysis was subsequently performed following a heuristic procedure with the TBR branch-swapping option, and using the GTR + I + Γ model selected from both hLRT and AIC. Robustness of MP and ML tree topologies was assessed from bootstrap proportions (BP) after 1000 replicates (Felsenstein 1985), following a branch-and-bound search in MP and under the NNI branch-swapping option in ML. Nodes for which bootstrap was lower than 50% were automatically collapsed. Bayesian Inference (BI) was conducted using the software MrBayes 3.04b (Huelsenbeck and Ronquist 2001). The analysis was conducted for 2 million generations on four MCMC chains, sampling trees every 100 generations (nst = 6; rates = invgamma). Bayesian posterior probabilities (BPP) were obtained

Table 1. Parasite species investigated, their host species, host family, geographic origin, infection site and GenBank accession numbers for 18S and partial 28S rDNA sequences.

Parasite species	Host species	Host family	Origin	Infection site	Accession number	
					18S	28S
Family Polystomatidae						
<i>Concinnocotyla australensis</i>	<i>Neoceratodus forsteri</i>	Ceratodontidae	Australia	skin and gills	AM157183	AM157197
<i>Diplorchis ranae</i>	<i>Rana rugosa</i>	Ranidae	Japan	urinary bladder	AM157184	AM157198
<i>Eupolystoma alluaudi</i>	<i>Bufo</i> sp.	Bufo	Togo	urinary bladder	AM051066	AM157199
<i>Eupolystoma vanasi</i>	<i>Schismaderma carens</i>	Bufo	South Africa	urinary bladder	AM157185	AM157200
<i>Neodiplorchis scaphiopi</i>	<i>Spea bombifrons</i>	Pelobatidae	USA	urinary bladder	AM051067	AM157201
<i>Neopolystoma palpebrae</i>	<i>Pelodiscus sinensis</i>	Trionychidae	Vietnam	palpebral cavities	FM992696	AF382065
<i>Neopolystoma spratti</i>	<i>Chelodina longicollis</i>	Chelidae	Australia	palpebral cavities	AJ228788	FM992702
<i>Parapolystoma bulliense</i>	<i>Litoria gracilentia</i>	Hylidae	Australia	urinary bladder	AM157186	AM157202
<i>Polystoma gallieni</i>	<i>Hyla meridionalis</i>	Hylidae	France	gills/urinary bladder	AM051070	AM157205
<i>Polystoma indicum</i>	<i>Rhacophorus maximus</i>	Rhacophoridae	India	urinary bladder	AM157193	AM157216
<i>Polystoma integerrimum</i>	<i>Rana temporaria</i>	Ranidae	France	gills/urinary bladder	AM051071	AM157206
<i>Polystoma nearcticum</i>	<i>Hyla versicolor</i>	Hylidae	USA	urinary bladder	AM051074	AM157210
<i>Polystomoides asiaticus</i>	<i>Cuora amboinensis</i>	Bataguridae	Malaysia	pharyngeal cavity	FM992697	FM992703
<i>Polystomoides malayi</i>	<i>Cuora amboinensis</i>	Bataguridae	Malaysia	urinary bladder	AJ228792	FM992704
<i>Polystomoides oris</i>	<i>Chrysemys picta marginata</i>	Emydidae	USA	pharyngeal cavity	FM992698	FM992705
<i>Polystomoides siebenrockiellae</i>	<i>Siebenrockiella crassicollis</i>	Bataguridae	Malaysia	urinary bladder	FM992699	FM992706
<i>Protopolystoma xenopodis</i>	<i>Xenopus laevis</i>	Pipidae	South Africa	urinary bladder	AM051078	AM157218
<i>Pseudodiplorchis americanus</i>	<i>Scaphiopus couchii</i>	Pelobatidae	USA	urinary bladder	AM051079	AM157219
<i>Pseudopolystoma dendriticum</i>	<i>Onychodactylus japonicus</i>	Hynobiidae	Japan	urinary bladder	FM992700	FM992707
<i>Sphyrnura oligorchis</i>	<i>Necturus maculosus</i>	Proteidae	USA	Skin and gills	FM992701	FM992708
Outgroup						
<i>Microcotyle erythrinii</i>	<i>Pagellus erythrinus</i>	Sparidae	France	gills	AM157195	AM157221
<i>Pseudaxine trachuri</i>	<i>Trachurus trachurus</i>	Carangidae	France	gills	AM157196	AM157222

after omitting the first 10,000 trees as the burn-in phase estimated on empirical evidence.

