

Molecular phylogeny and morphological evolution of laqueoid brachiopods

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Abstract. One of the virtues of molecular phylogeny for paleontology is that it can provide independent and often reliable sets of data from living relatives to test various evolutionary hypotheses inferred from fossil forms. In this study, we present results of a molecular phylogenetic analysis of 12 species of 7 genera belonging to the Laqueoidea, which is the most prolific of the brachiopod superfamilies in the seas around Japan. Onto a phylogenetic tree based on partial sequences (1218 bp) of the mitochondrial *cox1* gene, we superimposed various external and internal morphologic characters of both juveniles and adults for the taxa examined. The resulting patterns indicated that several lineages experienced paedomorphic evolution in terms of the brachidial (loop) morphology, and that, contrary to some traditional views, certain adult features, such as the bilateral loop, possession of a cardinal process, and a rectimarginate commissure, had homoplasious distributions. Examination of the character distributions also revealed, however, that anterior nonbifurcation of the septal pillar at the axial phase is a synapomorphy for a major clade recognized in the molecular analysis. Those results suggest that early loop ontogeny, information about which is still fragmentary, would be useful in assessing relationships among laqueoid brachiopods, including certain Mesozoic genera.

Key words: Brachiopoda, cytochrome c oxidase I (*cox1*) gene, Laqueoidea, loop ontogeny, mitochondrial DNA, molecular phylogeny, Recent

Introduction

The Laqueoidea is one of the larger terebratulide brachiopod superfamilies, being well represented in both present-day waters and Cenozoic strata of Japan. The members of this superfamily, as well as other superfamilies within the order Terebratulida, are characterized by the possession of calcareous internal skeleton known as the loop which continually undergoes considerable morphological change during growth before attaining the full adult stage. Due to its complexity and diversity, loop morphology has been the prime tool for the inference of phylogenetic relationships among laqueoid brachiopods and for the classification of long-looped brachiopods generally at various taxonomic levels (Hatai, 1936, 1940; Muir-Wood *et al.*, 1965; MacKinnon, 1993). However, the assumption that loop morphology reflects phylogeny has not been fully tested, nor has it been possible to discuss loop evolution without the risk of circular arguments. Ideally phylogenies should be based on characters that are completely independent of the loop. One such would be molecular characteristics.

Although molecular trees are only working hypotheses of the true evolutionary relationships of living species, they are useful in providing a basis for discussion of the likely history of a character of interest. In studies of morphological character states, predictions can be made about the probable direction of morphological character state transformations and combinations of characters in basal versus derived species, and therefore we can reassess the morphological characters that support the relationships. Because only morphological characters can be used to establish the phylogenetic affinities of ancient fossil taxa, the success of a morphological approach for fossils can be increased greatly if patterns of character state evolution are considered in the light of an independently estimated phylogeny.

Previous brachiopod molecular systematics have been based on immunological comparisons of shell macromolecules (Collins *et al.*, 1988; Curry *et al.*, 1991; Endo *et al.*, 1994) and on nucleotide sequence comparisons of nuclear 18S ribosomal ribonucleic acids (rRNA) and mitochondrial 12S rRNA (Cohen and Gawthrop, 1996, 1997; Cohen *et al.*, 1998a, 1998b). The novel overall patterns of loop evolution

that the immunological data indicated were largely unsupported by the results of 18S rRNA sequence comparisons (Cohen and Gawthrop, 1997; Cohen *et al.*, 1998b). Besides, it was difficult using the immunological data to resolve relationships among closely related genera. Even with the 18S rRNA data, which offered direct measurements of molecular similarity and thus are more reliable, detailed relationships among the long-looped terebratulide brachiopods remained unclear because the tempo of the 18S rRNA sequence evolution was considered too slow to provide adequate variations among these forms (Cohen *et al.*, 1997).

Both the nucleotide and amino acid sequences of the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene turned out to provide a potentially useful framework for shallower phylogenies, especially of the relationships among the long-looped laqueoid brachiopods (Saito *et al.*, 2000). In this paper, we report the phylogenetic relationships of laqueoid brachiopods inferred from the *cox1* sequences and discuss evolutionary processes of the loop and of other morphologic characters in laqueoid brachiopods, including some possibly basal Mesozoic fossils.

Material and methods

Brachiopod samples and molecular phylogenetic analysis.—Twenty-seven specimens representing a total of 16 species of terebratulide brachiopods including 11 laqueoid species were available for this study (Table 1).

Full details of DNA extraction, amplification and sequencing methods are described in Saito *et al.* (2000). In brief, *cox1* sequences (1218 bp or 406 amino acids in length) were obtained by the direct sequencing of DNA amplification products synthesized by PCR. Amino acid sequences were deduced by reference to the genetic code of brachiopod mitochondrial DNA (Saito *et al.*, 2000). Phylogenetic analysis by maximum-parsimony (MP) was performed with PAUP version 3.1 (Swofford, 1993), using the exhaustive search algorithm and equal weighting for all substitutions. To evaluate the robustness of the internal branches, 500 bootstrap replications were executed. Analysis by neighbor-joining (NJ; Saitou and Nei, 1997) and maximum-likelihood (ML) were performed with Molphy version 2.3 (Adachi and Hasegawa, 1996a) using the mtREV24-F model (Adachi and Hasegawa, 1996b) for amino acid data and HKY85 model for nucleotide data (Hasegawa *et al.*, 1985), using the "Local Rearrangement Search" option. For each internal branch, a local bootstrap probability (LBP) was estimated by the REL method (Kishino *et al.*, 1990) with 1000 replications. TreeView version 1.4 (Page, 1996) was used to draw trees.

Because of the low intraspecific nucleotide sequence variations in the examined individuals (less than 2%), and the lack of any amino acid difference within each species, one individual was selected to represent the species in the phylogenetic analysis. These representative nucleotide sequences will appear in the DDBJ nucleotide sequence database with the Accession Numbers AB026501–AB026516 shown in Table 1.

For analysis of deeper relationships within the Laqueoidea, both the amino acid and the 1st and 2nd codon

Table 1. Specimens used in this study and their sampling localities. Accession numbers refer to the DDBJ nucleotide sequence database.

Species	Locality	Accession number
Ingroup		
(Laqueoidea)		
<i>Laqueus rubellus</i> 1	Sagami Bay	AB026501
<i>L. rubellus</i> 2	Sagami Bay	
<i>L. rubellus</i> 3	Sagami Bay	
<i>L. blanfordi</i>	Otsuchi, Tohoku	AB026502
<i>L. quadratus</i> 1	SW of Oshima	AB026505
<i>L. quadratus</i> 2	SW of Oshima	
<i>L. californicus</i>	Monterey Bay, California, USA	AB026503
<i>L. c. vancouveriensis</i>	Monterey Bay, California, USA	AB026504
<i>Pictothyris picta</i> 1	off Mishima	AB026506
<i>Pictothyris picta</i> 2	Sagami Bay	
<i>Jolonica nipponica</i>	Izu Islands, W of Takase	AB026509
<i>Frenulina sanguinolenta</i> 1	Vava'u, Tonga	AB026510
<i>F. sanguinolenta</i> 2	Vava'u, Tonga	
<i>Shimodaia pterygiota</i> 1	Off Shimoda	AB026511
<i>S. pterygiota</i> 2	Off Shimoda	
<i>Terebratalia coreanica</i> 1	Otsuchi, Tohoku	AB026508
<i>T. coreanica</i> 2	Wakkanai, Hokkaido	
<i>Coptothyris grayi</i> 1	Otsuchi, Tohoku	AB026507
<i>C. grayi</i> 2	Wakkanai, Hokkaido	
Outgroup		
(long-looped forms)		
<i>Ecnomiosa</i> sp. 1	Izu Islands	AB026512
<i>Ecnomiosa</i> sp. 2	Izu Islands	
<i>Campages</i> sp.	SW of Yonejima	AB026513
Outgroup		
(short-looped forms)		
<i>Terebratulina crossei</i>	Otsuchi, Tohoku	AB026514
<i>Terebratulina pacifica</i>	off Oshima	AB026515
<i>Gryphus davidsoni</i>	S of Oshima	AB026516

position nucleotide sequences were used as data sets. Because little variation was detected in the amino acid sequences and the 1st and 2nd codon positions of nucleotide sequences, analyses of five *Laqueus* species were performed separately with full lengths of the nucleotide data.

