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MORPHOLOGICAL EVOLUTION OF CYTHEROCOPINE OSTRACODS INFERRED FROM 18S RIBOSOMAL DNA SEQUENCES

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A B S T R A C T

Nucleotide sequences for the 18S rDNA of 28 cytherocopine ostracods that represent 16 families were determined and compared with those of bairdioidean and cytherelloidean ostracods. Resulting molecular phylogenetic trees consistently indicated that cytheroideans formed a monophyletic group and that bythocytheroideans are paraphyletic outside of the cytheroideans. This relationship suggests that the diagnostic morphological features of the Bythocytheroidea, such as the five adductor muscle scars and the first antenna with seven articulated podomeres, are plesiomorphic. The molecular phylogenetic relationships among cytheroideans indicated polyphyly of the amphidont basic type hingement, which is distributed in four lineages, i.e., the loxoconchids, leptocytherids, schizocytherids, and a group of hemicytherids, thaerocytherids, and trachyleberidids, suggesting that the hinge structure of the amphidont basic type has evolved at least four times independently in cytheroidean ostracods. The ostracod hinge structures may have evolved in concert with the extent of carapace calcification.

Cytherocopina is one of the largest groups of Ostracoda, and is abundant in most marine bottom environments. The Bythocytheroidea and Cytheroidea are major extant cytherocopine superfamilies (Hinz-Schallreuter and Schallreuter, 1999). The cytherocopines generally have a strongly calcified carapace, hence the Bythocytheroidea and the Cytheroidea have an almost continuous fossil record since the Ordovician and the Permian, respectively (Whatley *et al*., 1993). The cytheroideans are considered to have derived from the bythocytheroideans in Late Paleozoic (e.g., McKenzie, 1969; Whatley and Boomer, 2000). The cytheroideans have then flourished in marine and freshwater bottom environments since the Middle Mesozoic (e.g., Brasier, 1980).

Ostracods have many morphological characters in the carapace, e.g., hingement, central muscle scars, normal pore canals, marginal pore canals, duplicature, surface ornamentation, and so on, some of which reflect the morphology, function, and physiology of soft parts. Of these characters, the hingement is generally regarded to be the most important for the family- and subfamily-level taxonomy of the post-Paleozoic Cytherocopina (e.g., Benson *et al*., 1961; Hanai, 1961; Scott, 1961; Hartmann, 1963; Hartmann and Puri, 1974; Cohen, 1982; Hinz-Schallreuter and Schallreuter, 1999). Well-developed hingements with complicated teeth and sockets are generally made up of three (merodont basic type) or four (amphidont basic type) elements. Such complicated cytherocopine hinge structures may have a function to close the two valves tightly to avoid danger (Tsukagoshi, 1996), or to support the attachment of valves, complementing the ligament, of which the thickness decreased for flexibility with increasing calcification and thickness of the carapace since the Ordovician (Hinz-Schallreuter and Schallreuter, 1999).

The origin of cytherocopine hingement is still poorly understood. Sylvester-Bradley (1956) suggested that the lophodont is the primitive cytherocopine hingement, because Devonian ostracods belonging to other suborders (Metacopina and Bairdiocopina) possess a lophodont hingement. On the other hand, Pokorny (1957) considered the holomerodont as the most primitive because certain platycopidan ostracods have the holomerodont hingement. Based on observations of several genera of the Cytherideinae, Sandberg (1964) regarded the ontogenetic change of hingement as representing evolutionary change and suggested that the entomodont and holomerodont evolved from the antimerodont. The cytherocopine hingements, thus, are generally thought to have an evolutionary trend from a simple one to a complicated one (Hartmann, 1963; Benson 1966). Sylvester-Bradley (1948) found this

pattern in the lineage from the Middle Jurassic *Oligocythereis* (entomodont) to the Tertiary and Recent *Trachyleberis* (amphidont). On the other hand, Triebel (1954) suggested that the amphidont has been achieved independently in the homeomorphic genera *Macrodentina* and *Amphicythere* in the Jurassic. Sylvester-Bradley (1956) also postulated parallel evolution from the entomodont to the amphidont in the lineage from the progonocytherid *Oligocythereis* in the Middle Jurassic to the trachyleberidid *Trachyleberis* in the Tertiary and Recent and in another ostracod lineage in the Middle Jurassic.

Meanwhile, Pokorny (1957) recognized the same pattern of hinge evolution from the entomodont to the amphidont in the lineage of hemicytherids, suggesting that this change in the hinge structure proceeds in correlation with overall changes of the carapace; the anterior half of the carapace becomes heavier as the anterior part of the hinge develops more strongly. Benson (1966) suggested that the hingement of the more complexly ornamented ostracod carapace underwent an increase in morphologic complexity with time, facilitating a more efficient union for more complicated and robust valves. Tsukagoshi (1996) indicated that all the basic hingement designs already appeared at least by the Paleogene and that the designs became modified exclusively by paedomorphosis in the Neogene. These views present conflicting ideas concerning the evolution of hingements. They must, of course, be reevaluated by phylogenetic analysis among cytherocopines based on other characters than hinge structures in order to avoid circular arguments. Thus, it is desired to take a new look at the problem of cytherocopine ostracod phylogeny based on a new methodology.

On the basis of a phenogram obtained from numerical taxonomic analyses of fifty morphological characters of the carapace among seven cytheroidean families (Hemicytheridae, Paradoxostomatidae, Cytheruridae, Xestoleberididae, Cytheridae, Loxoconchidae, and Leptocytheridae), Kaesler (1969) indicated that the loxoconchids and leptocytherids formed a cluster, the xestoleberidids, cytherids, and one of three cytherurid species formed a cluster, and the paradoxostomatids clustered with all the species except for hemicytherids. On the other hand, relying upon the distribution patterns of pore systems on the carapace among four families (Xestoleberididae, Loxoconchidae, Leptocytheridae, and Cytheridae), Kamiya (1997) suggested a close relationship among the three families other than the Xestoleberididae and that between the Loxoconchidae and the Cytheridae. However, a comprehensive study focusing on phylogenetic problems among cytherocopine families has not yet been done to our satisfaction.

The purpose of this study, therefore, is to reveal morphological evolution of cytherocopine ostracods by clarifying their phylogenetic relationships of almost all of the major extant cytherocopine families, relying on the comparison of 18S rDNA sequences.

Materials and Methods

Samples

The species investigated in this study are summarized in Table 1, with the descriptions of their sampling localities. The carapaces of the specimens examined except for *Limnocythere* sp. are shown in Figure 1. They are deposited in the collection of the University Museum, University of Tokyo (UMUT). The carapace of *Limnocythere* sp. was collapsed when the appendages were extracted from the specimen for the DNA extraction. The entire 18S rDNA gene was sequenced for 28 cytherocopine ostracods, representing 16 families. Two species, which seem to be the most phylogenetically distant among the collected specimens, were chosen as representatives of each family. When only a single species belonging to one family was able to be collected, the species was tentatively chosen as a representative of that family. In addition, two species of bairdioidean and cytherelloidean ostracods were used as outgroups for phylogenetic analysis.

DNA Extraction

For each DNA preparation, a single fresh or 100% ethanol-preserved specimen was used for each species. Each specimen was washed with distilled water, and appendages or eggs were extracted from the specimen and stored in a 0.6 ml microcentrifuge tube. The bottom of the tube was then immersed in liquid nitrogen, and the appendages or eggs were macerated by rotating the yellow pipette tip a few times within the microcentrifuge tube. Genomic DNA was prepared for PCR amplification by grinding the appendages or eggs in 50 μl of 5% (w/v) Chelex solution (Bio-Rad Laboratories, California), heating at 60°C for 30 min and at 94°C for 3 min. The resultant DNA preparation in Chelex was stored at 4°C.