Coding of developmental strategies

Polystome taxonomy is based mainly on anatomical characters of the attachment, digestive and reproductive organs. The configuration of the attachment organ (numbers of suckers and hooks), the branching pattern of the digestive tracts and the structure of both male and female genitalia (number of testicles, occurrence of vaginal apertures, and length of uterus) are the main criteria to assign genera within the Polystomatidae, while morphometrics of the haptor hooks and host systematics are usually used to discriminate species within the same genus. Unfortunately, original descriptions of polystomes are usually incomplete, high-quality drawings are lacking for some genera, and the terminology used to describe morphological features is inconsistent. Therefore, only two characters were used to investigate morphological homologies between the two patterns of life cycles: the occurrence of vaginal apertures and the length of the uterus.

We are able to distinguish two kinds of female reproductive systems among polystome genera that discriminate polystomes with similar transmission

strategies. The first kind combines the presence of vaginal apertures with a long uterus. It characterizes eight polystome genera, namely *Diplorchis*, *Eupolystoma*, *Neodiplorchis*, *Neopolystoma*, *Parapolystoma*, *Polystomoides*, *Pseudodiplorchis* and the bladder forms of *Polystoma*. All of them infest hosts with terrestrial ecology, which means limited parasite transmission opportunities during the year (see Fig. 1 and references therein). This pattern is referred to as the bladder type below. In the second kind of female reproductive system, vaginal apertures are lacking and the uterus is short. This characterizes five polystome genera, namely *Concinnocotyla*, *Protopolystoma*, *Pseudopolystoma*, *Sphyrnura* and the branchial mature forms of *Polystoma*. All of them infest hosts with aquatic ecology, which means permanent parasite transmission opportunities over the lifespan of the species (Fig. 1). This pattern is referred to as the branchial type below. The two patterns, which associate the shape of the female reproductive organ with transmission strategy, were ultimately encoded with binary characters (1 vs. 0). Based on the ontogenetic development of the haptor suckers, *Sphyrnura oligorchis* had been reported as a paedomorphic parasite infesting a neotenic host (Sinnappah et al. 2001). For this reason the shape of its female organ may also correspond to incomplete development. Consequently, the pattern for *S. oligorchis* was





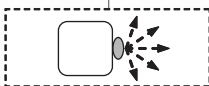



Parasites	Hosts	Parasite transmission pattern		References
		Host aquatic stage	Host terrestrial stage	
Fish monogeneans	Chondrichthyans and actinopterygians			Rohde 1994 (Review)
<i>Concinnocotyla</i>	Lungfish			Pichelin et al. 1991
<i>Polystomoides</i> <i>Neopolystoma</i>	Freshwater turtles			Paul 1938
<i>Protopolystoma</i> <i>Pseudopolystoma</i>	Pipidae Urodela	Tadpole	Adult	Price 1943 Ozaki 1948 Yamaguti 1963
		Uninfected		
<i>Sphyranura</i>	Perrenibranchiate			Alvey 1936 Paul 1938
<i>Pseudodiplorchis</i> , <i>Neodiplorchis</i> <i>Diplorchis</i> , <i>Parapolystoma</i>	Pelobatidae Neobatrachia	Uninfected		Ozaki 1935 Yamaguti 1963 Vande Vusse 1976 Pichelin 1995
<i>Eupolystoma</i>	Bufonidae, Ranidae	Uninfected		De Beauchamp 1913 Euzet and Combes 1967 Du Preez et al. 2003
<i>Polystoma</i>	Neobatrachia and <i>Pelobates</i>	Young 		Gallien 1935 Williams 1961 Combes 1968
		Old 		

Fig. 1. Parasite transmission patterns in fish monogeneans and polystomatids depending upon host ecologies. Larger, squarish boxes represent hosts; smaller, oval boxes represent parasites (white oval = branchial type, black oval = bladder type, grey oval = undetermined type; arrows = reproductive output). Alvey, 1936; De Beauchamp, 1913; Du Preez et al., 2003; Euzet and Combes, 1967; Ozaki, 1935; Ozaki, 1948; Paul, 1938; Pichelin et al., 1991; Pichelin, 1995; Price, 1943; Vande Vusse, 1976; Yamaguti, 1963.

considered in both ways, either as the bladder type (1) or as the branchial type (0).