Morphological observations.—Observation of juvenile loop morphologies of two laqueoid species, *Jolonica nipponica* and *Terebratalia coreanica*, was carried out on a Hitachi S-2400S Scanning Electron Microscope using the methods described by Saito (1996).

Results

Laqueoid relationships

Of the terebratulides analyzed, *Ecnomiosa* sp. and *Campages* sp. clustered basal to the laqueoids and monophyly of laqueoids were strongly supported (100% LBP, Figure 1). Therefore, we used *Ecnomiosa* sp. and *Campages* sp. as the outgroups for the analyses of all laqueoids sampled and within the species of *Laqueus*. Analyses were also made for an ingroup comprising the 6 genera of Laqueoidea using *Laqueus rubellus* as outgroup.

Analyses based on different tree-building methods (NJ, ML, and MP) and different data sets converged to indicate four possible topologies for the relationships among laqueoid genera (Trees 1 to 4; Figure 2). The results of the molecular phylogenetic analysis are summarized in Figure 3.

All resulting trees clearly indicated the basal placement of *Laqueus* in the Laqueoidea. The local bootstrap support of this node is high in all analysis (99–100%). Among the remaining six genera (*Terebratalia*, *Coptothyris*, *Shimodaia*, *Frenulina*, *Pictothyris* and *Jolonica*), the close relationship between *Jolonica* and *Pictothyris* is consistently supported by high bootstrap values (82–99%). Three of the four trees (Trees 1 to 3) show very similar topologies: the close association of *Shimodaia*-*Frenulina*-*Pictothyris*-*Jolonica*, with *Terebratalia* and *Coptothyris* left outside. The positions of *Terebratalia* and *Coptothyris* differ slightly in each tree but

they are generally positioned close to one another in the four cladograms. Tree 4 shows early branching of *Shimodaia* within the 6 genera.

The NJ analysis consistently supported Tree 1 (Figure 2). The ML analysis supported Tree 1 or Tree 2, however, the log-likelihood differences among Trees 1 to 3 are very small when amino acid sequences are used. The LBP support for the branch including *Terebratalia* or *Coptothyris* is low (19–

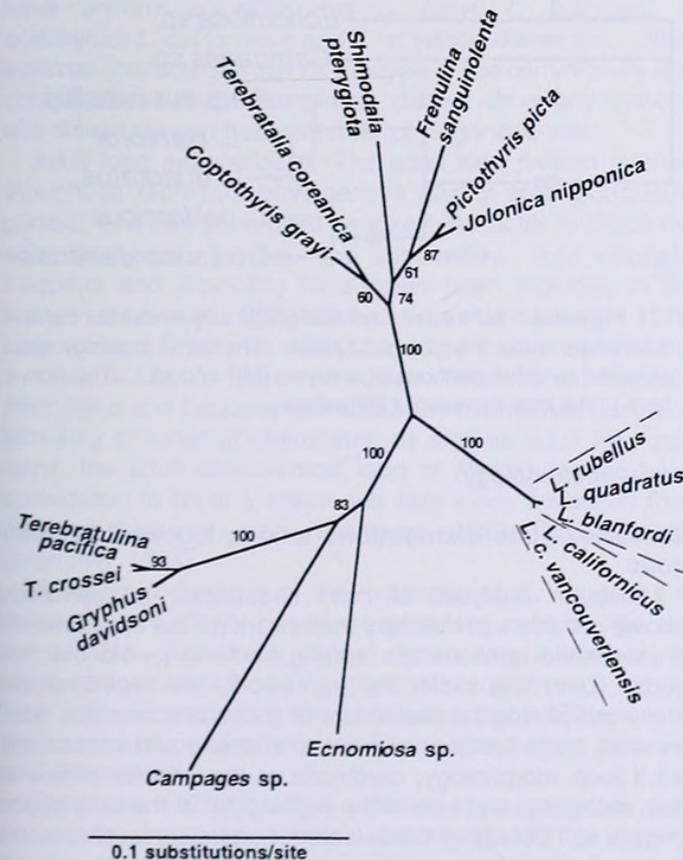
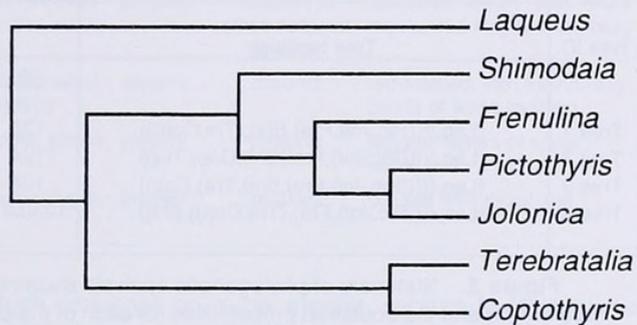
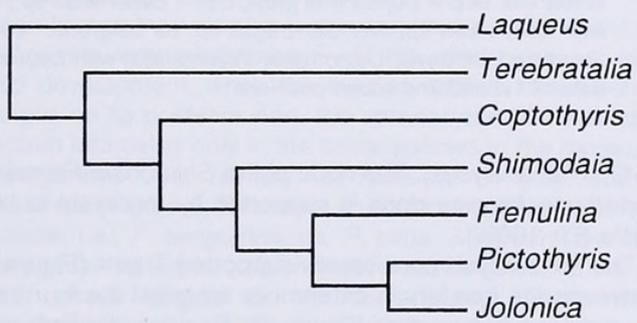


Figure 1. NJ tree based on amino acid sequences. The number at each internal node of the tree indicates the percentage of node occurrence in 500 bootstrap replicates.

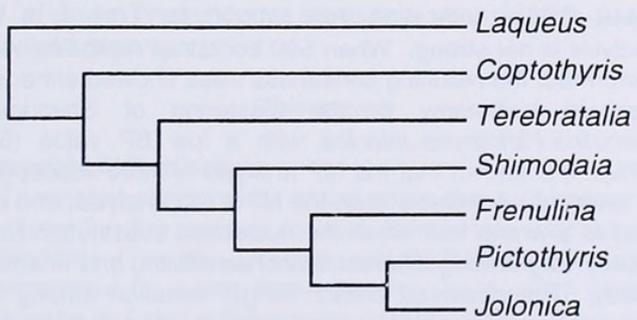
Tree 1



Tree 2



Tree 3



Tree 4

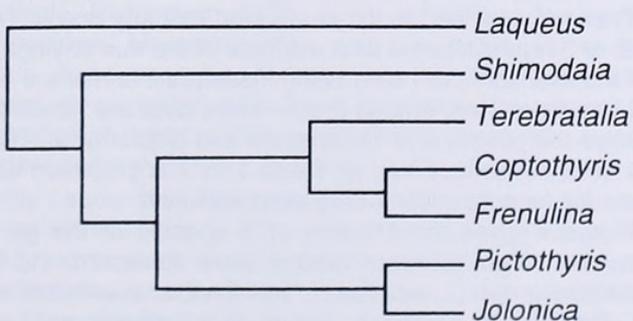


Figure 2. Four possible topologies for the relationships among laqueoid genera.