PCR Amplification

Amplification of an about 1,800 bp region of the 18S ribosomal RNA gene was carried out in a 100 μl reaction solution containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 50 μM of each dNTP, 0.5 μM of each primer, 1.0 μl of the Chelex solution suspending the genomic DNA as template and 2.5 units of Taq DNA polymerase (Toyobo Co., Tokyo). The primer pair of 18S-F1 and 18S-R9 were used for the initial amplification of all sequences (Table 2). These primers amplify almost the entire region of the 18S rRNA gene, missing only three nucleotide pairs at the 3′ end. The PCR was performed over 30 to 35 cycles. Each cycle consisted of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 1 min. The

Table 1. Taxa from which 18S rDNA sequences used in this study were derived. Podocopidan and platycopidan classification is based on Hinz-Schallreuter and Schallreuter (1999); bythocytheroidean classification is adopted from Athersuch *et al*. (1989); schizocytherid classification from Benson *et al*. (1961). Abbreviation: N = Number of bases sequenced. The sequence of *Keijia* cf. *demissa* has about 50 missing sites.

Species	Locality	Latitude	Longitude	Water depth (m)	Accession number	*N*
Order Podocopida						
Suborder Cytherocopina						
Superfamily Bythocytheroidea						
Family Bythocytheridae						
Bythoceratina hanejiensis Nohara, 1987	Off Wakayama Pref.	33°42.2′N	135°16.6′E	71	AB076619	1808
Schlerochilus oshoroensis Hiruta, 1976	Oshoro Beach, Otaru-shi, Hokkaido	43°12.2′N	140°52.6′E	0.5	AB076620	1812
Superfamily Cytheroidea						
Family Encytheridae						
Kotoracythere inconspicua (Brady, 1880)	Arumi Cove, Higashi-son, Okinawa Pref.	26°35.2′N	128°09.6′E	0.5	AB076621	1816
Keijia cf. *demissa* (Brady, 1868)	Arumi Cove, Higashi-son, Okinawa Pref.	26°35.2′N	128°09.6′E	0.5	AB076622	1752
Family Paradoxostomatidae						
Paradoxostoma setoense Schornikov, 1975	Aburatsubo Inlet, Misaki-cho, Kanagawa Pref.	35°08.9′N	139°36.6′E	0.5	AB076623	1810
Xiphichilus sp.	Off Wakayama Pref.	33°39.6′N	135°09.8′E	146	AB076624	1809
Family Cytheruridae						
Hemicytherura kajiyamai Hanai, 1957	Aburatsubo Inlet, Misaki-cho, Kanagawa Pref.	35°09.3′N	139°36.9′E	0.5	AB076627	1817
Cytheropteron subuchioi Zhao, 1988	Off Kanagawa Pref.	35°08.3′N	139°34.9′E	83	AB076628	1810
Family Loxoconchidae						
Loxocorniculum mutsuense Ishizaki, 1971	Tanabe Cove, Shirahama-cho, Wakayama Pref.	33°41.3′N	135°20.3′E	0.5	AB076629	1810
Cytheromorpha acupunctata (Brady, 1880)	Tsukumo Cove, Uchiura-cho, Ishikawa Pref.	37°18′N	137°14′E	1	AB076630	1806
Family Leptocytheridae						
Leptocythere lacertosa (Hirschmann, 1912)	Pegwell Bay, Kent, U.K.	51°19.1′N	1°22.7′W	0.5	AB076631	1816
Ishizakiella miurensis (Hanai, 1957)	Mouth of the Natori River, Natori-shi, Miyagi Pref.	38°11.3′N	140°56.3′E	0.5	AB076632	1815
Family Xestoleberididae						
Xestoleberis hanaii Ishizaki, 1968	Aburatsubo Inlet, Misaki-cho, Kanagawa Pref.	35°09.3′N	139°36.9′E	0.5	AB076633	1814
Cobanocythere? *japonica* Schornikov, 1975	Tanabe Cove, Shirahama-cho, Wakayama Pref.	33°41.3′N	135°20.4′E	0.5	AB076634	1813
Family Limnocytheridae						
Limnocythere sp.	Hatchet Pond, Hampshire, U.K.	50°48.6′N	1°28.9′W	1	AB076635	1808
Family Cytheridae						
Cythere lutea Müller, 1785	Palm Bay, Kent, U.K.	51°23.4′N	1°25.3′W	0.5	AB076636	1812
Family Schizocytheridae						
Neomonoceratina microreticulata Kingma, 1948	Yagachi Cove, Nago-shi, Okinawa Pref.	26°36.8′N	128°01.3′E	0.5	AB076637	1810
Spinileberis quadriaculeata (Brady, 1880)	Yagachi Cove, Nago-shi, Okinawa Pref.	26°36.8′N	128°01.3′E	0.5	AB076638	1810
Family Krithiidae						
Parakrithella pseudadonta (Hanai, 1959)	Aburatsubo Inlet, Misaki-cho, Kanagawa Pref.	35°08.9′N	139°36.6′E	0.5	AB076639	1815
Family Cushmanideidae						
Pontocythere subjaponica (Hanai, 1959)	Off Wakayama Pref.	33°40.7′N	135°19.7′E	30	AB076640	1814
Pontocythere sp.	Oshoro Beach, Otaru-shi, Hokkaido	43°12.2′N	140°52.6′E	0.5	AB076641	1813

Table 1. Continued.

Species	Locality	Latitude	Longitude	Water depth (m)	Accession number	*N*
Family Cytherideidae						
Perissocytheridea japonica Ishizaki, 1968	Mouth of the Natori River, Natori-shi, Miyagi Pref.	38°11.3′N	140°56.3′E	0.5	AB076642	1814
Family Hemicytheridae						
Aurila disparata Okubo, 1980	Aburatsubo Inlet, Misaki-cho, Kanagawa Pref.	35°09.3′N	139°36.9′E	0.5	AB076643	1814
Caudites asiaticus Zhao and Whatley, 1989	Arumi Cove, Higashi-son, Okinawa Pref.	26°35.2′N	128°09.6′E	0.5	AB076646	1814
Family Thaerocytheridae						
Bradleya nuda Benson, 1972	Off Wakayama Pref.	33°37.1′N	136°01.5′E	147	AB076647	1814
Tenedocythere transoceanica (Teeter, 1975)	Arumi Cove, Higashi-son, Okinawa Pref.	26°35.2′N	128°09.6′E	0.5	AB076648	1815
Family Trachyleberididae						
Bicornucythere bisanensis (Okubo, 1975)	Aburatsubo Inlet, Misaki-cho, Kanagawa Pref.	35°08.9′N	139°36.6′E	0.5	AB076649	1818
Actinocythereis cf. *scutigera costata* Hartmann, 1978	Tanapag Lagoon, Saipan, Northern Mariana Islands	15°14.8′N	145°44.0′E	3	AB076652	1813
Suborder Bairdiocopina						
Superfamily Bairdioidea						
Neonesidea oligodentata (Kajiyama, 1913)	Aburatsubo Inlet, Misaki-cho, Kanagawa Pref.	35°08.9′N	139°36.6′E	0.5	AB076615	1807
Order Platycopida						
Superfamily Cytherelloidea						
Cytherella leizhouensis Gou, 1983	Off Wakayama Pref.	33°37.1′N	136°01.5′E	147	AB076611	1805

reaction was completed with a final 5 min incubation at 72°C. A volume of 5 μl of PCR products was electrophoresed in 2% agarose gels to confirm whether the specific DNA fragment was amplified. If not, a second PCR was carried out in a reaction solution containing 1.0 μl of the first PCR products as the template under the same condition as the first PCR, using each of the four pairs of forward and reverse primers listed in Table 2. These procedures yielded double-stranded segments of approximately 1,800 bp (18S-F1–18S-R9), 1,450 bp (18S-F1–18S-R8), 1,050 bp (18S-F1–18S-R7), and 800 bp (18S-F3–18S-R9) in length.

DNA Sequencing

The PCR products were electrophoresed in 2% agarose gels, excised, and purified for sequencing reactions, using the GENECLEAN II KIT (BIO 101 Inc., California) following the guidelines provided with the kit. Double-stranded DNA was sequenced directly using a Perkin Elmer ABI PRISM 377 automated DNA sequencer. Dideoxy terminal cycle sequencing was performed using a Thermo Sequenase dye terminator cycle sequencing pre-mix kit, v2.0 (Amersham Pharmacia Biotech Inc., Ohio) following the recommended protocols. Both strands were sequenced using four forward and four reverse primers listed in Table 2.