Reconstruction of ancestral developmental strategies

Character states for the most ancestral nodes regarding Malmberg's and Murith's hypotheses were investigated using the BayesTraits program (Pagel et al. 2004; Pagel and Meade 2006; program available at: <http://www.evolution.rdg.ac.uk/BayesTraits.html>). The Multi-State procedure was conducted following the program's authors' instructions and the recommendations by Keever and Hart (2008) for both theoretical and practical aspects. An ML analysis was carried out first, to estimate model parameters with or without restrictions on rates of character changes. The MCMC analysis was performed following 3 million iterations, sampling every 100 generations after a burn-in phase

of 500,000 iterations. We used the 'addmrca' command to estimate the proportion of likelihood associated with each character state. Analysis was performed twice, once each with either character state encoding for *S. oligorchis*.

Results

Polystomatid phylogeny

The combined 18S and 28S rDNA sequences consisted of 2717 molecular characters, 602 of which were informative in MP. The ML settings were as follows: nucleotide frequencies (π [A] = 0.2249; π [C] = 0.2108; π [G] = 0.2934; π [T] = 0.2709); rate matrix ([A,C] = 0.6742; [A,G] = 3.7108; [A,T] = 2.1263; [C,G] = 0.2519; [C,T] = 4.7163; [G,T] = 1.0000); invariable sites = 0.5147; gamma shape parameter = 0.6346. The ML tree

with MP and ML bootstrap proportions as well as Bayesian posterior probabilities is shown in Fig. 2.

Within the ingroup *Concinnocotyla australensis*, which infests the Australian lungfish, is in a highly supported basal position (BP = 99% in MP and 76% in ML; BPP = 0.99). Three noteworthy groups are well supported: the clade of chelonian polystomes which includes species of *Neopolystoma* and *Polystomoides* (BP = 100% in MP and 99% in ML; BPP = 1.00); the clade of amphibian polystomes (BP = 88% in MP and 89% in ML; BPP = 1.00); and the clade of neobatrachian polystomes that groups *Diplorchis*, *Eupolystoma*, *Parapolystoma* and *Polystoma* (BP = 100% in MP and 99% in ML; BPP = 1.00). In contrast, relationships within chelonian polystomes are poorly resolved, as are the basal relationships among amphibian polystomes. *Sphyrnura oligorchis* and *Protopolystoma xenopodis* appear in a basal polytomy, and the phylogenetic position of the clade grouping *Pseudodiplorchis americanus* and *Neodiplorchis scaphiopi* is tenuous. *Pseudopolystoma dendriticum* results as closely related to the neobatrachian polystomes, but with moderate bootstrap values. Finally, the phylogenetic placement of *Polystoma indicum* and

Eupolystoma within neobatrachian polystomes remains controversial. Thus, branching patterns within polystomatids mirror host phylogenetic relationships at the early stages of their evolution and suggest a long coevolutionary history between sarcopterygians and their parasites.

Ancestral developmental strategies

The proportion of likelihood associated with each pattern is depicted directly in Fig. 2 for the two nodes of interest, X and Y. The first node corresponds to the split between *C. australensis* and all other polystomes. It fits well with the early origin of tetrapods. The second node corresponds to the split between chelonian and amphibian polystomes and is linked to the early diversification of tetrapods. When *Sphyrnura oligorchis* is coded with the branchial type, the proportion of likelihood associated with this type is 0.7 and 0.5 for nodes X and Y, respectively. On the other hand, when *S. oligorchis* is coded with the bladder type, the proportion of likelihood associated with the branchial type is 0.5 and 0.4 for nodes X and Y, respectively.