Tree ID.	Tree topology	MP		ML			
				aa		1st+2nd	
		aa	1st+2nd	InL	BP	InL	BP
Tree 1	((Laq,(((Pic,Jol),Fre),Shi),(Tra,Cop))),Ecn,Cam)	228	349	-2418.64 ± 109.10(ML)	0.2887	-2855.96 ± 101.53(ML)	0.4484
Tree 2	((Laq,(((Pic,Jol),Fre),Shi),Cop),Tra),Ecn,Cam)	230	351	-2419.42 ± 108.87(-0.8)	0.2626	-2863.78 ± 102.18(-7.8)	0.0369
Tree 3	((Laq,(((Pic,Jol),Fre),Shi),Tra),Cop),Ecn,Cam)	228	349	-2419.54 ± 109.61(-0.9)	0.2410	-2859.35 ± 101.83(-3.4)	0.2996
Tree 4	((Laq,(((Pic,Jol),Fre),(Tra,Cop)),Shi),Ecn,Cam)	226(MP)	348(MP)	-2423.61 ± 109.48(-5.0)	0.2077	-2860.59 ± 101.85(-4.6)	0.2151

Tree ID.	Tree topology	MP		ML			
				aa		1st+2nd	
		aa	1st+2nd	InL	BP	InL	BP
Tree 1	(Laq,(((Pic,Jol),Fre),Shi),(Tra,Cop)))	125	206	-1852.65 ± 77.85(-5.5)	0.0715	-2183.02 ± 75.88(-3.9)	0.0891
Tree 2	(Laq,(((Pic,Jol),Fre),Shi),Cop),Tra)	124	206	-1847.14 ± 77.19(ML)	0.6903	-2179.14 ± 75.71(ML)	0.4130
Tree 3	(Laq,(((Pic,Jol),Fre),Shi),Tra),Cop)	124	206	-1852.30 ± 78.06(-5.2)	0.1245	-2182.64 ± 75.92(-3.5)	0.1915
Tree 4	(Laq,(((Pic,Jol),Fre),(Tra,Cop)),Shi)	123(MP)	205(MP)	-1861.79 ± 79.67(-14.6)	0.1137	-2182.97 ± 76.15(-3.8)	0.3064

Figure 3. Summary of phylogenetic analysis showing the total maximum parsimony tree length, the log-likelihood (\pm standard errors) and the bootstrap probabilities for each of the plausible trees. Species name abbreviations: Laq = *Laqueus rubellus*, Pic = *Pictothyris picta*, Jol = *Jolonica nipponica*, Fre = *Frenulina sanguinolenta*, Shi = *Shimodaia pterygiota*, Tra = *Terebratalia coreanica*, Cop = *Coptothyris grayi*, Ecn = *Ecnomiosa* sp., Cam = *Campages* sp. Upper Box: relationships of 7 laqueoid genera with *Ecnomiosa* sp. and *Campages* sp. as outgroup. Lower Box: relationships of 6 laqueoid genera (*Shimodaia*, *Frenulina*, *Jolonica*, *Pictothyris*, *Coptothyris*, *Terebratalia*) with *Laqueus rubellus* as outgroup. aa: amino acid data. 1st + 2nd: nucleotide data of 1st and 2nd codon positions.

66%) in all analyses. The node of the *Shimodaia-Frenulina-Pictothyris-Jolonica* clade is supported by moderate to high LBPs (70–100%).

The MP analysis consistently supported Tree 4 (Figure 2). However, the tree length differences amongst the four trees are only one or two steps (Figure 3). Besides, the Retention Index (RI) for all four topologies was also relatively low (RI = 0.446–0.663), indicating that support for Tree 4 in MP analysis is not strong. When 500 bootstrap replicates were performed, the resulting consensus trees showed either unresolved trichotomy or the clustering of *Shimodaia-Frenulina-Pictothyris-Jolonica* with a low BP value (51–56%). It is known that the MP analysis is more susceptible to 'unequal rate effects' than the NJ or ML analysis, and can lead to a wrong tree when the nucleotide substitution rates greatly vary among different branches (Saitou and Imanishi, 1989). The observed branch length variation among the ingroup taxa (Figure 1) suggests that the tree indicated by the MP analysis may not be reliable.

These results lead to the conclusion that any one of Tree 1, 2, or 3 represents the best estimate of the true phylogeny, but the available *cox1* data being inadequate to make a final determination from among them. More data are needed to resolve the positions of *Terebratalia* and *Coptothyris*. Thus, the strict consensus tree of Trees 1 to 3 is proposed as a basis for reconstruction of laqueoid evolution.

Figure 4 gives the ML tree of 5 species of the genus *Laqueus*. Two coherent groups were assessed; (1) the Japanese group (*L. rubellus*, *L. blanfordi*, *L. quadratus*) and (2) the North American group (*L. californicus*, *L. c. vancouveriensis*). Each cluster was supported by a high LBP value (100%). The NJ and MP analyses yielded the

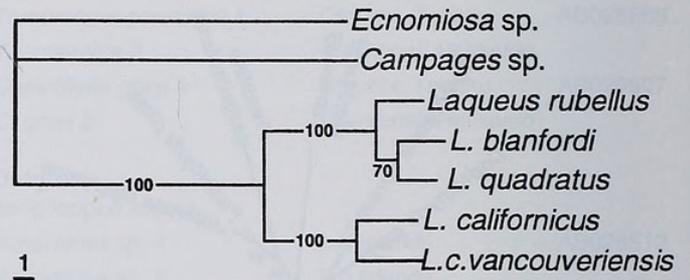


Figure 4. NJ tree of *cox1* nucleotide sequences for the relationships within the genus *Laqueus*. The same topology was obtained by other methods of analysis (MP and ML). The numbers in the tree represent LBP values.

same tree topology.

Character state distributions among laqueoid brachiopods

Molecular analyses of *cox1* sequences, as described above, provide a preliminary framework for the elucidation of phylogenetic relationships among some laqueoid brachiopods. Onto this molecular framework, we superimposed some morphological characters of those brachiopods, such as shell traits (outline, commissure shape and coloration), adult loop morphology, cardinalia at the annular phase of loop ontogeny, and type of the septal pillar at the axial phase (Figure 5). Details of the selected morphological characters for each species are summarized in Table 2. Loop ontogenetic series of laqueoid species are shown in Figure 6. Figure 7 illustrates hitherto undescribed early loop stages of *Terebratalia coreanica* and *Jolonica nipponica*.

Table 2. Comparisons of selected morphological characters of 7 species of the Laqueoidea.

