



Assessing the molluscan hypothesis Serialia (Monoplacophora + Polyplacophora) using novel molecular data

Nerida G. Wilson^{a,*}, Greg W. Rouse^a, Gonzalo Giribet^b

^a Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0202, USA

^b Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

ARTICLE INFO

Article history:

Received 2 April 2009

Revised 22 July 2009

Accepted 24 July 2009

Available online 30 July 2009

Keywords:

Monoplacophora

Aplacophora

Bivalvia

Gastropoda

Conchifera

ABSTRACT

A consensus on molluscan relationships has yet to be achieved, largely because of conflicting morphological and molecular hypotheses. Monoplacophora show marked seriality of ctenidia, atria, muscles and nephridia and this has been interpreted as plesiomorphic for Mollusca, reflecting a segmented ancestry. More recently this seriality, also partly seen in Polyplacophora, has been seen as a derived condition. Analysis of the first published monoplacophoran DNA sequence from *Laevipilina antarctica* Warén & Hain, 1992 [Giribet, G., Okusu, A., Lindgren, A.R., Huff, S., Schrödl, M., Nishiguchi, M.K., 2006. Evidence for a clade composed of molluscs with serially repeated structures: Monoplacophorans are related to chitons. *Proc. Natl. Acad. Sci. USA* 103, 7723–7728. [10.1073/pnas.0602578103](https://doi.org/10.1073/pnas.0602578103)], showed Monoplacophora inside Polyplacophora. These taxa were then grouped under the name Serialia, reflecting the hypothesis that their seriality is a synapomorphy. Subsequent examination revealed that part of the *L. antarctica* published sequence was the result of contamination with Polyplacophora (Giribet, Supplementary Material S1). We collected and sequenced another monoplacophoran, *Laevipilina hyalina* McLean, 1979, resulting in the first multi-gene dataset representing all molluscan classes. Our analyses did not show unambiguous support for Serialia. Model-based approaches strongly supported Serialia as a clade, however, parsimony analyses under dynamic and static homology did not resolve the position of Monoplacophora. Although our study provides support for Serialia and none for Conchifera, it appears that further resolution of molluscan relationships will require large increases of data.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Despite recent progress in understanding relationships among and within most major animal phyla (e.g. Bourlat et al., 2006; Dunn et al., 2008; Helmkamp et al., 2008), many questions remain. Among the most vexing of these problems is the resolution of relationships among the extant molluscan classes. Although monophyly of Mollusca has been recently supported in a phylogenomic analysis of metazoan relationships (Dunn et al., 2008), molluscan internal phylogeny remains a recalcitrant problem (Giribet et al., 2006; Haszprunar, 2008; Passamanek et al., 2004; Winnepenninckx et al., 1996).

Monoplacophoran fossils appear in the early Cambrian (Lindberg, 2009), and were thought to be extinct until Lemche's (1957) discovery of a living tryblid. We use the name Monoplacophora here, as it is in common usage, although the extant forms are referred to as Tryblidia and the former name is generally thought to not represent a clade (see Haszprunar, 2008; Schwabe, 2008).

Discoveries of living Monoplacophora (e.g. Haszprunar and Schaefer, 1997; Lemche, 1957; Warén and Gofas, 1996) have generated considerable interest in addressing longstanding questions regarding the evolution of molluscs. Bearing a simple limpet-like shell, Monoplacophora show serial repetition of atria, dorsoventral muscles, nephridia and ctenidia (Haszprunar and Schaefer, 1997; Lemche and Wingstrand, 1959; Wingstrand, 1985). The majority view (see Haszprunar, 2008) would argue that Monoplacophora is the sister group to Conchifera, a group which includes all other shell-bearing molluscs except Polyplacophora.

The Conchifera hypothesis was recently challenged by the results of the most comprehensive DNA-based study of molluscan relationships to that point, which included a partial 28S rRNA gene sequence of a monoplacophoran species, *Laevipilina antarctica* (Giribet et al., 2006). Giribet et al. (2006) found parsimony jackknife support for *L. antarctica* nested inside Polyplacophora (chitons). This somewhat surprising outcome resulted in the establishment of a new taxon, Serialia, for the clade comprised of Monoplacophora and Polyplacophora. The Serialia hypothesis contradicted the widespread view that molluscs with a true shell formed a clade (Conchifera). Serialia was deemed questionable by some authors (Steiner in Haszprunar, 2008), who considered

* Corresponding author. Fax: +1 858 534 7313.

E-mail addresses: ngwilson@ucsd.edu (N.G. Wilson), grouse@ucsd.edu (G.W. Rouse), ggiribet@oeb.harvard.edu (G. Giribet).

the possibility that the included monoplacophoran DNA sequence was chimeric. This has now been confirmed (see [Supplementary Material S1](#)) and it appears that 472 bp of that sequence represented *L. antarctica*, and the remaining 553 bp were of polyplacophoran origin (97% blast-n match to *Acanthopleura granulata* AY839252, deposited October 2007).

Given the controversy, and that the amount of genetic data available for *L. antarctica* was very small, further assessment of the Serialia hypothesis and placement of Monoplacophora was imperative. We undertook new fieldwork to collect live Monoplacophora for molecular study. The new monoplacophoran sequence data was generated independently in two laboratories, added to

Table 1
Accession numbers of samples used in this study.