Sequence Analysis

The DNA sequences were assembled and edited using the software program SeqPup version 0.6d (Gilbert, 1996), and preliminary alignment was achieved using CLUSTAL W (Thompson *et al*., 1994) with default gap penalties. The output was later improved manually using the SeqPup version 0.6d (Gilbert, 1996). Regions that included indels and missing sites and ones that could not be unambiguously aligned were both excluded from subsequent phylogenetic analyses.

Phylogenetic analyses were carried out using three different methods, (1) maximum likelihood (ML) (quartet puzzling method; Strimmer and von Haeseler, 1996); (2) maximum parsimony (MP) (Swofford, 1993); and (3) neighbor-joining (NJ) (Saitou and Nei, 1987) to verify whether the same topology was supported by different tree-building methods. Sequence data of a bairdioidean (*Neon-*

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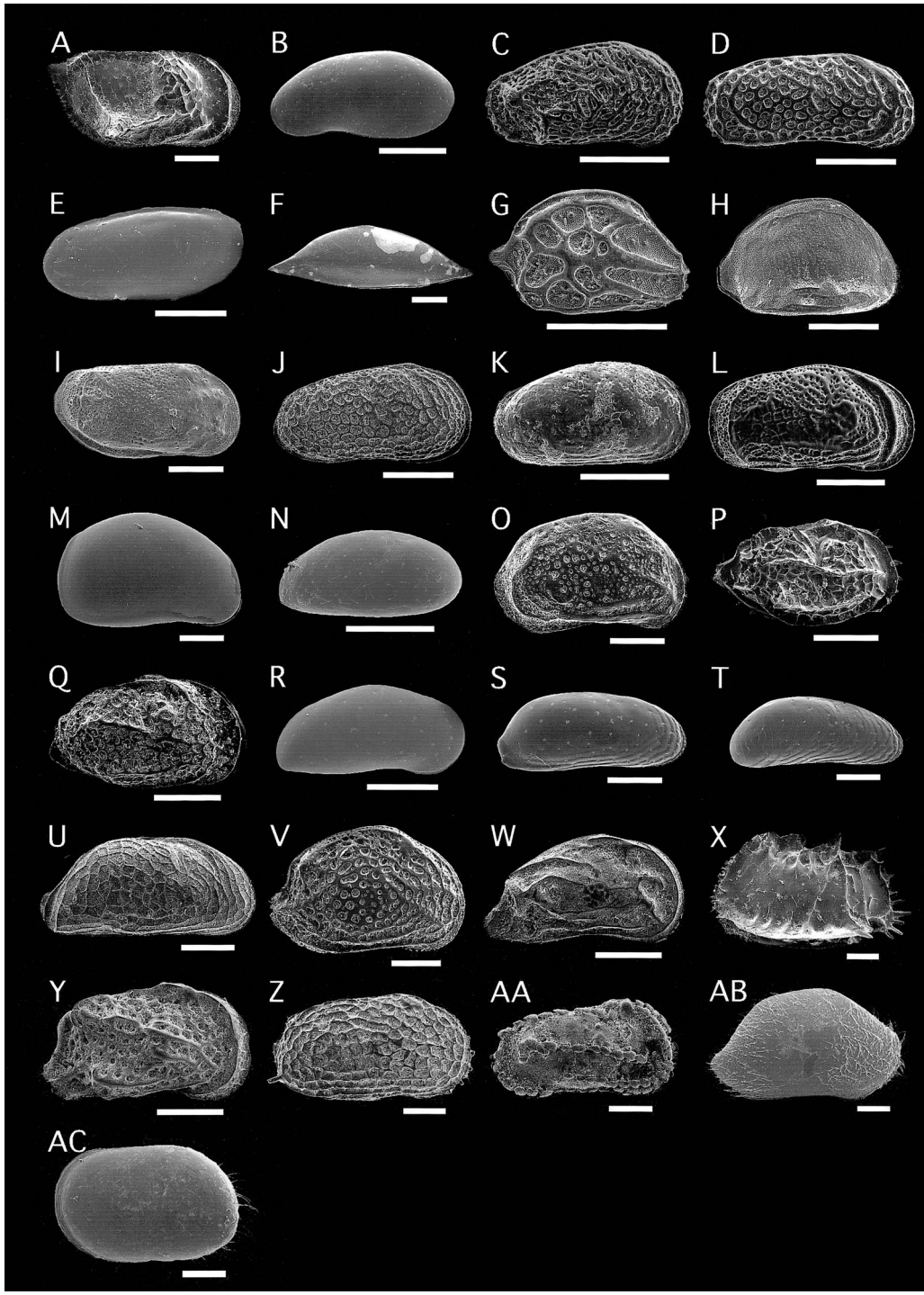
Fig. 1. Carapaces in external lateral view of the ostracods used for phylogenetic analysis. A, Right valve (RV) of *Bythoceratina hanejiensis* (UMUT RA 27981). B, Left valve (LV) of *Sclerochilus oshoroensis* (UMUT RA 27982). C, RV of *Kotoracythere inconspicua* (UMUT RA 27984). D, RV of *Keijia* cf. *demissa* (UMUT RA 28013). E, LV of *Paradoxostoma setoense* (UMUT RA 28007). F, RV of *Xiphichilus* sp. (UMUT RA 28008). G, RV of *Hemicytherura kajiyamai* (UMUT RA 28011). H, RV of *Cytheropteron subuchioi* (UMUT RA 28012). I, RV of *Loxocorniculum mutsuense* (UMUT RA 28014). J, RV of *Cytheromorpha acupunctata* (UMUT RA 28015). K, RV of *Leptocythere lacertosa* (UMUT RA 28016). L, RV of *Ishizakiella miurensis* (UMUT RA 28017). M, RV of *Xestoleberis hanaii* (UMUT RA 28018). N, LV of *Cobanocythere*? *japonica* (UMUT RA 28019). O, RV of *Cythere lutea* (UMUT RA 27983). P, RV of *Neomonoceratina microreticulata* (UMUT RA 28020). Q, RV of *Spinileberis quadriaculeata* (UMUT RA 28021). R, RV of *Parakrithella*



pseudadonta (UMUT RA 28022). S, RV of *Pontocythere subjaponica* (UMUT RA 28023). T, RV of *Pontocythere* sp. (UMUT RA 28024). U, RV of *Perissocytheridea japonica* (UMUT RA 28025). V, RV of *Aurila disparata* (UMUT RA 27996). W, RV of *Caudites asiaticus* (UMUT RA 27999). X, LV of *Bradleya nuda* (UMUT RA 28000). Y, RV of *Tenedocythere transoceanica* (UMUT RA 28001). Z, RV of *Bicornucythere bisanensis* (UMUT RA 28002). AA, RV of *Actinocythereis* cf. *scutigera costata* (UMUT RA 28005). AB, RV of *Neonesidea oligodentata* (UMUT RA 27989). AC, LV of *Cytherella leizhouensis* (UMUT RA 27990). Scale bar indicates 200 μm.

Table 2. Sequences of oligonucleotide primers used in this study. The direction of the primers is either forward (F) or reverse (R). Nucleotide ambiguities are represented by one-letter codes proposed by the International Union of Biochemistry. The location of each primer corresponds to the position given in the 18S rRNA sequence of *Artemia salina* Linnaeus, 1758 (Nelles *et al*., 1984).

Primer	Direction	Sequence (5′ to 3′)	Location
18S-F1	F	TACCTGGTTGATCCTGCCAG	1–20
18S-R6	R	TYTCTCRKGCTBCCTCTCC	388–406
18S-F2	F	CCTGAGAAACGGCTRCCACAT	398–418
18S-R7	R	GYYARAACTAGGGCGGTATCTG	1,011–1,032
18S-F3	F	GYGRTCAGATACCRCCSTAGTT	1,006–1,027
18S-R8	R	ACATCTRAGGGCATCACAGACC	1,437–1,458
18S-F4	F	GGTCTGTGATGCCCTYAGATGT	1,437–1,458
18S-R9	R	GATCCTTCCGCAGGTTCACCTAC	1,784–1,806

esidea oligodentata) and a cytherelloidean (*Cytherella leizhouensis*) were added to those of cytherocopines as the outgroups before the phylogenetic analyses.