Species	maximum size (cm)	adult loop pattern	axial/annular septal pillar	cardinalia	cardinal process	deltidial plates in adult	ornament and coloration
<i>Laqueus rubellus</i>	3.5	bilateral	bifurcate	inner and outer hinge plates, inner resting on the median septum	absent	conjunct	yellowish red with stripes
<i>Pictothyris picta</i>	3.5	latero-vertical	nonbifurcate	no inner hinge plates, the cardinalia are divergent	present	conjunct	irregular divaricating stripes of white upon red background
<i>Jolonica nipponica</i>	2.5	bilacunar	nonbifurcate	divided hinge plates, with high crural-bases, hinge-sockets deep	present	conjunct	rose-red; some have intervening bands of light yellow mottling.
<i>Frenulina sanguinolenta</i>	1.5	bilateral	nonbifurcate	the cardinalia bear disjunct, inner hinge-plates elevated well above the valve floor	present	disjunct in small conjunct in large	yellowish brown with short irregular red stripes marginally
<i>Shimodaia pterygiota</i>	0.7	incomplete annular	nonbifurcate	steeply dipping inner hinge plates which converge on a low median septum	absent	disjunct	red-mottled, with intervening bands of white mottling.
<i>Terebratalia coreanica</i>	5.9	trabecular	bifurcate	callus between the socket-ridges joined to septum	present	conjunct	red with layers of white, rather dull
<i>Coptothyris grayi</i>	5.0	teloform	bifurcate	callus deposit between the socket-ridges with which septum unites	present	disjunct	dull red with radial ribs

Shell shape and coloration.—Externally, laqueoid species exhibit great variability in shell size and shape. In this study, it became apparent that the only external shell character that supported phylogenetic relationships was the pattern of shell coloration (Figure 5; top and second row). All the species in the clade of *Shimodaia-Frenulina-Pictothyris-Jolonica* exhibit irregular red stripes or mottling patterns, while shells of others are radially striped (in *L. rubellus* and *T. coreanica*) or have uniform coloration (in *C. grayi*, *L. blanfordi*, *L. quadratus*, *L. californicus* and *L. c. vancouveriensis*). Other external characters, such as the type of the commissure and completeness of deltidial plates, did not show any systematic distribution on the molecular phylogenetic tree.

Adult loop morphology.—The adult loop pattern is often thought to represent phylogenetic affinity among laqueoid genera, and has been used as a key character to divide the superfamily into families and subfamilies. For example, *Laqueus* and *Frenulina* have often been included in the same subfamily (Kingeninae in Richardson, 1975; Laqueinae in Smirnova, 1984) based on their possession of a bilateral adult loop. A close relationship between *Pictothyris* and *Laqueus* has also been maintained based on similarity of external characters, as well as adult loop patterns; the adult laterovertical loop of *Pictothyris* has been considered to be at a stage one step more advanced than that of *Laqueus* (see Thomson, 1927; Hatai, 1940; Smirnova, 1984).

However, the results of the molecular study suggest that these interpretations, based on adult loop morphology, are not reliable. The three genera possessing a bilateral, or laterovertical, adult loop (Figure 5; third row; boxed) did not form a clade, supporting the conclusion that a bilateral loop appeared independently in the lineage leading to *Laqueus* and *Frenulina*.

Cardinalia.—The cardinal process is prominent in most laqueoids; however, species belonging to the genera *Laqueus* and *Shimodaia* lack it. The molecular phylogenetic tree indicates that the cardinal process may have been lost at least twice independently (Figure 5; fourth row). *S. pterygiota* possesses steeply dipping inner hinge plates

which converge on a low median septum to form a sessile septalium, comparable to that occurring in young *L. rubellus* and *Laqueus* sp. (Saito, 1996; MacKinnon *et al.*, 1997). This resemblance may also have resulted from parallel evolution.

Juvenile loop morphology.—At the axial phase of early loop development, when the median septum has a pair of flanges on its posterior part, the anterior part of the median septum bifurcates only in the basal species in the molecular phylogenetic tree, namely, *L. rubellus*, *C. grayi* and *T. coreanica* (Figure 5; fifth row). In all other species that form a clade, i.e., *F. sanguinolenta*, *P. picta*, *J. nipponica* and *S. pterygiota*, the septal pillar remain nonbifurcate until the annular phase (Figure 5; fifth row; boxed; Saito, 1996; MacKinnon *et al.*, 1997). Although the adult loop patterns may be misleading, early bifurcation of the septal pillar may be a useful character in assessing relationships among laqueoid genera.

Discussion

Laqueoid classification

Taxonomic assignments of the seven laqueoid genera investigated in this paper (*Laqueus*, *Terebratalia*, *Coptothyris*, *Shimodaia*, *Frenulina*, *Jolonica* and *Pictothyris*) have been controversial for a long time. Opinions as to which genera should be included in the family Laqueidae varied depending on the features that each author conjectured important. For example, Richardson (1973, 1975) considered the families Kingenidae, Macandreviidae and Laqueidae as synonymous, and proposed uniting them in the family Laqueidae, based on resorption patterns in loop development and the presence of dental plates. In this view, the seven ingroup genera compared in our molecular study are included in the family Laqueidae. Smirnova (1984) defined the Laqueidae as those with a loop of the late frenuliniiform stage (i.e. bilacunar loop) or of more advanced stages, in which the connections between the ascending and descending branches remain joined to the septum at all stage. In so doing, she included a number of lower Cretaceous genera in the Laqueidae (*Zittellina*, *Zeuschneria*, *Tulipina*, *Waconella*),