	Species	18S rRNA	28S1 rRNA	28S2 rRNA	28S3 rRNA	28S4 rRNA	H3	COI	16S rRNA
Nemertea	<i>Lineus bilineatus</i>	DQ279932	DQ279947	DQ279947	DQ279947	DQ279947	DQ279996	DQ280014	DQ280022
Annelida	<i>Paranereis limicola</i>	DQ279933	DQ279948	DQ279948	DQ279948	DQ279948			DQ280023
Brachiopoda	<i>Novocrania anomala</i>	DQ279934	DQ279949	DQ279949	DQ279949	DQ279949	DQ279997		DQ280024
Entoprocta	<i>Loxosomella murmanica</i>	AY218100	DQ279950	DQ279950	DQ279950	DQ279950	AY218150		
Sipuncula	<i>Phascolion strombi</i>	DQ299984	AY210468	AY210468	AY210468	AY210468	DQ279998		
Cycliophora	<i>Symbion americanus</i>	AY218107	AY210472	AY210472	AY210472	AY210472	AY218153	AY218085	DQ280025
Caudofoveata	<i>Chaetoderma nitidulum</i>	AY377658			FJ445775		AY377763	AY377726	AY377612
	<i>Scutopus ventrolineatus</i>	X91977							
Solenogastres	<i>Helicoradomenia</i> sp.	AY145377	AY145409	AY145409	AY145409	AY145409	AY377764		AY377613
	<i>Epimania cinerea</i>						AY377765	AY377723	AY377615
Polyplacophora	<i>Lepidopleurus cajetanus</i>	AF120502	FJ445776	FJ445776	FJ445776		AY377735	AF120626	AY377585
	<i>Leptochiton asellus</i>	AY377631	AY145414	AY145414	AY145414	AY145414	AY377734	FJ461256	AY377586
	<i>Callochiton septemvalvis</i>	AY377632		DQ279952	DQ279952		AY377736	AY377700	
	<i>Chaetopleura apiculata</i>	AY377636	AY145398	AY145398	AY145398	AY145398	AY377741	AY377704	AY377590
	<i>Ischnochiton comptus</i>	AY377639	AY145412	AY145412	AY145412	AY145412	AY377744	AY377709	AY377593
	<i>Callistochiton antiquus</i>	AY377645	DQ279953	DQ279953	DQ279953		AY377749	AY377712	AY377599
	<i>Lorica volvox</i>	AY377647	DQ279954	DQ279954	DQ279954		AY377751		AY377601
	<i>Chiton olivaceus</i>	AY377651		DQ279955	DQ279955		AY377755	AY377716	AY377605
	<i>Mopalia muscosa</i>	AY377648	DQ279956	DQ279956	DQ279956		AY377752	AY377713	AY377602
	<i>Tonicella lineata</i>	AY377635		AY377665			AY377739	AF377702	AY377588
	<i>Acanthochitona crinita</i>	AF120503	DQ279957	DQ279957	DQ279957		AY377759	AF120627	AY377609
	<i>Cryptochiton stelleri</i>		AY377686			AY377686	AY377760	AY377720	AY377610
	<i>Cryptoplax japonica</i>	AY377656	AY145402	AY145402	AY145402	AY145402	AY377761	FJ445780	AY377611
Monoplacophora	<i>Laevipilina antarctica</i>			DQ279958					
	<i>Laevipilina hyalina</i>	FJ445774	FJ445777	FJ445777	FJ445777	FJ445777	FJ445778	FJ445781	FJ445782
	<i>Laevipilina hyalina</i>	FJ449542	FJ449541	FJ449541	FJ449541	FJ449541		FJ449540	FJ449543
Scaphopoda	<i>Dentalium inaequicostatum</i>	DQ279935		DQ279959	DQ279959		DQ279999	DQ280015	DQ280026
	<i>Rhabdus rectius</i>	AF120523		AF120580			AY377772	AF120640	AY377619
	<i>Antalis pilsbryi</i>	AF120522		AF120579				AF120639	
	<i>Antalis entalis</i>	DQ279936	AY145388	AY145388	AY145388	AY145388	DQ280000	DQ280016	DQ280027
	<i>Fustiaria rubescens</i>	AF490597							
	<i>Entalina tetragona</i>	AF490598							
	<i>Pulsellum affine</i>	AF490600							
	<i>Siphonodentalium lobatum</i>	AF490601							
	<i>Cadulus subfusiformis</i>	AF490603							
Bivalvia	<i>Solemya velum</i>	AF120524	AY145421	AY145421	AY145421	AY145421	AY070146	U56852	DQ280028
	<i>Nucula sulcata</i>	DQ279937	DQ279960	DQ279960	DQ279960	DQ279960	DQ280001	DQ280017	DQ280029
	<i>Nuculana minuta</i>	DQ279938	DQ279961	DQ279961	DQ279961	DQ279961	DQ280002	DQ280018	DQ280030
	<i>Yoldia limatula</i>	AF120528	AY145424	AY145424	AY145424	AY145424	AY070149	AF120642	
	<i>Mytilus galloprovincialis</i>	L33452	AB103129	AB103129	AB103129	AB103129	AY267748	AY497292	AY497292
	<i>Arca imbricata/ventricosa</i>	AY654986	AB101612	AB101612	AB101612	AB101612	AY654989	AY654988	
	<i>Pteria hirundo/loveni</i>	AF120532	AB102767	AB102767	AB102767	AB102767		AF120647	DQ280031
	<i>Ostrea edulis</i>	L49052	AF137047/	AF137047/	AF137047/		AY070151	AF120651	DQ280032
			AF120596	AF120596					
	<i>Limaria hians/fragilis</i>	AF120534	AB102742	AB102742	AB102742	AB102742	AY070152	AF120650	
	<i>Anomia ehippium/sinensis</i>	AF120535	AB102739	AB102739	AB102739	AB102739			
	<i>Chlamys varia</i>	DQ279939	DQ279962	DQ279962	DQ279962	DQ279962	DQ280003		DQ280033
	<i>Neotrigonia margaritacea</i>	AF411690	DQ279963	DQ279963	DQ279963	DQ279963	AY070155	U56850	DQ280034
	<i>Margaritifera auricularia</i>	AY579097		AY579113			AY579137	AY579125	DQ280035
	<i>Anodonta</i> sp.	AY579090	DQ279964	DQ279964			AY579132	AY579122	
	<i>Cardita calyculata</i>	AF120549		AF120610			AY070156	AF120660	
	<i>Astarte castanea</i>	AF120551	AF131001	AF131001			DQ280004	AF120662	
	<i>Abra nitida</i>	DQ279940	DQ279965	DQ279965	DQ279965	DQ279965	DQ280005		
	<i>Phaxas pellucidus</i>	DQ279941	AY145420	AY145420	AY145420	AY145420	DQ280006	DQ280019	DQ280036
	<i>Parvicardium minimum</i>	DQ279942	DQ279966	DQ279966	DQ279966	DQ279966	DQ280007		DQ280037
	<i>Dreissena polymorpha</i>	AF120552	AF131006	AF131006			AY070165	AF120663	DQ280038
	<i>Corbicula fluminea/japonica</i>	AF120557	AB126330	AB126330	AB126330	AB126330	AY070161	AF120666	DQ280039
	<i>Mercenaria mercenaria</i>	AF120559	AF131019	AF131019			DQ280008	DQ399403	DQ280040
	<i>Chamelea striatula</i>	DQ279943		DQ279967	DQ279967	DQ279967	DQ280009	AF120668	DQ280041
	<i>Mya arenaria</i>	AF120560	AB126332	AB126332	AB126332	AB126332	AY377770	AY070140	AY377618