The phylogenetic relationships were analyzed by means of three methods: (1) the ML method using PUZZLE version 4.02 (Strimmer and von Haeseler, 1999), based on the HKY model (Hasegawa *et al*., 1985) with the exact parameter estimates option; (2) the MP method using PAUP version 3.1.1 (Swofford, 1993) with the heuristic or branch and bound option; and (3) the NJ method (Saitou and Nei, 1987) using the NEIGHBOR program based on the Kimura's two-parameter distance (Kimura, 1980), which was computed using the DNADIST program in PHYLIP version 3.57c (Felsenstein, 1995). The quartet puzzling (QP) procedures with 1,000 QP steps were performed for the estimation of the branch supports of the ML trees. Bootstrap analyses (Felsenstein, 1985) with 1,000 iterations for MP and NJ trees were conducted using the bootstrap option in PAUP and the SEQBOOT program in PHYLIP, respectively. A skewness test statistic (g1) (Hillis and Huelsenbeck, 1992) in MP analysis was calculated based on the distribution of tree lengths of a random sample of 10,000 topologies. Transitions (TS) were downweighted relative to transversions (TV) by a factor of 2.0 (TS:TV = 1:2) in NJ analysis.

Nucleotide sequences of a subset of the ingroup, for which a consistent topology was not generated by ML, MP, and NJ analyses, were realigned and reanalyzed separately. In these analyses, two ostracod species, which were shown to be paraphyletic and most closely related to the reanalyzed group in the first analyses, were chosen as an outgroup in order to increase the number of unambiguously aligned sites so as to enhance the resolution of their relationships. The sequence alignments used for the phylogenetic analyses are available on request from the author. The nucleotide sequences determined in this study are available from the DDBJ/EMBL/GenBank database with the accession numbers shown in Table 1.

Results

Sequences of the 1,752 to 1,818 bp 18S rDNA fragment were obtained for 30 specimens from sixteen cytherocopine families and two outgroup ostracods (Bairdioidea and Cytherelloidea) (Table 1). The sequence of *Keijia* cf. *demissa* has about 50 missing sites. The full alignment resulted in a character matrix consisting of 1,864 positions owing to numerous inferred insertion and/or deletion events. Of the aligned sequences, regions of 259, 212, 93, and 67 sites, which included gaps and/or missing sites and could not be unambiguously aligned, were discarded, resulting in a total of 1,605, 1,652, 1,771, and 1,797 bp from the 18S rDNA fragment being used for the phylogenetic analyses of sixteen cytherocopine families, those of fifteen cytheroidean families, those of twelve cytheroidean families, and those of ten cytheroidean families, respectively (Sequence 1, Sequence 2, Sequence 3, and Sequence 4, respectively). The 1,605 bp Sequence 1 sequences showed a base composition that slightly differs among taxa (Table 3). The average base composition deviated slightly from 25%, with A = 26.0%, C = 21.1%, G = 27.2%, and T = 25.7%. Of the 1,605 bp Sequence 1 sequences, the number of variable sites and that of phylogenetically informative sites were 423 (26.4%) and 270 (16.8%), respectively. Of the 1,652 bp Sequence 2 sequences, the number of variable sites and that of phylogenetically informative sites were 425 (25.7%) and 277 (16.8%), respectively. Of the 1,771 bp Sequence 3 sequences, the number of variable sites and that of phylogenetically informative sites were 406 (22.9%) and 257 (14.5%), respectively. Of the 1,797 bp Sequence 4 sequences, the number of variable sites and that of phylogenetically informative sites were 322 (17.9%) and 203 (11.3%), respectively (Table 4).

Table 5 summarizes pairwise comparisons of sequence divergence corrected for multiple hits by the Kimura's two-parameter method (Kimura, 1980) among the sixteen cytherocopine families and two outgroup ostracods (Bairdioi-

Table 3. Comparison of the nucleotide composition (ranges of percentage values) among the sixteen cytherocopine families and the two outgroups (Bairdioidea and Cytherelloidea) analyzed. Comparisons are based on the 1,605 bp of unambiguously aligned sequences (Sequence 1).

		A	C	G	T
Bythocytheroidea	Bythocytheridae	25.8–25.9	21.1–21.9	27.2–27.4	25.0–25.7
Cytheroidea	Eucytheridae	25.9–26.2	21.2	27.2–27.5	25.4
	Paradoxostomatidae	26.4	20.4–20.7	26.9–27.1	25.8–26.2
	Cytheruridae	26.2–26.3	20.7	27.3–27.4	25.7
	Loxoconchidae	25.6–26.0	21.2–21.6	26.9–27.0	25.7–26.0
	Leptocytheridae	25.9–26.0	21.0–21.1	27.1	25.7–26.0
	Xestoleberididae	25.8–26.3	20.9–21.1	27.3–27.6	25.5
	Limnocytheridae	26.0	21.0	27.1	25.9
	Cytheridae	25.9	21.1	27.1	25.9
	Schizocytheridae	25.9–26.2	21.1	26.9–27.1	25.9
	Krithiidae	25.9	20.8	27.4	25.9
	Cushmanideidae	25.7	21.0–21.1	27.4–27.6	25.7–25.8
	Cytherideidae	26.1	20.9	27.2	25.7
	Hemicytheridae	25.7–25.8	20.7–20.9	27.3–27.5	25.9–26.2
	Thaerocytheridae	25.8–25.9	21.0	27.3–27.4	25.8
	Trachyleberididae	26.1–26.4	20.7	27.0	25.9–26.1
Outgroup	Bairdioidea	26.2	21.6	27.4	24.9
	Cytherelloidea	26.7	21.2	27.4	24.7
Average		26.0	21.1	27.2	25.7

dea and Cytherelloidea) based on the 1,605 bp of unambiguously aligned sequences (Sequence 1). The distances ranged from 0.004 to 0.095 between the sequences of cytherocopine ostracods, and from 0.068 to 0.120 between the sequences of cytherocopine ostracods and that of outgroup ostracods.

Scatter plot of the absolute number of transitions (TS) and transversions (TV) *versus* the pairwise genetic distance among individuals of each taxon based on the 1,605 bp of unambiguously aligned sequences (Sequence 1) is shown in Fig. 2. The numbers of transitions (TS) and transversions (TV) increased almost linearly with the increase of the genetic distance, and were clearly separated into two linear trends (Fig. 2). This suggests that there is no marked saturation in nucleotide substitutions in the aligned sequences.

Phylogenetic relationships among the sequences were inferred using maximum likelihood (ML) (Strimmer and von Haeseler, 1996), maximum parsimony (MP) (Swofford, 1993), and neighbor joining methods (NJ) (Saitou and Nei, 1987) (Figs. 3–6). For these phylogenetic analyses, the 16 cytherocopine families were divided into four nested groups, i.e., 16 cytherocopine families and 15, 12, and 10 cytheroidean families, in order to enhance resolution by progressively increasing the number of safely aligned sites to be subjected to analyses. Firstly, all these molecular phylogenetic analyses were performed using the sequences of 30 specimens from 16 cytherocopine families and two outgroup ostracods (Bairdioidea and Cytherelloidea) based on the 1,605 bp Sequence 1 sequences. Resulting molecular phylogenetic trees among them consistently indicated that each of the cytherocopines and cytheroideans formed a cluster, and that bythocytheroideans were paraphyletic outside of the cytheroideans (Fig. 3). However, the position of eucytherids was obscure in the cluster of cytheroidean ostracods (Fig. 3). There was no remarkable long branch on the resulting molecular phylogenetic trees.

Secondly, ML, MP, and NJ analyses were performed to ascertain the position of eucytherids using the sequences of 28 specimens from 15 cytheroidean families as ingroup and the two bythocytheroideans as outgroup based on the 1,652 bp Sequence 2 sequences. Resulting molecular phylogenetic trees among them con-

Table 4. Number of variable and phylogenetically informative sites of unambiguously aligned sequences (Sequence 1, 2, 3, and 4).