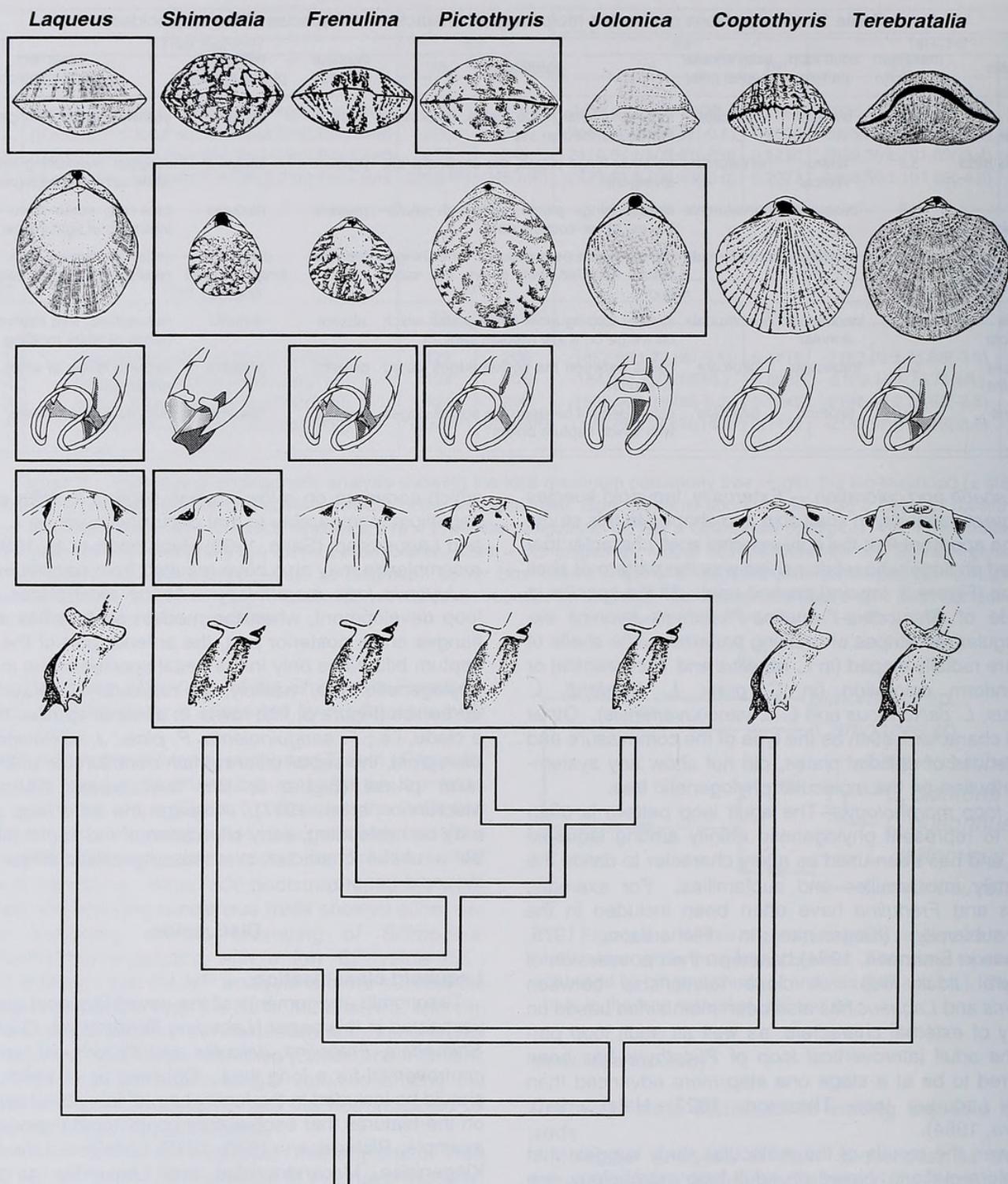


Figure 5. Morphological characters of laqueoid species superimposed on the molecular phylogenetic tree. The tree topology represents the consensus of the Trees 1, 2, and 3 (cf. Figure 3). Vertical lengths of the branches are arbitrary. Morphological characters (from top to bottom): anterior view of the shells; dorsal view of the shells; adult loop pattern; cardinalia in the annular phase; septal pillar at the axial phase. The drawings are not strictly to scale. The rectimarginate commissure of *Laqueus* and *Pictothyris* (top row; boxed) which was previously considered to be evidence uniting these genera, appeared separately in the molecular phylogenetic tree. In the shell external features, the red-white dot coloration (second row; boxed) supports a close relationship between *Shimodaia*, *Frenulina*, *Pictothyris* and *Jolonica*. Characters such as the adult bilateral or latero-vertical loop pattern (third row; boxed), and the absence of cardinal process (fourth row; boxed), do not reflect phylogeny. The non-bifurcation of the septal pillar in the axial phase (bottom row; boxed) supports the *Shimodaia*-*Frenulina*-*Jolonica*-*Pictothyris* clade.

but excluded certain genera such as *Terebratalia* and *Coptothyris*, which exhibited a trabecular or teloform adult loop pattern. Zezina (1984) elevated the subfamily Terebrataliinae (Richardson, 1975) to family status, and distinguished it from the Laqueidae that accommodated such genera as *Laqueus*, *Frenulina*, *Aldingia*, *Jolonica*, *Pictothyris*, *Compsoria* and *Ecnomiosa*. More recently, in summarizing the biogeography of articulated brachiopods, Richardson (1997) included 13 living genera in the family Laqueidae (*Coptothyris*, *Jolonica*, *Pictothyris*, *Terebratalia*, *Laqueus*, *Tythyris*, *Simplicithyris*, *Frenulina*, *Ecnomiosa*, *Compsoria*, *Aldingia*, and two other undiscussed genera), but she did not provide explicit criteria for this classification.

Concerning the familial groupings of the seven Recent genera, the following two points can be drawn from the results of our *cox1* study. Firstly, in the rooted monophyletic cluster of laqueoids that included *Terebratalia* and *Coptothyris*, *Laqueus* branched off first, followed by a trichotomous cluster comprised of *Terebratalia*, *Coptothyris* and the subcluster of *Shimodaia*, *Frenulina*, *Jolonica*, and *Pictothyris* (Figure 5). Therefore, if *Terebratalia* and *Coptothyris* are excluded from the Laqueidae and included in the Terebrataliidae, then *Laqueus* and the remaining four genera (*Shimodaia*, *Frenulina*, *Jolonica*, and *Pictothyris*) should be accommodated in at least two separate families. A grouping including *Laqueus*, *Shimodaia*, *Frenulina*, *Jolonica*, and *Pictothyris* to the exclusion of *Terebratalia* and *Coptothyris* would be paraphyletic at best.

Secondly, in analyses of all the available terebratulide forms including other than laqueoids, *Ecnomiosa* branched off outside not only of the laqueoids, but also of the terebratuloids of the Southern Hemisphere (Saito et al., in press). Thus, on molecular grounds, the view of including *Ecnomiosa* in the family Laqueidae (Richardson, 1997) is not supported. MacKinnon and Gaspard (1996) reported that the descending branches of *Ecnomiosa* grow only from the crura unlike other long-looped brachiopods, justifying our conclusion based on loop ontogeny.

Inclusion of *Terebratalia* and *Coptothyris* and exclusion of *Ecnomiosa* imply that the adult loop morphology alone cannot be used as the prime character to define the Laqueoidea. Instead, presence of a pair of flanges on the septal pillar at the axial stage of loop ontogeny (Figure 8; Saito, 1996) and also the presence of dental plates in the ventral valve appear to be more explicit and better-suited character states to define this superfamily, and are to be incorporated as such in the diagnosis of the Laqueoidea in the forthcoming revised Treatise (MacKinnon, pers. comm., 2000).

Processes of loop evolution

Paedomorphosis.— It is evident from comparison of the ontogenetic sequences of the loop morphology (Figure 6) with phylogenetic relationships (Figure 5), that paedomorphic loop evolution occurred at least twice among laqueoids, in the lineages that produced *Shimodaia* and *Jolonica*. As discussed in MacKinnon et al. (1997), adult individuals of *Shimodaia* have an incomplete annular loop, the brachial ring being incomplete due to resorption of the very narrow transverse band. Adult individuals of *Jolonica* dis-

play a bilacunar loop, a loop with two pairs of connecting bands (lateral and mediovertical), although the width of the bands are different from that in the bilateral loop such as that found in *Laqueus*. The adult loop phases of both *Shimodaia* and *Jolonica* are comparable with juvenile loop phases in other laqueoid members, and based on the molecular cladograms (Figure 5), it is more parsimonious to consider the abbreviated ontogenies of *Shimodaia* and *Jolonica* as independent synapomorphies.

Williams and Hurst (1977) pointed out that the most significant trend within the post-Paleozoic long-looped terebratulids is the neotenus elimination of later stages of loop ontogeny and a simultaneous simplification of the lophophore. Our results indicate that such complex evolutionary processes have indeed been at work in laqueoids.

Bifurcation.— As reported by Richardson (1975) and Saito (1996), the loop ontogenies of laqueoid species appear to be roughly the same until the bilacunar phase. However, at the earlier axial phase, characterized by the development of septal flanges, two types of septal pillar can be recognized; in one form of septal pillar the anterior edge becomes bifurcate whereas in the other form of septal pillar the anterior edge is nonbifurcate (Saito, 1996; Figure 6). In *Laqueus* sp. (Figures 8.1, 8.2), *T. coreanica* (Figure 8.3) and *C. grayi* (Figure 8.4), the septal pillar is anteriorly bifurcate. On the other hand, *Pictothyris* sp. (Figure 8.5), *Jolonica nipponica* (Figure 8.6), *F. sanguinolenta* (Figure 8.7) and *Shimodaia pterygiota* (Figure 8.8) all exhibit a nonbifurcate septal pillar and retain remains of projections until the annular phase (Figure 9.5–9.8).

The results of molecular phylogeny indicate paraphyly for those with the bifurcate septal pillar (Figure 5). Thus, bifurcation is considered as the ancestral state and nonbifurcation a synapomorphy. Two Mesozoic laqueoid genera (*Gemmarcula* and *Trigonosemus*) show the anterior bifurcation of the septal pillar at the annular phase (Elliott 1947; Cooper 1955; Steinich 1965). This observation accords well with our contention that anterior bifurcation of the septal pillar is an ancestral character (Figure 5).