Table 1 (continued)

	Species	18S rRNA	28S1 rRNA	28S2 rRNA	28S3 rRNA	28S4 rRNA	H3	COI	16S rRNA	
Cephalopoda	<i>Nautilus pompilius</i>	AY557455	AY145417	AY145417	AY145417	AY145417		AY557514	AY377628	
	<i>Nautilus scrobiculatus</i>	AF120504		AF120567			AF033704		U11606	
	<i>Stauroteuthis syrtensis</i>	AY557457		DQ279968		DQ279968	AY557406	AF000067	DQ280042	
	<i>Vampyroteuthis infernalis</i>	AY557459	AH012197	AH012197		AH012197	AH012197	AF000071	DQ280043	
	<i>Bathypolypus arcticus</i>	AY557465		AY557554				AF000029	DQ280044	
	<i>Sepia officinalis</i>	AY557471		AY557560			AY557415	AF000062	DQ093491	
	<i>Sepioloa affinis</i>	AY557474		AY557562			AY557418	AY557523	AY293667	
	<i>Heteroteuthis hawaiiensis</i>	AY557472		DQ279969			AY557416	AF000044	AY293680	
	<i>Rossia palpebrosa</i>	AY557473		AY557561			AY557417	AF000061	DQ280045	
	<i>Spirula spirula</i>	AY557476		AY557563			AY557420	AY293709	AY293659	
	<i>Idiosepius pygmaeus</i>	AY557477		AY293684			AY557421	AY293708	AY293658	
	<i>Loligo pealei</i>	AY557479	AH012196	AH012196		AH012196	AY557423	AF120629	AF110079	
	<i>Architeuthis dux</i>	AY557482	DQ279970	DQ279970			AY557426	AF000027	AY377629	
	<i>Cranchia scabra</i>	AY557487		AY557571			AY557430	AF000035	DQ280046	
	<i>Histioteuthis hoylei</i>	AY557500		AY557584			AY557442	AF000045	DQ280047	
	<i>Lepidoteuthis grimaldii</i>	AY557503		AY557587			AY557445	AF000049	DQ280048	
	<i>Ommastrephes bartrami</i>	AY557510		AY557594			AY557451	AF000057	DQ280049	
	<i>Moroteuthis knipovitchi</i>	AY557512		AY557596			AY557453	AY557543	DQ280050	
	Gastropoda	<i>Cellana nigrolineata</i>	DQ013353		DQ279971			DQ093493	DQ093515	DQ093467
		<i>Eulepetopsis vitrea</i>	DQ093427	DQ279972	DQ279972			DQ093495	DQ093516	DQ093468
<i>Cocculina messingi</i> /sp.		AF120508	DQ279973	DQ279973		DQ279973	AY377777	AY377731	AY377624	
<i>Alcacia dysonia</i>		DQ093428		DQ279974		DQ279974	DQ093496		DQ093469	
<i>Theodoxus fluviatilis</i>		AF120515		DQ279975		DQ279975		AF120633	DQ093470	
<i>Nerita fumulata</i>		DQ093429		DQ279976		DQ279976	DQ093497	DQ093517	DQ093471	
<i>Cyathernia naticoides</i>		DQ093430	DQ279977	DQ279977		DQ279977	DQ093498	DQ093518	DQ093472	
<i>Depressigya globulus</i>		DQ093431	DQ279978	DQ279978		DQ279978	AF033689	DQ093519	DQ093473	
<i>Perotrochus midas</i>		AF120510		DQ093453			DQ093500	AY296820	DQ093474	
<i>Entemnotrochus adansonianus</i>		AF120509	DQ279979	DQ279979		DQ279979	AY377774		AY377621	
<i>Lepetodrilus elevatus</i>		DQ093432	AY145413	AY145413		AY145413	DQ093501	DQ093520	DQ093475	
<i>Diodora graeca</i>		AF120513	DQ279980	DQ279980		DQ279980	DQ093502	AF120632	DQ093476	
<i>Haliotis tuberculata</i> /discus		AF120511	AY145418	AY145418		AY145418	AY070145	AY377729	AY377622	
<i>Sinezona confusa</i>		AF120512	DQ279981	DQ279981		DQ279981		AY377773	AF120631	
<i>Bathymargarites symplector</i>		DQ093433	DQ279982	DQ279982		DQ279982	DQ279982	DQ093503	DQ093521	
<i>Aperostoma palmeri</i>		DQ093435	DQ279983	DQ279983		DQ279983		DQ093505	DQ093523	
<i>Pomacea bridgesi</i>		DQ093436		DQ279984		DQ279984		DQ093506	DQ093524	
<i>Viviparus georgianus</i>		AF120516		AF120574				AY377779	AF120634	
<i>Balcis eburnea</i>		AF120519		AF120576					AF120636	
<i>Crepidula fornicata</i>		AY377660	AY145406	AY145406		AY145406	AY145406	AY377778	AF353154	
<i>Littorina littorea</i>		DQ093437	DQ279985	DQ279985				DQ093507	DQ093525	
<i>Truncatella guerini</i>		AF120518		AF120575					AF120635	
<i>Bolinus brandaris</i>		DQ279944	DQ279986	DQ279986		DQ279986		DQ280010	DQ280020	
<i>Raphitoma linearis</i>		DQ279945		DQ279987				DQ280011	DQ280053	
<i>Philina aperta</i>		DQ093438	DQ279988	DQ279988			DQ279988	DQ093508	DQ093482	
<i>Creseis</i> sp.		DQ279946	DQ279989				DQ279989	DQ280012	DQ280021	
<i>Peltodoris atromaculata</i>		AF120521		DQ279990				DQ280013	AF120637	
<i>Salinator solida</i>		DQ093440	DQ279991	DQ279991		DQ279991		DQ093510	DQ093528	
<i>Onchidella</i> sp.		DQ093441		DQ279992				DQ093511	DQ093529	
<i>Siphonaria pectinata</i>		X91973	DQ279993	DQ279993				AY377780	AF120638	
<i>Ophicardelus ornatus</i>		DQ093442	DQ279994	DQ279994		DQ279994		DQ093512	DQ093530	
<i>Micromelo undatus</i>		DQ093443		DQ279995				DQ093513	DQ093487	