	Total	Variable	Phylogenetical
Sequence 1	1605	423	270
Sequence 2	1652	425	277
Sequence 3	1771	406	257
Sequence 4	1797	322	203

Table 5. Pairwise comparisons of sequence divergence among the sixteen cytherocopine families and the two outgroups (Bairdioidea and Cytherelloidea) analyzed. Comparisons are based on the 1,605 bp of unambiguously aligned sequences (Sequence 1). Genetic distances were corrected for multiple hits using Kimura's two-parameter model (Kimura, 1980).

	Bythocytheroidea	Cytheroidea								
	Bythocytheridae	Eucytheridae	Paradoxostomatidae	Cytheruridae	Loxoconchidae	Leptocytheridae	Xestoleberididae	Limnocytheridae	Cytheridae	Schizocytheridae
Bythocytheridae	0.067	0.059–0.072	0.078–0.093	0.078–0.093	0.083–0.095	0.084–0.087	0.079–0.083	0.081–0.083	0.082–0.090	0.086–0.092
Eucytheridae		0.015	0.049–0.053	0.042–0.063	0.057–0.070	0.049–0.056	0.046–0.055	0.047–0.052	0.048–0.051	0.057–0.063
Paradoxostomatidae			0.018	0.041–0.049	0.076–0.083	0.065–0.070	0.063–0.066	0.062–0.063	0.064–0.067	0.069–0.080
Cytheruridae				0.050	0.070–0.087	0.061–0.081	0.059–0.078	0.059–0.075	0.059–0.076	0.067–0.093
Loxoconchidae					0.032	0.038–0.052	0.041–0.052	0.041–0.050	0.043–0.051	0.049–0.058
Leptocytheridae						0.008	0.035–0.036	0.033–0.035	0.036–0.037	0.042–0.048
Xestoleberididae							0.016	0.023–0.026	0.029–0.030	0.032–0.042
Limnocytheridae								—	0.025	0.031–0.037
Cytheridae									—	0.029–0.034
Schizocytheridae										0.020
Krithiidae										
Cushmanideidae										
Cytherideidae										
Hemicytheridae										
Thaerocytheridae										
Trachyleberididae										
Bairdioidea										
Cytherelloidea										

	Cytheroidea						Outgroup	
	Krithriidae	Cushmanideidae	Cytherideidae	Hemicytheridae	Thaerocytheridae	Trachyleberididae	Bairdioidea	Cytherelloidea
Bythocytheridae	0.088–0.092	0.083–0.086	0.083–0.087	0.093–0.094	0.088–0.093	0.091–0.092	0.068–0.088	0.082–0.101
Eucytheridae	0.059–0.061	0.048–0.053	0.050–0.055	0.065–0.069	0.055–0.061	0.057–0.063	0.077–0.082	0.091–0.095
Paradoxostomatidae	0.074–0.075	0.061–0.065	0.063–0.064	0.077–0.083	0.067–0.072	0.071–0.074	0.090–0.093	0.104–0.106
Cytheruridae	0.074–0.087	0.061–0.078	0.064–0.079	0.076–0.093	0.067–0.086	0.067–0.088	0.092–0.103	0.103–0.114
Loxoconchidae	0.056–0.062	0.042–0.050	0.047–0.055	0.056–0.067	0.052–0.062	0.048–0.063	0.093–0.100	0.105–0.107
Leptocytheridae	0.046–0.047	0.033	0.039–0.040	0.045–0.052	0.041–0.042	0.042–0.046	0.096–0.098	0.109–0.111
Xestoleberididae	0.034–0.038	0.026–0.027	0.030–0.031	0.042–0.047	0.032–0.034	0.035–0.037	0.092–0.093	0.101–0.103
Limnocytheridae	0.034	0.022	0.026	0.039–0.044	0.029–0.031	0.033	0.093	0.106
Cytheridae	0.031	0.022–0.024	0.028	0.042–0.043	0.033	0.034	0.090	0.107
Schizocytheridae	0.033–0.038	0.029–0.034	0.033–0.035	0.042–0.048	0.036–0.043	0.035–0.046	0.098–0.102	0.111–0.116
Krithiidae	—	0.027–0.028	0.031	0.048–0.051	0.036–0.040	0.042–0.044	0.107	0.113
Cushmanideidae		0.004	0.015–0.016	0.035–0.040	0.027–0.028	0.027–0.033	0.092–0.094	0.106–0.107
Cytherideidae			—	0.040–0.044	0.031–0.033	0.033–0.039	0.095	0.111
Hemicytheridae				0.011	0.019–0.024	0.031–0.040	0.106	0.118–0.120
Thaerocytheridae					0.008	0.023–0.027	0.099–0.104	0.114
Trachyleberididae						0.033	0.099–0.103	0.112–0.113
Bairdioidea							—	0.065
Cytherelloidea								—

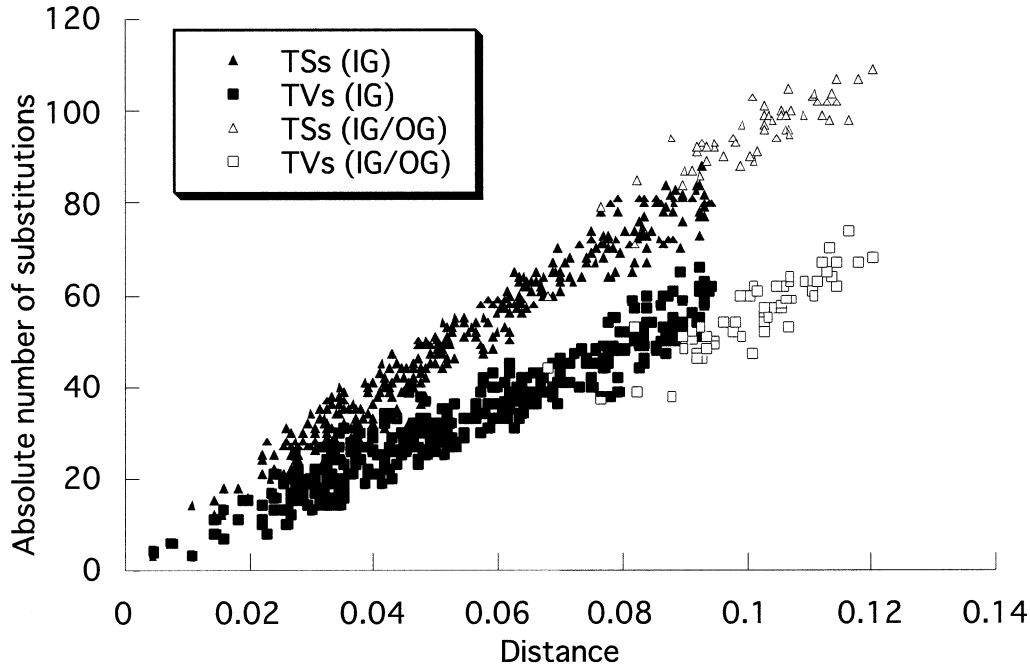
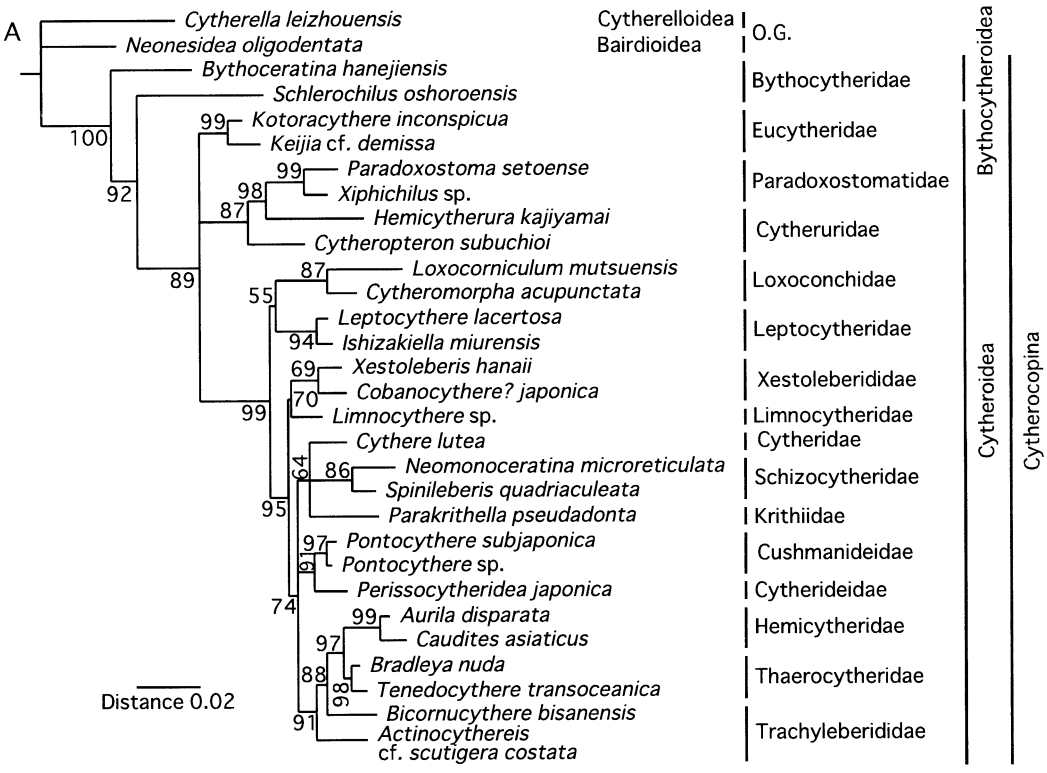