Evolution of Bilateral Loop.— As discussed earlier, the *cox1* results indicated that species with a bilateral adult loop did not form a monophyletic cluster (Figure 5). Two interpretations are possible for the evolution of the bilateral loop; one is that parallel evolution occurred, i.e., the bilateral loop evolved twice independently, and the other is that the bilateral loop is a plesiomorphic character.

The former interpretation tends to be supported by the fact that the two Cretaceous laqueoid genera, *Gemmarcula* and *Trigonosemus*, possess a trabecular loop. But the latter interpretation becomes equally possible if another genus such as *Waconella* from the Lower Cretaceous that has a bilateral loop in the adult phase is taken into consideration. *Waconella* has been considered as one of the members of the ancestral group from which *Laqueus* is derived, because of the possession of the same type of adult loop, cardinalia and shell shape, as well as the close geographical distribution with other laqueoid genera (Owen, 1970; Smirnova, 1984). Since a deep diversification between *Laqueus* and other laqueoid genera is inferred from the *cox1* analysis, this connection between *Waconella* and *Laqueus* seems quite

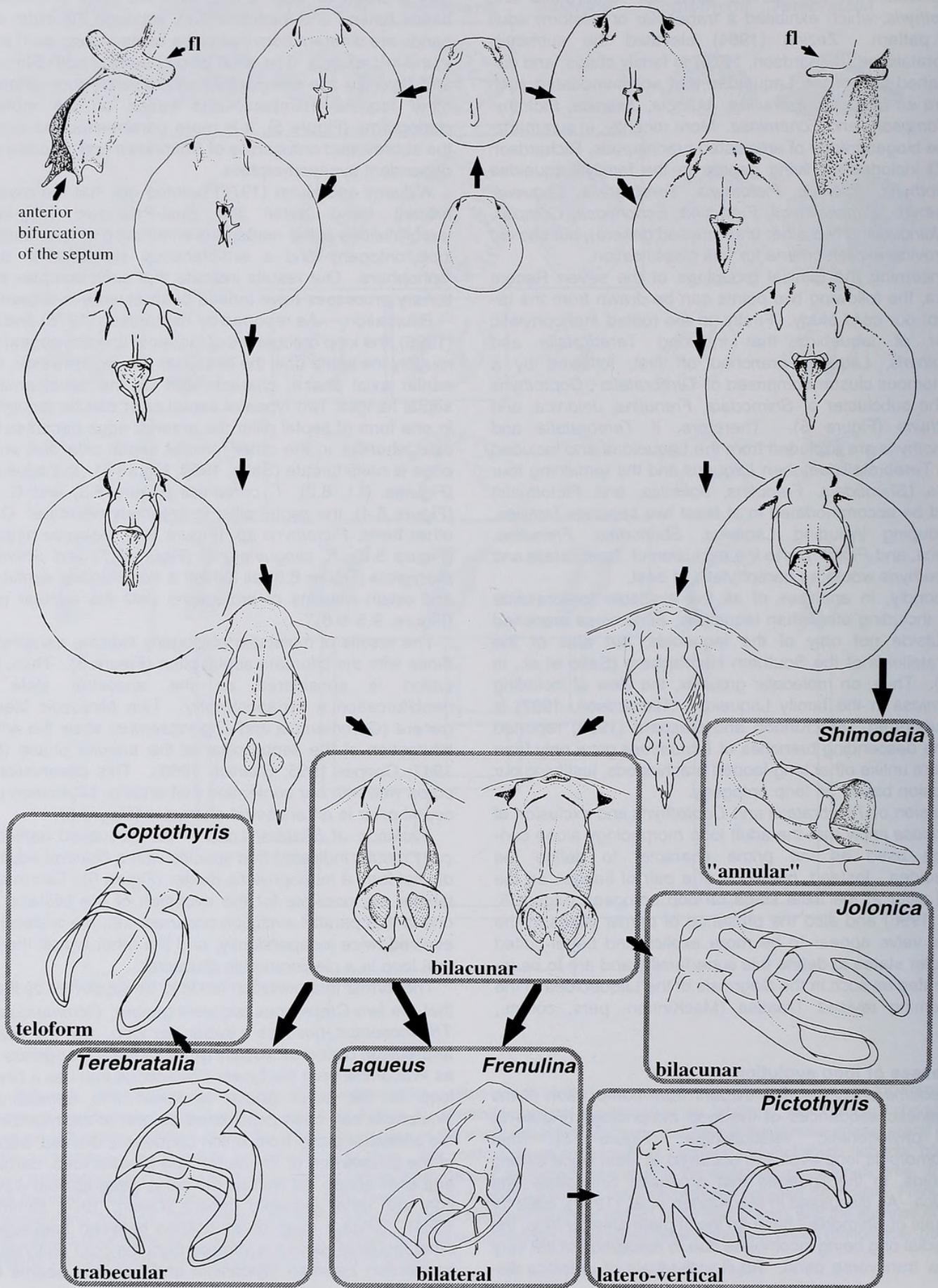


Figure 6.