the previous data set of Giribet et al. (2006) with some important updates (see Table 1), and analyzed using static and dynamic homology methods under different optimality criteria.

2. Materials and methods

2.1. New data

We collected ~50 live specimens of *Laevipilina hyalina* McLean, 1979 from dredged rock nodules in the Santa Rosa-Cortes Ridge of the Southern California continental borderland at depths ca. 400 m (see Wilson et al., 2009 for details). Direct sequencing of two individuals of *L. hyalina* was carried out in respective laboratories (SIO and Harvard) to ensure fidelity of data. We extracted genomic DNA with a DNeasy blood & tissue extraction kit (Qiagen, USA) and amplified genes (the nuclear genes 18S rRNA, 28S rRNA and

histone H3 and the mitochondrial genes 16S rRNA and cytochrome c oxidase subunit I) using primers listed in Giribet et al. (2006). We then added our new data to an updated version of the multi-gene data set used by Giribet et al. (2006) (Table 1 and Supplementary Material S2 and S3). Novel sequences have also been added for three chitons *Leptochiton asellus*, *Lepidopleurus cajetanus* and *Cryptoplax japonica*.

2.2. Static homology analyses

We examined the data set with a broad range of analytical criteria. MUSCLE v3.7 (Edgar, 2004) was used to generate alignments for each partition using default settings, and then data were combined. We also parsed the MUSCLE alignments through the Gblocks server (Castresana, 2000) allowing for the least stringent exclusion options. This procedure retained 54% of positions for the 16S

partition, 37% of 18S, 38% of 28s1, 39% of 28s2, 63% of 28s3 and 42% of 28s4. Finally, we also generated alignments using default parameters in TCOffee (Notredame et al., 2000).

We carried out heuristic searches under parsimony in PAUP* (Swofford, 2002) with 100 random sequence additions and TBR branch swapping, and estimated nodal support with 100 jackknife replicates (37% deletion; Farris et al., 1996). Bayesian inference was implemented in MrBayes (6 chains, 5 million generations, sampling one tree every 1000 generations) with appropriate models of evolution (GTR + I + Γ for each unlinked partition) chosen via the Akaike Information Criterion with MrModeltest v2.3 (Nylander, 2004; Posada, 2005). Data were partitioned by gene (and 28S rRNA into four fragments corresponding to amplification described in detail under the dynamic homology section in Supplementary Material S4). Burn-in was set *a posteriori*, after using Tracer v1.3 (Rambaut and Drummond, 2005). We used RaxML v7.0.4 (Stamatakis, 2006) for maximum likelihood analyses, selecting joint branch length optimization of parameters under a GTR + Γ model, with 1000 bootstrapped replicate searches (Stamatakis et al., 2008) on the CIPRES web portal (Cyberinfrastructure for Phylogenetic Research at the San Diego Supercomputer Center).

2.3. Dynamic homology analyses

The data files used in these analyses were partitioned as in Giribet et al. (2006) with a few changes, specified in Supplementary Material S4. Dynamic homology analyses were conducted with the program POY 4.0.0 rc 2885 (Varón et al., 2008). Analyses consisted of a timed search that lasted up to 24 h on 200 processors, or stopping after hitting minimum tree length 20 times, whatever came first. Tree searches were conducted using an opening indel cost of 3, indel extension cost of 1, and nucleotide substitution cost of 2, as in the preferred tree of Giribet et al. (2006), using the command 'transform (tcm:(2, 1), gap_opening:2)' (De Laet, 2005). An initial Wagner tree was built and swapped using SPR and TBR branch swapping, followed by tree fusing (Goloboff, 1999) and parsimony ratchet (Nixon, 1999). This process was repeated using the trees obtained in the first round of analyses as input for the next round of searching (Giribet, 2007). Nodal support was assessed via 1000 replicates of jackknifing (Farris et al. 1996) using auto sequence partition to select the fragments to be deleted. A probability of deletion of 0.36 was used.