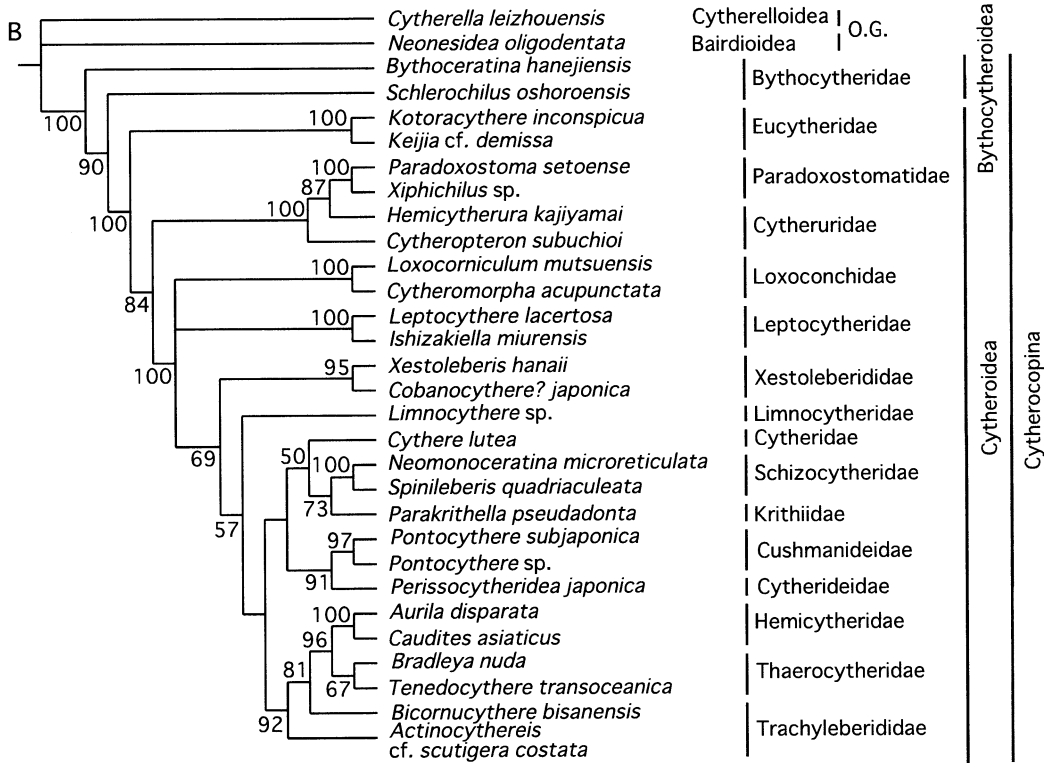
Fig. 2. Scatter plot showing pairwise comparisons of absolute number of transitions (TSs) and transversions (TVs) against Kimura's two-parameter distance for each sequence pair among the individuals of each group of taxa based on the 1,605 bp of unambiguously aligned sequences (Sequence 1). Solid symbols represent ingroup (IG) comparisons; open symbols represent ingroup/outgroup (IG/OG) comparisons.

sistently indicated that in the cluster of cytheroidean ostracods, eucytherids branched out first, followed by cytherurids plus paradoxostomatids, and other ostracods belonging to the remaining 12 families (Loxoconchidae, Leptocytheridae, Xestoleberididae, Limnocytheridae, Cytheridae, Schizocytheridae, Krithiidae, Cushmanideidae, Cytherideidae, Hemicytheridae, Thaerocytheridae, Trachyleberididae) (Fig. 4). The cytherurids are paraphyletic outside of the cluster of paradoxostomatids (Fig. 4). However, the position of loxoconchids was obscure in the cluster of ostracods belonging to the above 12 families (Fig. 4).

Thirdly, ML, MP, and NJ analyses were performed for revealing the position of loxoconchids using the sequences of 22 specimens from the 12 cytheroidean families as ingroup and the two ostracods (Eucytheridae and Paradoxostomatidae) as outgroup based on the 1,771 bp Sequence 3 sequences. Resulting molecular phylogenetic trees among them consistently indicated that, in the cluster of ostracods belonging to the 12 families, loxoconchids branched out first, followed by leptocytherids, and other ostracods belonging to the remaining 10 families (Xestoleberididae, Limnocytheridae, Cytheridae, Schizocytheridae, Krithiidae, Cushmanideidae, Cytherideidae, Hemicytheridae, Thaerocytheridae, Trachyleberididae) (Fig. 5). The relationships among the remaining 10 families were obscure (Fig. 5).

Lastly, molecular phylogenetic analyses were performed to obtain the exact relationships among the 10 families using the sequences of 18 specimens from the 10 cytheroidean families as ingroup and the two leptocytherids as outgroup based on the 1,797 bp Sequence 4 sequences. Although the loxoconchids, which came outside of the 10 families, could also have been used as an outgroup, they were not taken as the outgroup, because their sequences included a number of insertion/deletion sites against those of 10 families and did not increase the number of unambiguously alignable sites. Resulting molecular phylogenetic trees of the cytheroidean ostracods belonging to the 10 families consistently indicated a still unresolvable polychotomy of the following four clusters: the xestoleberidids, the limnocytherid, the cytherid, and the others belonging to the seven families (Fig. 6). The last cluster of seven families was further divided into three clusters, i.e., the cluster of schizocytherids and the