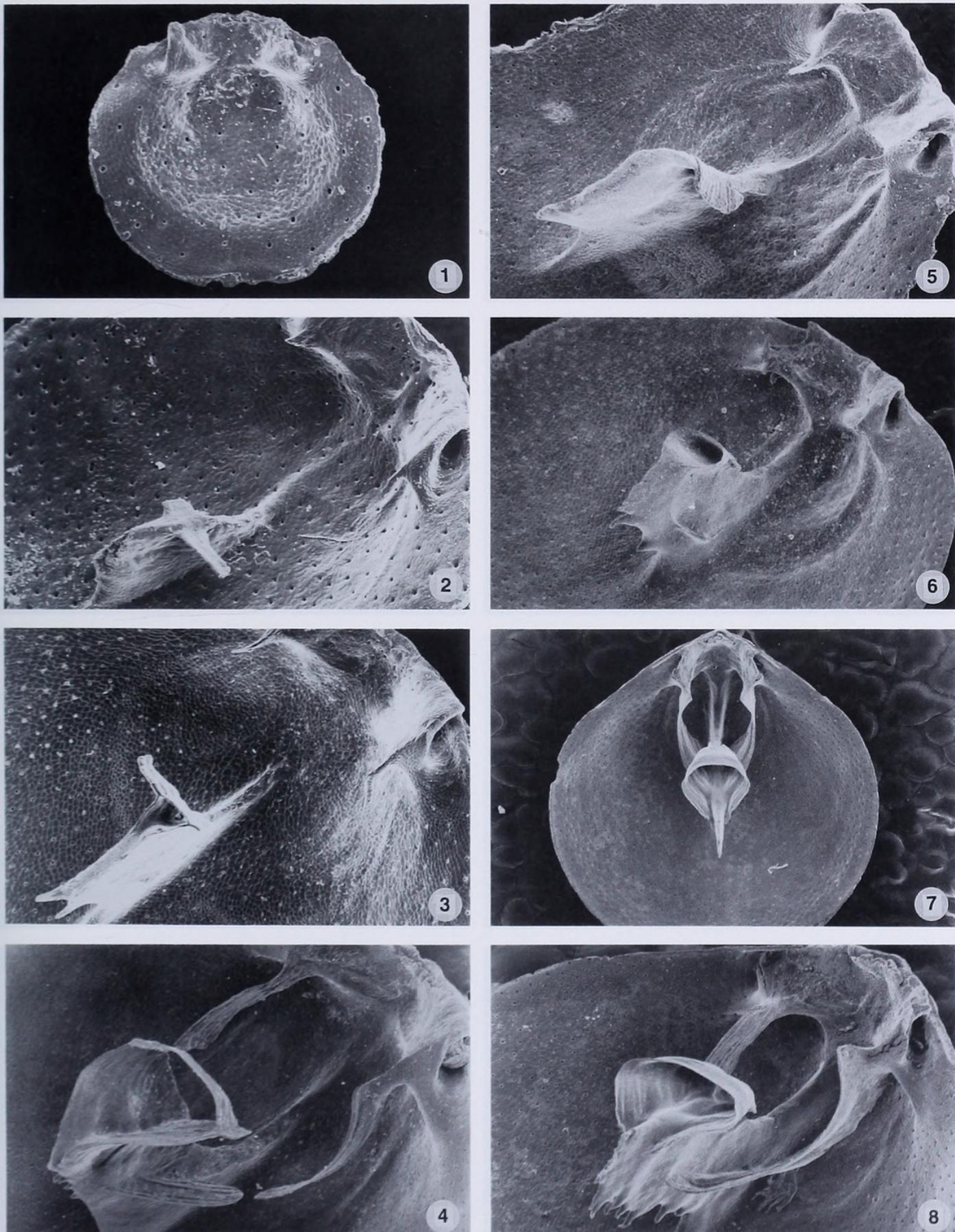


Figure 7.

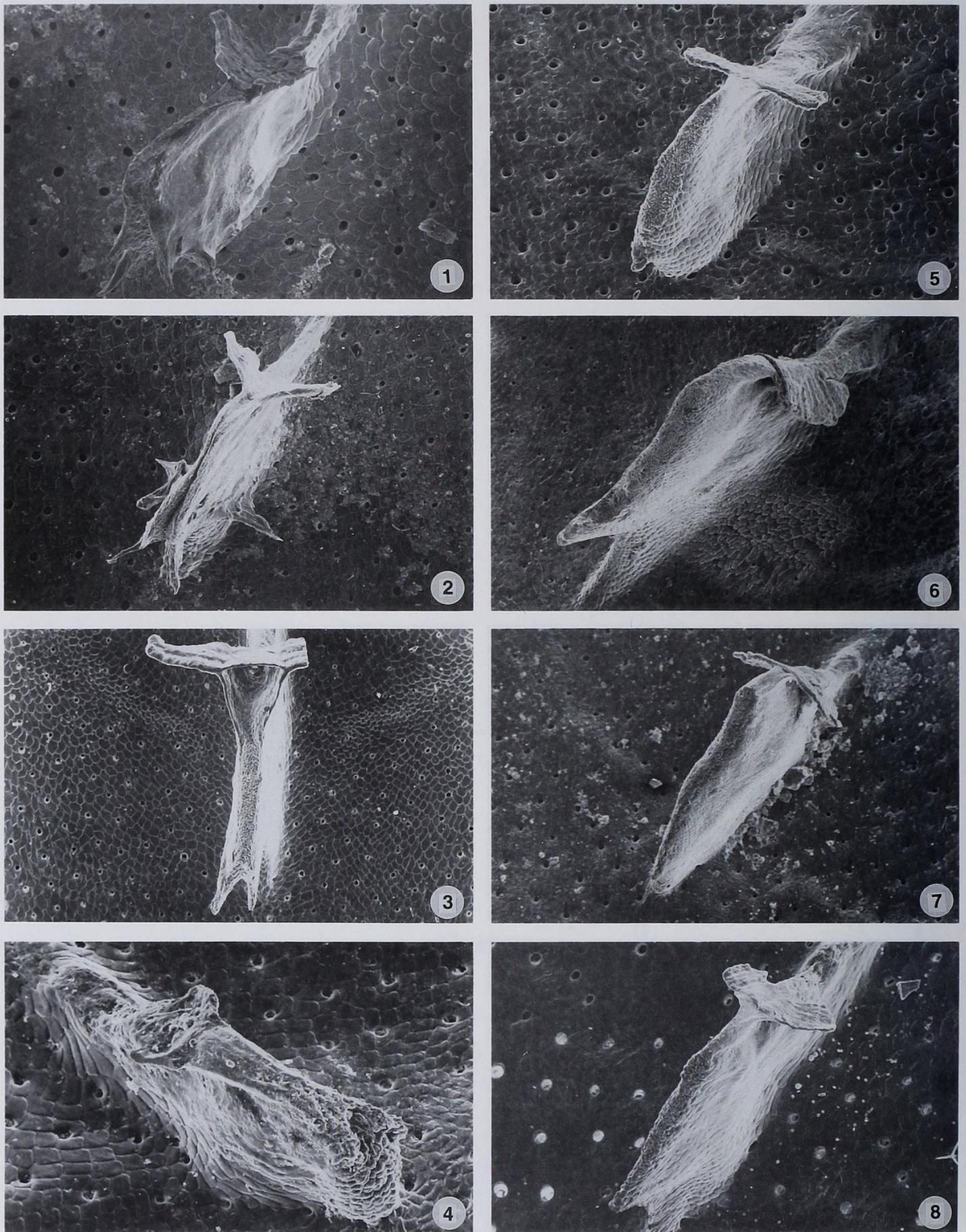


Figure 8.