3. Results and discussion

3.1. The Serialia hypothesis

A comparison of all analytical approaches (Table 2) suggests Serialia is supported by model-based approaches, but not by parsimony implemented with either static or dynamic homology approaches. Analyses based on a MUSCLE alignment strongly supported the clade Serialia (ML bootstrap 93%; Bayesian posterior probability [PP] 1.0), consisting of Monoplacophora and Polyplacophora as reciprocally monophyletic sister taxa (Figs. 1 and 2) that was sister to the rest of Mollusca. Use of a TCOffee alignment also recovered Serialia (Table 2), but with weaker support under ML (ML bootstrap 59%; Bayesian PP 1.00) (Fig. 2). Using Gblocks to remove areas of the MUSCLE alignment considered ambiguous lowered support values, but maintained them in the range considered as significant (ML bootstrap 69%; Bayesian PP 0.98). In contrast, parsimony analyses, using static (MUSCLE or MUSCLE + Gblocks alignment, Fig. 2) or dynamic homology (POY, Supplementary Material S5) approaches, did not recover Serialia. The alignment created with TCOffee retrieved Serialia in the shortest tree, but this did receive any statistical support via jackknifing. These results are

Table 2

Support for Serialia under different alignment and analytical approaches.

Support values	Alignment generator		
	MUSCLE	MUSCLE + Gblock	TCoffee
PAUP* jackknife	NA	NA	NA ^a
RaxML bootstrap	93	69	59
MrBayes posterior probability	1.00	0.98	1.00

POY analysis did not recover Serialia.

^a Serialia was recovered in the shortest tree, but had no jackknife support.

somewhat surprising as Giribet et al. (2006) found Serialia using a POY analysis, and also with a Bayesian analysis of an implied alignment generated by POY, but this was likely caused by the inclusion of the chimeric sequence for the monoplacophoran terminal (see Supplementary Material S1).

Most malacologists suggest that Monoplacophora is the sister group to the remaining Conchifera (Haszprunar, 2008). Accordingly, the identification of Serialia uniting Monoplacophora with Polyplacophora by Giribet et al. (2006) was a revolutionary hypothesis in molluscan systematics. Its introduction was not without controversy; Steiner (in Haszprunar, 2008) suggested there were potential contamination issues without giving details, and others insisted on the need for more data (Haszprunar et al., 2008). Our results indicate reciprocal monophyly of Monoplacophora with Polyplacophora, and it is likely that the nesting of Monoplacophora inside Polyplacophora by Giribet et al. (2006) was due to the chimeric nature of the 28S rRNA gene fragment they used. Nevertheless, our new data do provide support for the Serialia hypothesis, but statistical support was only forthcoming under analytical methods that incorporated model-based (maximum-likelihood and Bayesian) approaches. In summary, we conclude that the Serialia hypothesis is supported by increased data, but this support is not unambiguous. Nevertheless, support for the long-standing Conchifera hypothesis was not found in any of the analyses performed here.

3.2. Molluscan relationships

The three major molluscan lineages recovered here with high support were Serialia, Bivalvia and Gastropoda + Scaphopoda + Caudofoveata + Cephalopoda (Fig. 2). The results from multiple methods differed significantly only with respect to the position of the gastropod group Patellogastropoda. In general, most methods recovered a Patellogastropoda–Cephalopoda clade. Exceptions were found when the MUSCLE + Gblock alignment was subjected to maximum likelihood, which resulted in a Patellogastropoda–Caudofoveata clade, or Bayesian analyses, which recovered a Patellogastropoda–Cephalopoda–Caudofoveata polytomy (also seen using the TCOffee alignment). The only combination to retrieve Gastropoda as monophyletic was Bayesian inference on the MUSCLE alignment, which did not garner much statistical support (Fig. 1). If Patellogastropoda is excluded from Gastropoda, the latter generally receives strong support from likelihood (58–99) and the Bayesian analyses (0.86–1.00). Even when excluding Patellogastropoda, parsimony analyses rarely recover support for Gastropoda. On examination of the alignments we identified large inserts in the patellogastropod ribosomal data that overlap with large inserts also found in many cephalopods. This suggests that processes such as long-branch attraction and heterotachy (site specific substitution rate changing through time) interacting with secondary structure may be responsible for topology and support shifts under different reconstruction scenarios (Baele et al., 2006; Philippe et al., 2005), and we regard the Patellogastropoda and Cephalopoda relationship as artifactual.