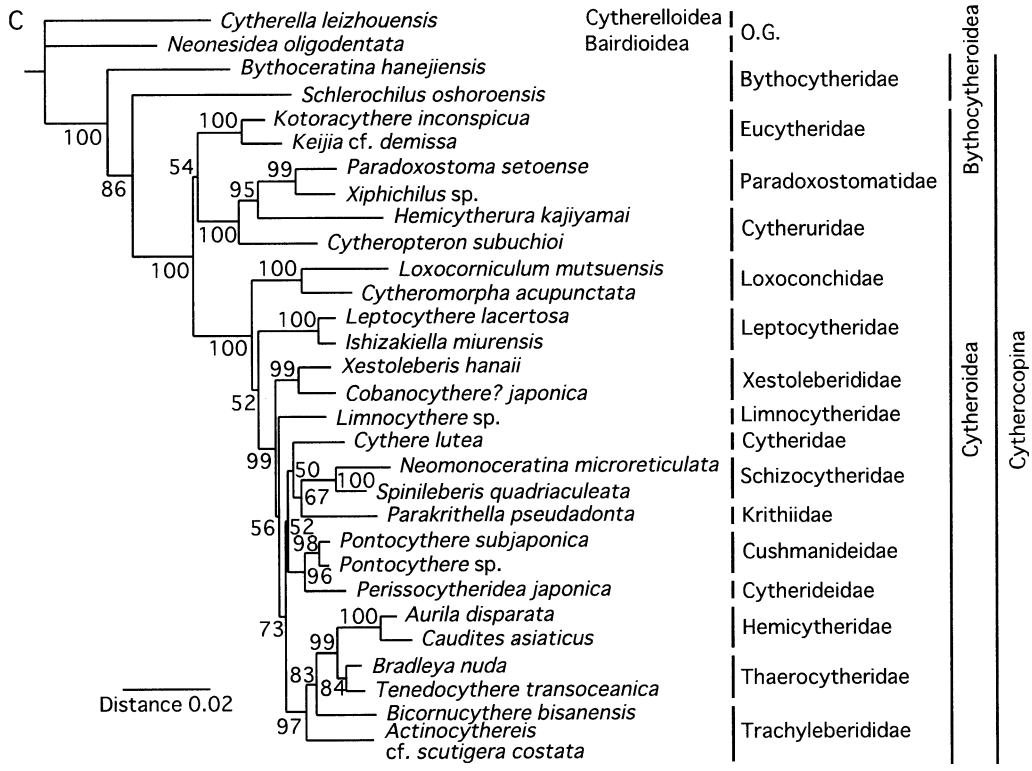


Fig. 3. Continued.

krithiid, that of cushmanideids and cytherideid, and that of hemicytherids, thaerocytherids, and trachyleberidids (Fig. 6). The trachyleberidids are paraphyletic outside of the cluster of hemicytherids and thaerocytherids (Fig. 6).

The results of molecular phylogenetic analyses mentioned above are summarized in Fig. 7. Maximum parsimony analysis was also carried out, using the sequences of 29 specimens except for that of *Keijia* cf. *demissa* from 16 cytherocopine families and two outgroup ostracods (Bairdioidea and Cytherelloidea) based on the 1,864 sites of full alignment sequences, and counting indels as characters. As the sequence of *Keijia* cf. *demissa* has about 50 missing sites, the sequence was not used. This resulted in two maximum parsimonious trees. The consensus tree of them has the same topology of the summary tree shown in Fig. 7.

Discussion

There are many different kinds of ornamentation on the external surface of carapace, for example, ridge, tubercle, reticulation and so on (see Fig. 1). On the other hand, some cytherocopines, such as the *Sclerochilus* species (Bythocytheridae), paradoxostomatids, xestoleberidids, and krithiids, have a carapace with smooth surface (Fig. 1B, E, F, M, N, R). The

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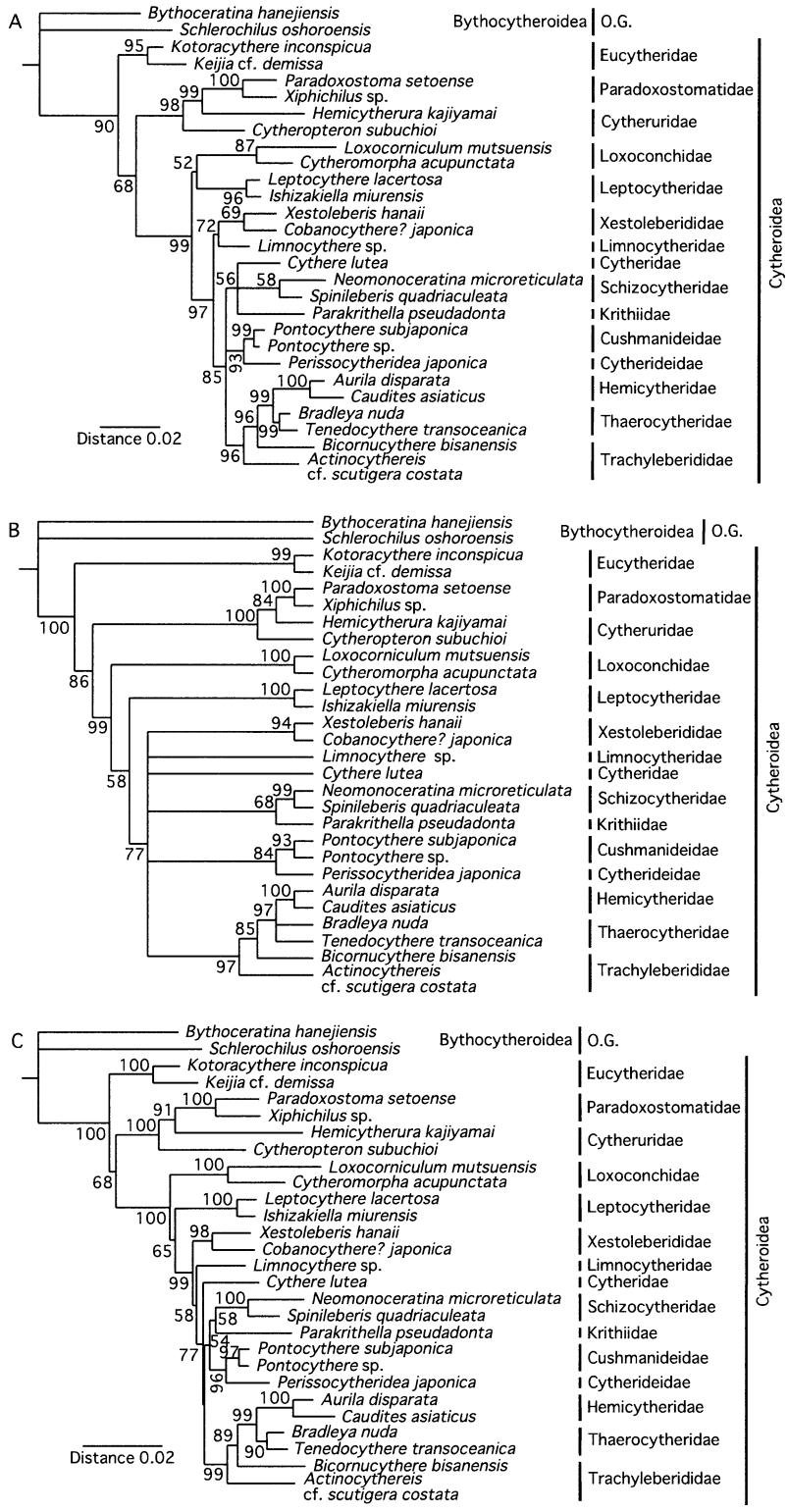
Fig. 3. Molecular phylogenetic trees based on the 18S rDNA sequences of 30 specimens from the sixteen cytherocopine families and two outgroups (Bairdioidea and Cytherelloidea) based on the 1,605 bp of unambiguously aligned sequences (Sequence 1). A, ML tree. The quartet puzzling support value (Strimmer and von Haeseler, 1996) based on 1,000 puzzling steps is shown at each branching point. B, MP tree. Strict consensus of three equally parsimonious trees found in the heuristic search [tree length = 950 steps, CI = 0.59, RI = 0.66, g1 = –0.68]. C, NJ tree based on Kimura's two-parameter evolutionary distance. The bootstrap confidence level (Felsenstein, 1985) based on 1,000 replications is shown at each branching point of MP and NJ trees. Only quartet puzzling support values and bootstrap confidence level above 50% are shown.



most parsimonious reconstruction of the surface features of the ostracods used for phylogenetic analysis using the inferred branching topology shown in Fig. 7 suggests that the carapace with smooth surface evolved at least three times independently in the lineages leading to paradoxostomatids, xestoleberidids, and krithiids (Fig. 8).

The Bythocytheridae had generally been placed in the Superfamily Cytheroidea (e.g., Benson *et al*., 1961; Hartmann and Puri, 1974; Bowman and Abele, 1982; Cohen, 1982; Athersuch *et al*., 1989), but Hinz-Schallreuter and Schallreuter (1999) included this family in the Superfamily Bythocytheroidea. The Bythocytheroidea and Cytheroidea are both classified in the Suborder Cytherocopina (Hinz-Schallreuter and Schallreuter, 1999). It is generally thought that the Cytheroidea was derived from the Bythocytheroidea in Late Paleozoic (e.g., McKenzie, 1969; Whatley and Boomer, 2000). The extant Bythocytheroidea differ from the extant Cytheroidea in several morphological features of carapace and appendage; the bythocytheroideans have a carapace with five adductor muscle scars in a vertical row and a pair of first antenna (antennula) with seven articulated podomeres, whereas the cytheroideans have a carapace with four adductor muscle scars in a vertical row and a pair of first antenna with five or six articulated podomeres (Athersuch *et al*., 1989) (Fig. 9A, B).