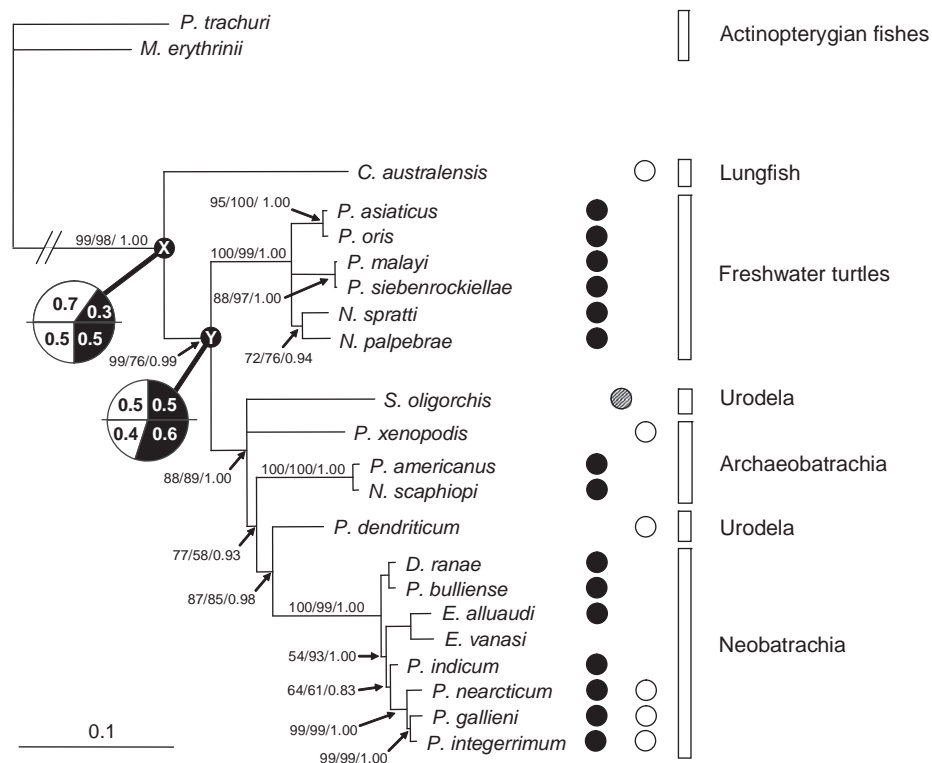


Fig. 2. Maximum likelihood (ML) tree inferred from complete 18S and partial 28S rDNA sequences (2717 characters). Numbers at nodes (left to right): Maximum parsimony (MP) bootstrap proportions/ML bootstrap proportions/Bayesian posterior probabilities. Broken line = branch not drawn to scale. Host lineages and encoding developmental patterns indicated at right; white circles = branchial type, black circles = bladder type, grey circle = undetermined type. Pie symbols at left show respective proportion of likelihood associated with branchial (white) and bladder types (black), with *S. oligorchis* referenced as branchial type (values in upper half) or as bladder type (lower half). Nodes X and Y correspond to early diversification of polystomatids and to split between chelonian and amphibian polystomes, respectively.

Discussion

Branchial phenotype: neotenic or not?

The proportion of likelihood associated with both bladder and branchial types at the deepest nodes of the phylogenetic tree (nodes X and Y in Fig. 2) is always close to 0.5, regardless of the coding for *S. oligorchis*. In fact, the MultiState procedure showed two likely reconstructions for the ancestral state, suggesting that the ancestor of the whole family could have developed both bladder and branchial types, as is the case within *Polystoma*. Both types could have coexisted during the early stages of polystome evolution, thus neither of them should be considered as the ancestral pattern any longer. This is in accordance with Malmberg's hypothesis, which stated that neither of the two life cycles was derived from the other (Malmberg 1981, 1990). The two life cycles may have arisen independently prior to polystome diversification. Accordingly, the branchial type in *Polystoma* would not reflect neotenic development, as suggested by Gallien (1935) and Murith (1979, 1981), and the bladder type would not reflect peramorphic development either. Consequently, the occurrence of both life cycles associated with discrete developmental pathways likely reflects the ancestral condition in *Polystoma*, in which an alternative phenotype constitutes a reappearing trait comparable to an atavism (see Hall 2003). The two developmental pathways would reflect two ontogenetic programs that are alternatively expressed depending upon host ecologies as a consequence of a very early gene functional diversification in the polystomatid ancestor. The expression of one single phenotype across almost all polystomatid genera would be the effect of different selective pressures according to parasite transmission opportunity. As a result one may expect that all species of the family that show only one of the two developmental strategies should be able to restore the ancestral condition, as is the case in some species of *Polystoma*. Similarly, one would expect that *Polystoma* species can ultimately preserve a single phenotype only, as exemplified by *P. indicum*, which shows only the bladder phenotype (Diengdoh and Tandon 1991).