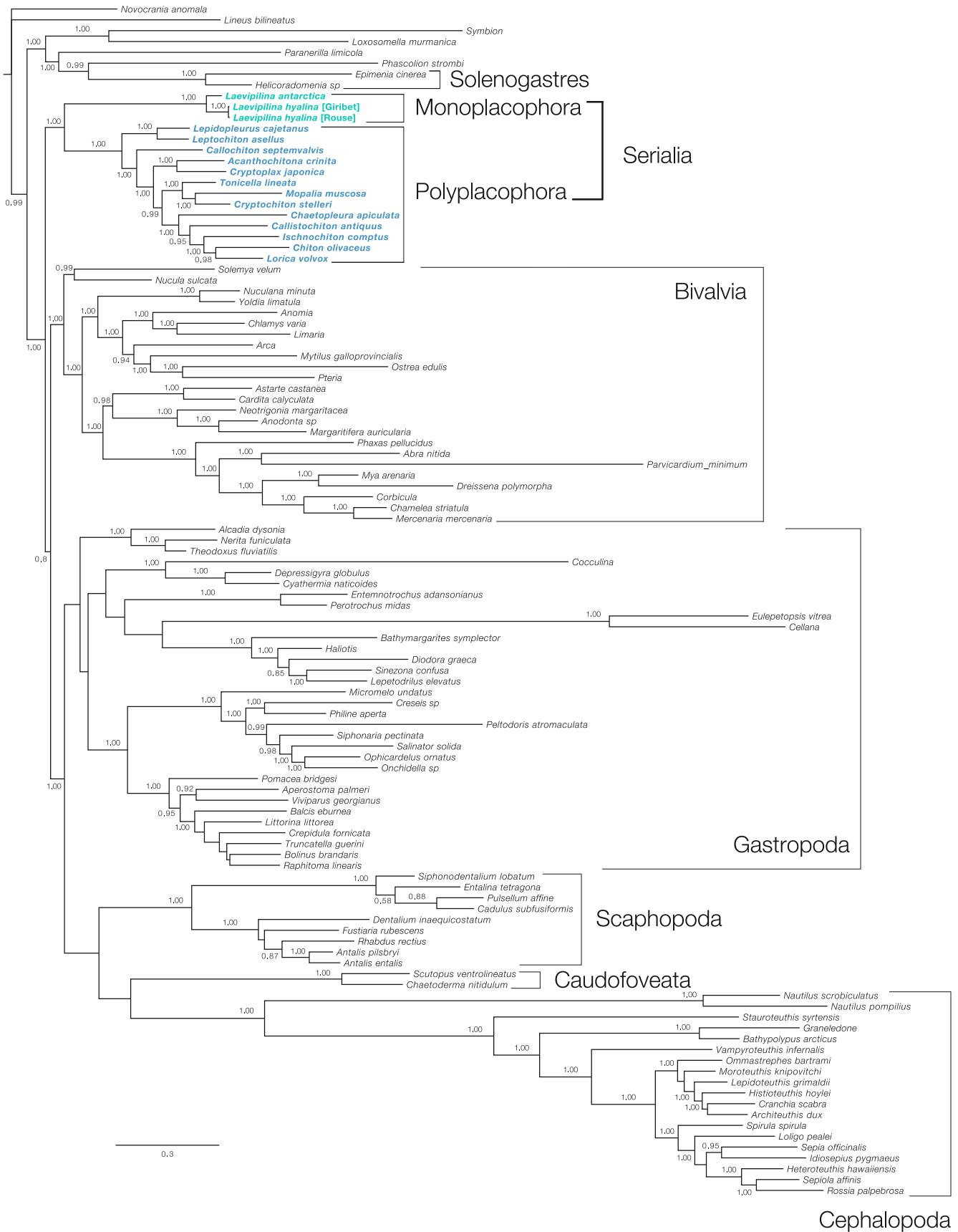


Fig. 1. Bayesian consensus tree of relationships within Mollusca generated by MrBayes v3.1.2 from an alignment generated with MUSCLE, with posterior probabilities assessed from 3000 trees. A Serialia clade is recovered with strong support and is indicated by colored font.