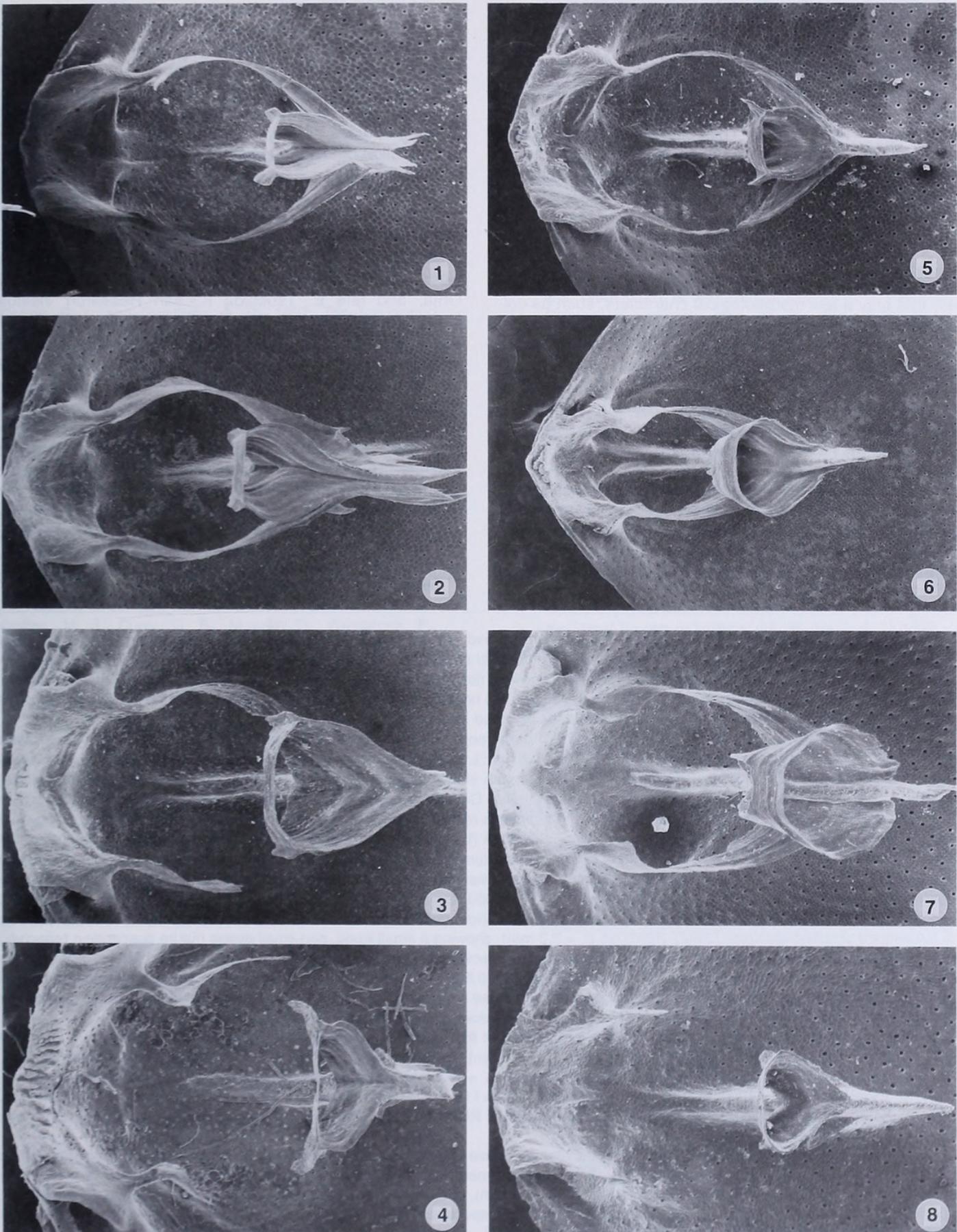


Figure 9.

probable, although the oldest fossil record of *Laqueus* is middle Miocene (Hatai, 1938). However, the early loop ontogeny of this genus is not reported, and it is not known whether *Waconella* has the laqueoid character of the flanges at the axial phase. The ancestral state of the laqueoid loop, therefore, cannot be established at present based on the morphology of fossil forms. The relationships of Mesozoic genera to the Cenozoic ones should become clearer when the early loop ontogenies, especially at the axial and annular phases of Mesozoic genera, are further examined.

Conclusions

Molecular phylogenetic analysis using the *cox1* gene suggests that only a few morphological characters, such as coloration of the shell and bifurcation of the septal pillar, may be of use in deciphering the phylogenetic relationships of laqueoids. Other characters like cardinalia, external morphologies of the shell, notably, adult loop patterns, all of which have previously been considered as taxonomically important, are likely to have undergone a complex evolutionary history, and thus have to be treated with caution when used in taxonomic studies.

Reconstruction of the relationships of fossil and Recent terebratulide brachiopods is reliant mainly on the morphology of hard parts such as the shell, the loop and occasionally spicules, i.e., characters that can relatively easily be preserved in fossils. In addition, careful observations of

early loop development, especially of the fossil taxa, would be useful in filling existing gaps in the fossil record of the Laqueoidea and other superfamilies, and in resolving evolutionary relationships among fossil and Recent species. In any event, it appears important to evaluate the taxonomic value of each character, by means of molecular phylogeny of living species, to help clarify the phylogenetic history of terebratulide brachiopods and of other organisms in general.

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Figure 6. Loop ontogeny of the living laqueoid genera studied. Drawings are not to scale. The Laqueoidea is characterized by the presence of a pair of flanges (fl) on the septal pillar at the axial phase, and the patterns of ring resorption to produce the bilacunar loop pattern. The route to the bilacunar phase is two-fold; one with (left) and one without (right) anterior bifurcation of the median septum. The common bilacunar pattern for two types and adult patterns are boxed. Note that ontogenetic sequences to the adult patterns do not necessarily correspond with the phylogenetic relationships revealed by the molecular data.

Figure 7. Scanning electron microscopic images of early loop morphologies for *Terebratalia* and *Jolonica*. 1–4. *Terebratalia coreanica*. 1. Dorsal view, specimen UMUT RB28050 (L = 0.6 mm; L is the length of the dorsal valve), displays no loop-supporting structure (Prebrachial phase), $\times 77$. 2. A specimen 1.5 mm in length shows septal flanges (Axial phase; oblique view), specimen UMUT RB28051, $\times 63$. The anterior part of the septal pillar is bifurcate. Cardinal process begins to develop during this stage. 3. Specimen UMUT RB28052 (L = 2.2 mm) displays a small hood with rudiments of the flanges, $\times 48$. Crura extend from areas at the base of the inner socket ridges. 4. Oblique view of the specimen UMUT RB28053 (L = 4.0 mm), showing the ring which retains the rudiments of the flanges (Annular phase), $\times 32$. The anterior part of the median septum is bifurcate. The descending branches extend further toward one another, albeit still unconnected. Further development of *Terebratalia coreanica* as those figured in Saito (1996). 5–8. *Jolonica nipponica*. 5. Lateral view of the smallest specimen UMUT RB28054 (L = 2.7 mm) displaying flanges (Axial phase), $\times 37$. Note that the ventral edge of the septal pillar is non-bifurcate. Crura project out from near the base of each inner socket ridge. 6. Posterodorsal view of the second smallest specimen UMUT RB28055 (L = 3.2 mm), showing a small hood, and small plates (future descending branches) on the septal pillar (Cucullate phase), $\times 30$. The crura and the descending branches extend further toward one another. 7–8. Annular phase. 7. Dorsal view of a larger specimen UMUT RB28056, (L = 3.8 mm) showing the annular phase loop and well developed cardinal process, $\times 15$. 8. Oblique view of the annular phase loop of the same specimen as in Fig. 8.6, showing the anteriorly spinose septal pillar and the ring with rudiments of the flanges. The septal pillar is spinous, but remains non-bifurcate, $\times 31$.

Figure 8. Comparison of the median septum morphology at the axial phase among laqueoid species. 1. *Laqueus* sp. (L = 1.9 mm), specimen UMUT RB28057, $\times 155$. 2. *Laqueus* sp. (L = 1.9 mm), specimen UMUT RB19819, $\times 114$. 3. *Terebratalia coreanica* (L = 2.2 mm), specimen UMUT RB28052, $\times 73$. 4. *Coptothyris grayi* (L = 1.3 mm), specimen 28498 in the collection of Tohoku University, $\times 228$. 5. *Pictothyris* sp. (L = 1.5 mm), specimen UMUT RB19830, $\times 113$. 6. *Jolonica nipponica* (L = 2.7 mm), specimen UMUT RB28054, $\times 63$. 7. *Frenulina sanguinolenta* (L = 2.0 mm), specimen UMUT RB28058, $\times 77$. 8. *Shimodaia pterygiota* (L = 1.54 mm), specimen UMUT RB28059, $\times 120$.

Figure 9. Comparative views of cardinalia at the annular phase in eight laqueoid species. 1. *Laqueus* sp. (L = 2.7 mm), specimen UMUT RB19821, $\times 39$. 2. *Laqueus blanfordi* (L = 3.7 mm), specimen UMUT RB28060, $\times 30$. 3. *Terebratalia coreanica* (L = 4.0 mm), specimen UMUT RB28053, $\times 30$. 4. *Coptothyris grayi* (L = 2.3 mm), specimen UMUT RB28061, $\times 31$. 5. *Pictothyris* sp. (L = 2.5 mm), specimen UMUT RB19836, $\times 37$. 6. *Jolonica nipponica* (3.8 mm), specimen UMUT RB28056, $\times 24$. 7. *Frenulina sanguinolenta* (L = 3.9 mm), specimen UMUT RB28062, $\times 30$. 8. *Shimodaia pterygiota* (L = 2.2 mm), specimen UMUT RB28063, $\times 48$.

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