Evolution of the two developmental strategies within polystomatids

It has been shown that modifications of the developmental strategies among parasitic platyhelminths could be linked to the evolution of transmission opportunities, especially within the Digenea that show complex life cycles (Poulin and Cribb 2002; Lefebvre and Poulin 2005; Lagrue and Poulin 2007). In these host-parasite associations, the evolutionary forces that may have

shortened or extended life cycles are correlated mainly to the modifications of interspecific relationships between intermediate and definitive host species through changes in the ecosystems (see Combes 2001; Thomas et al. 2002). Within parasitic flatworms with direct life cycles, the evolution of developmental strategies may also be correlated with the evolution of transmission opportunities (Tinsley 1990, 1993, 2004). However, modifications in direct life cycles can be explained only by the evolutionary ecology of a single host, as the routes of parasite transmission do not depend on interspecific host relationships within ecosystems.

Polystomatids are sarcopterygian parasites assumed to be derived from an ancestral stock of monogeneans which originally infested chondrichthyan and actinopterygian fish (Littlewood et al. 1999; Park et al. 2007). As exemplified by the coevolutionary patterns between sarcopterygian hosts and their parasites (Fig. 2), the latter would have originated and diversified very soon after the evolution of their specific hosts in the Palaeozoic period (Verneau et al. 2002). During the ecological fish-tetrapod transition, which is well documented between 380 Myr and 340 Myr ago (see Long and Gordon 2004 for a review), the evolution of host ecologies from strictly aquatic to more terrestrial habits may have shaped the transmission opportunities of parasites across more terrestrial vertebrate hosts. Because fish monogeneans are mainly gill parasites, it can be hypothesized that the plesiomorphic monogenean life cycle moved towards the bladder and branchial types, within ancestral polystomatids, when strictly aquatic sarcopterygians attempted colonization of temporary freshwater environments.

Subsequently both life cycles and developmental pathways may have been maintained as two separate ontogenetic programs, and activated according to the transmission opportunities within ecosystems. Parasites that infest host species whose ecology favors permanent transmission are branchial-like polystomes that develop rapidly and lay eggs continuously (Fig. 1). This is exemplified by *Protopolystoma xenopodis*, the specific parasite of the African Clawed Toad, *Xenopus laevis*. In fact, this polystome is found only in the bladder of its fully aquatic adult host and has never been recorded from the gills of tadpoles (Tinsley 2004). On the other hand, parasites that infest host species with terrestrial ecology are bladder-like polystomes that lay eggs only temporarily during the short contact between hosts and freshwater environments (Fig. 1). This is illustrated by *Pseudodiplorchis americanus*, the specific parasite of the desert-dwelling Couch's Spadefoot Toad, *Scaphiopus couchii*. This polystome is also recovered from the bladder of its host but, unlike *P. xenopodis*, lays fully developed eggs during only two or three nights per year (Tinsley 1990). The occurrence of the two patterns within some species of the genus *Polystoma* may

reveal an equilibrium situation that allows success from either developmental strategy, depending on shifts in the environmental conditions. For instance *P. gallieni*, the specific parasite of the Stripeless Tree Frog, *Hyla meridionalis*, can be recovered either from the gills of tadpoles or from the bladder of the adult host. Frogs usually reproduce in temporary ponds in which tadpoles develop over four to five months. Tadpoles still quite far from metamorphosis are suitable for the development of branchial-type parasites that reproduce permanently until the pond dries up. In contrast, tadpoles nearer metamorphosis drive parasite development towards the bladder type seen later in the terrestrial adult frogs. As a result, the ecology of the host species is the main factor controlling the developmental strategies within polystomatids. *Polystoma* is the only genus in which an ancestral pattern as old as about 400 Myr is restored.

Conclusions and prospects

Such hypotheses on the origins of developmental processes, even where they are well-supported by phylogenetic analyses and homology assessments, remain tentative and cannot be tested experimentally. However, in the light of recent advances in molecular biology, our knowledge of genes and processes behind development has greatly increased. For instance, the family of homeotic genes encodes master transcription factors controlling the anteroposterior patterning and segment identity in the early developmental stages of all metazoans, including parasitic platyhelminths (Balavoine 1998; Pierce et al. 2005; Baguña et al. 2008). Therefore, the expression patterns of these genes should be investigated within *Polystoma* and other species of the Polystomatidae, in order to unravel the genetic mechanisms activating the two developmental pathways. The need for such comparative molecular analysis has been emphasized recently by Olson (2008), who provided a phylogenetic scaffold of *Hox* genes within flatworms.

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