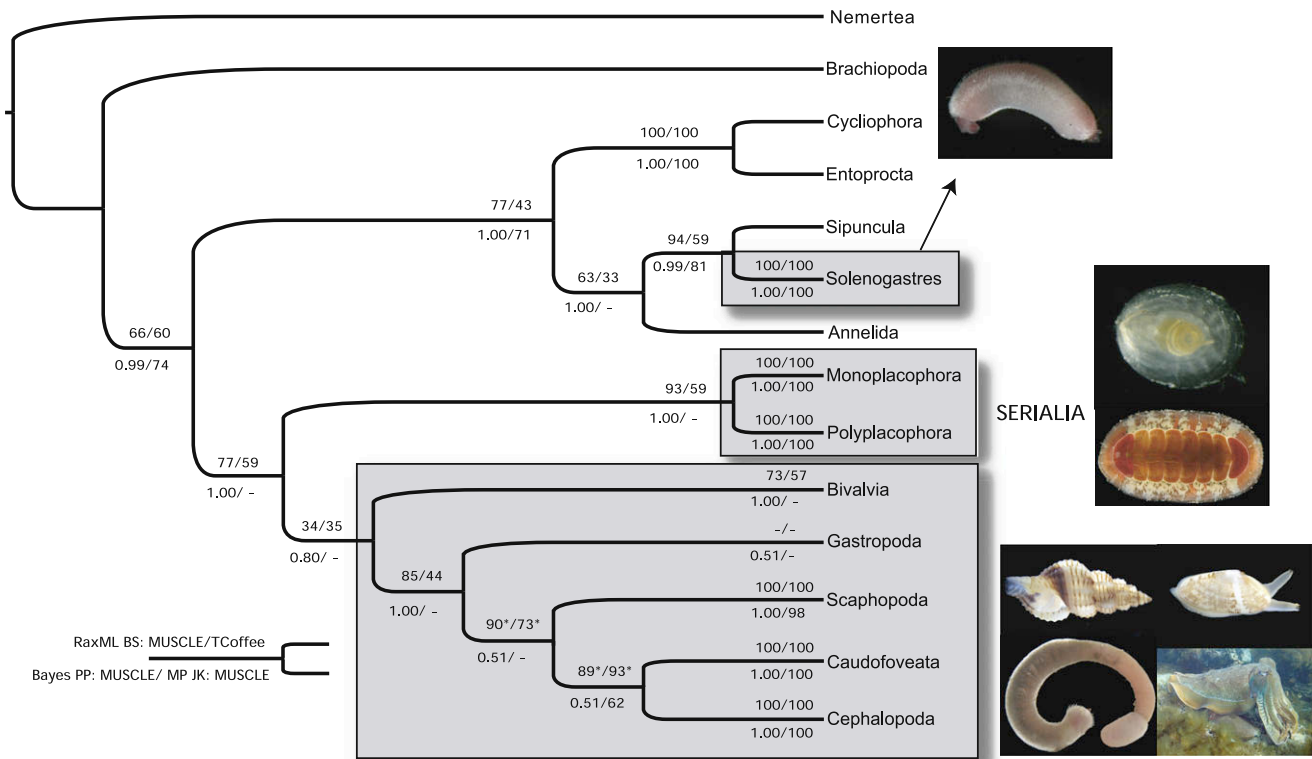


Fig. 2. Cladogram of molluscan relationships comparing analytical approaches. Weakly-supported nodes are not collapsed to allow comparison between different phylogenetic reconstruction methods. Support for clades marked with an asterisk also include Patellogastropoda. BS, bootstrap; PP, posterior probability; JK, jackknife. All photographs by Greg Rouse.

The aplacophoran group Caudofoveata, was placed intriguingly close to Cephalopoda (sometimes also including Patellogastropoda) in all of our analyses, though with varying levels of support (Figs. 1 and 2). But for this placement, no putative long-branch attraction can be invoked based on examination of the sequence alignments. Some evidence for a Caudofoveata + Cephalopoda relationship has previously been recovered via direct sequencing phylogenetics (Giribet et al. 2006), a phylogenomic analysis consisting of 150 genes (Dunn et al., 2008), and a haemocyanin gene phylogeny (Lieb and Todt, 2008). If this relationship is further corroborated, there are interesting implications for shell evolution, suggesting the shell-less vermiform body of Caudofoveata is secondarily derived, and not plesiomorphic as is widely accepted (e.g. Haszprunar et al., 2008; Todt et al., 2008).

The monophyly of Mollusca as traditionally formulated was not recovered here because Solenogastres were nested inside an Annelida (including Sipuncula) clade (Figs. 1 and 2) or the sipunculan and brachiopod sequences were nested within molluscs (Supplementary Material S5). Although there is increasing morphological evidence indicating Aplacophora (Solenogastres + Caudofoveata) is not monophyletic (Haszprunar, 2000; Salvini-Plawen, 1980; Salvini-Plawen and Steiner, 1996), we note here that Solenogastres are notorious for presenting exogenous DNA contamination problems (Okusu and Giribet, 2003). The only available 18S and 28S sequences for Solenogastres in this study (*Helicoradomenia* sp. AY145377 and AY145409, respectively) blast closely to polychaete sequences in GenBank, but not unambiguously enough to support their exclusion here. The high support for inclusion of Solenogastres in Annelida in this study is almost certainly caused by these two sequences, and thus this result should be reassessed. Moreover, such persistent contamination has generally limited the available data for Solenogastres, perhaps contributing to its

non-traditional placement here. New generation sequencing of Expressed Sequence Tags and analytical techniques have provided promising preliminary data (Dunn, Wilson and Giribet, unpublished results), and will undoubtedly aid in future resolution of molluscan relationships.

Acknowledgments

The captain and crew of the *R/V Robert Gordon Sproull*, Cambria Colt, Eddie Kisfaludy and volunteers were essential for efficient sampling. We acknowledge a grant from UC Ship Funds Panel to NGW to lead the collection cruise. This study is based on work supported by NSF Assembling the Tree of Life Program (Grant 0334932 to GG) and SIO start-up funds to GWR. We also acknowledge the NSF-funded CIPRES project for computational resources.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ympmv.2009.07.028](https://doi.org/10.1016/j.ympmv.2009.07.028).

References

- Baele, G., Raes, J., Van de Peer, Y., Vansteelandt, S., 2006. An improved statistical method for detecting heterotachy in nucleotide sequences. *Mol. Biol. Evol.* 23, 1397–1405.
- Bourlat, S.J., Juliusdottir, T., Lowe, C.J., Freeman, R., Aronowicz, J., Kirschner, M., Lander, E.S., Thorndyke, M., Nakano, H., Kohn, A.B., Heyland, A., Moroz, L.L., Copley, R.R., Telford, M.J., 2006. Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. *Nature* 444, 85–88.
- Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analyses. *Mol. Biol. Evol.* 17, 540–552.
- De Laet, J.E., 2005. Parsimony and the problem of inapplicables in sequence data. In: Albert, V.A. (Ed.), *Parsimony, Phylogeny, and Genomics*. Oxford University Press, Oxford, pp. 81–116.

- Dunn, C.W., Hejnol, A., Matus, D.Q., Pang, K., Browne, W.E., Smith, S.A., Seaver, E.C., Rouse, G.W., Obst, M., Edgecombe, G.D., Sørensen, M.V., Haddock, S.H.D., Schmidt-Rhaesa, A., Okusu, A., Kristensen, R.M., Wheeler, W.C., Martindale, M.Q., Giribet, G., 2008. Broad taxon sampling improves resolution of the animal tree of life. *Nature* 452, 745–749.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797.
- Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D., Kluge, A.G., 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12, 99–124.
- Giribet, G., 2007. Efficient tree searches with available algorithms. *Evol. Bioinform.* 3, 1–16.
- Giribet, G., Okusu, A., Lindgren, A.R., Huff, S., Schrödl, M., Nishiguchi, M.K., 2006. Evidence for a clade composed of molluscs with serially repeated structures: Monoplacophorans are related to chitons. *Proc. Natl. Acad. Sci. USA* 103, 7723–7728.
- Goloboff, P.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15, 415–428.
- Haszprunar, G., 2000. Is the Aplousobranchia monophyletic? A cladistic point of view. *Am. Mal. Bull.* 15, 115–130.
- Haszprunar, G., 2008. Monoplacophora (Tryblidia). In: Ponder, W.F., Lindberg, D.R. (Eds.), *Phylogeny and Evolution of the Mollusca*. University of California Press, Berkeley, pp. 97–104.
- Haszprunar, G., Schaefer, K., 1997. Anatomy and phylogenetic significance of *Micropilina arntzi* (Mollusca, Monoplacophora, Micropilinidae fam. nov.). *Acta Zool. Stockholm* 77, 315–334.
- Haszprunar, G., Schander, C., Halanych, K.M., 2008. Relationships of higher molluscan taxa. In: Ponder, W.F., Lindberg, D.R. (Eds.), *Phylogeny and Evolution of the Mollusca*. University of California Press, Berkeley, pp. 19–32.
- Helmkamp, M., Bruchhaus, I., Hausdorf, B., 2008. Phylogenomic analyses of lophophorates (brachiopods, phoronids and bryozoans) confirm the Lophotrochozoa concept. *Proc. R. Soc. Lond. B* 275, 1927–1933.
- Lemche, H., 1957. A new living deep-sea mollusc of the Cambro-Devonian class Monoplacophora. *Nature* 179, 413–416.
- Lemche, H., Wingstrand, K., 1959. The anatomy of *Neopilina galathea* Lemche, 1957 (Mollusca, Tryblidiacea). *Galathea Rep.* 3, 9–72.
- Lieb, B., Todt, C., 2008. Hemocyanin in mollusks – a molecular survey and new data on hemocyanin genes in Solenogastres and Caudofoveata. *Mol. Phylogenet. Evol.* 49, 382–385.
- Lindberg, D.R., 2009. Monoplacophorans and the origin and early relationships of Mollusks. *Evo. Edu. Outreach* 2, 191–203.
- Nixon, K.C., 1999. The Parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics* 15, 407–414.
- Notredame, C., Higgins, D.G., Heringa, J., 2000. T-Coffee: a novel method for multiple sequence alignments. *J. Mol. Biol.* 302, 205–217.
- Nylander, J.A.A., 2004. MrModeltest. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Okusu, A., Giribet, G., 2003. New 18S rRNA sequences from neomeniid aplousobranchs and the possible origin of persistent exogenous contamination. *J. Mollus. Stud.* 69, 385–387.
- Passamaneck, Y.J., Schander, C., Halanych, K.M., 2004. Investigation of molluscan phylogeny using large-subunit and small-subunit nuclear rRNA sequences. *Mol. Phylogenet. Evol.* 32, 25–38.
- Philippe, H., Zhou, Y., Brinkmann, H., Rodrigue, N., Delsuc, F., 2005. Heterotachy and long-branch attraction in phylogenetics. *BMC Evol. Biol.* 5, 50.
- Posada, D., 2005. Modeltest Version 3.7 documentation. Available from: <<http://darwin.uvigo.es/software/modeltest.html/>>.
- Rambaut, A., Drummond, A., 2005. Tracer: MCMC Trace Analysis Tool. University of Oxford.
- Salvini-Plawen, L.v., 1980. A reconsideration of systematics in the Mollusca (phylogeny and higher classification). *Malacologia* 19, 249–278.
- Salvini-Plawen, L.v., Steiner, G., 1996. Synapomorphies and plesiomorphies in higher classification of Mollusca. In: Taylor, J. (Ed.), *Origin and Evolutionary Radiation of the Mollusca*. Oxford University Press, Oxford, pp. 29–51.
- Schwabe, E., 2008. A summary of reports of abyssal and hadal Monoplacophora and Polyplacophora. In: Martínez Arbizu, P., Brix, S. (Eds.), *Bringing Light into Deep-sea Biodiversity*. Magolia Press, Zootaxa, pp. 205–222.
- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web-servers. *Syst. Biol.* 57, 758–771.
- Swofford, D.L., 2002. PAUP[®]. Sinauer Associates Inc., Sunderland, Massachusetts.
- Todt, C., Okusu, A., Schander, C., Schwabe, E., 2008. Solenogastres, Caudofoveata, and Polyplacophora. In: Ponder, W.F., Lindberg, D.R. (Eds.), *Phylogeny and Evolution of the Mollusca*. University of California Press, Berkeley, p. 469.
- Varón, A., Vinh, L.S., Bomash, I., Wheeler, W.C., 2008. POY. Available from: <<http://research.amnh.org/scicomp/projects/poy.php/>>. American Museum of Natural History, New York.
- Warén, A., Gofas, S., 1996. A new species of Monoplacophora, redescription of the genera *Veloropilina* and *Rokopella*, and new information on three species of the class. *Zool. Scr.* 25, 215–232.
- Wilson, N.G., Huang, D., Goldstein, M.C., Cha, H., Giribet, G., Rouse, G.W., 2009. Field collection of *Laevipilina hyalina* McLean, 1979 from southern California, the most accessible living monoplacophoran. *J. Mollus. Stud.* 75, 195–197.
- Wingstrand, K.G., 1985. On the anatomy and relationships of recent Monoplacophora. *Galathea Rep.* 16, 7–94.
- Winnepenninckx, B., Backeljau, T., de Wachter, R., 1996. Investigation of molluscan phylogeny on the basis of 18S rRNA sequences. *Mol. Biol. Evol.* 13, 1306–1317.