Phylogenetic analyses using 18S rDNA sequences suggested that the cytherocopines and cytheroideans are monophyletic respectively, and the bythocytheroideans are paraphyletic outside of the cytheroideans (Figs. 3, 7). The paraphyletic relationship seems to reflect that the ancestor of extant cytheroideans had derived from an ancestral bythocytheroidean. The Paleozoic bythocytheroideans, such as *Monoceratina*, *Editia*, and *Adeditia*, have a carapace with five adductor muscle scars (e.g., Sohn, 1988; Gramm, 1992). Thus, it is probable that in the extant cytherocopine ostracods, the carapace with five adductor muscle scars and the first antenna with seven articulated podomeres are plesiomorphic features, and that the carapace with four adductor muscle scars and the first antenna with five or six articulated podomeres are apomorphic ones.

All cytherocopine ostracods possess a well-developed hingement which consists of complicated teeth and sockets. The hingement has generally been regarded as the most important taxonomic character to distinguish cytherocopine families (e.g., Hinz-Schallreuter and Schallreuter, 1999). On the other hand, the evolution of hingement is still speculative. Evolutionary processes of the hinge structures of cytherocopine ostracods used in the molecular phylogenetic analyses (Figs. 9, 10) are most parsimoniously reconstructed using the phylogenetic tree inferred from the 18S rDNA sequences (Fig. 11). This evidence indicates that the lophodont is plesiomorphic in the cytherocopine ostracods, that the lophodont, merodont (hemimerodont and antimerodont), or pentodont is plesiomorphic in the cytheroidean ostracods, and that the remaining other hingements, i.e., the gongylodont, entomodont, schizodont, pseudadont, desmodont, and amphidont (holamphidont and hemiamphidont), are apomorphic (Fig. 11). Therefore, various kinds of hingement such as the pentodont, gongylodont, entomodont, desmodont, and amphidont, probably evolved from a merodont basic type, such as the lophodont and merodont.

Hanai (1961) suggested that loxoconchids and leptocytherids are monophyletic, respectively, based on their possession of unique hingement. Based on the representatives of each family used for this study, resulting molecular phylogenetic trees among cytherocopine families consistently indicated that each cytherocopine family is either a monophyletic group or a paraphyletic group (Figs. 3–7). The most parsimonious mapping of the hinge structures in the molecular phylogenetic tree suggests that gongylodont-, entomodont-, and desmodont-

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Fig. 4. Molecular phylogenetic trees based on the 18S rDNA sequences of 28 specimens from the fifteen cytheroidean families and outgroup (Bythocytheroidea) based on the 1,652 bp of unambiguously aligned sequences (Sequence 2). A, ML tree. The quartet puzzling support value (Strimmer and von Haeseler, 1996) based on 1,000 puzzling steps is shown at each branching point. B, MP tree. Strict consensus of 8 equally parsimonious trees found in the heuristic search [tree length = 965 steps, CI = 0.59, RI = 0.64, g1 = –0.72]. C, NJ tree based on Kimura's two-parameter evolutionary distance. The bootstrap confidence level (Felsenstein, 1985) based on 1,000 replications is shown at each branching point of MP and NJ trees. Only quartet puzzling support values and bootstrap confidence level above 50% are shown.

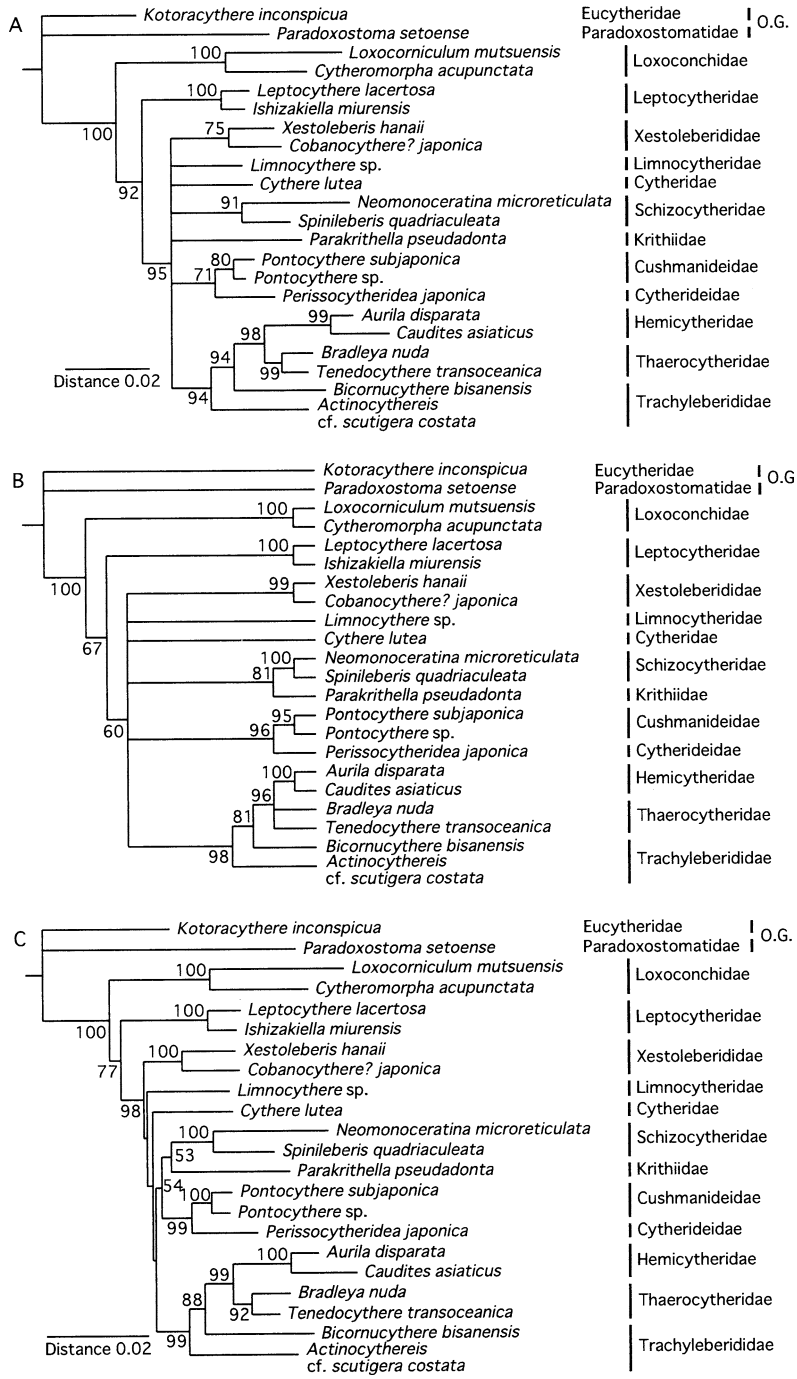

Fig. 5. Molecular phylogenetic trees based on the 18S rDNA sequences of 22 specimens from the twelve cytheroidean families and two outgroups (Eucytheridae and Paradoxostomatidae) based on the 1,771 bp of unambiguously aligned sequences (Sequence 3). A, ML tree. The quartet puzzling support value (Strimmer and von Haeseler, 1996) based on 1,000 puzzling steps is shown at each branching point. B, MP tree. Strict consensus of 12 equally parsimonious trees found in the heuristic search [tree length = 875 steps, CI = 0.62, RI = 0.58, g1 = –0.95]. C, NJ tree based on Kimura's two-parameter evolutionary distance. The bootstrap confidence level (Felsenstein, 1985) based on 1,000 replications are shown at each branching point of MP and NJ trees. Only quartet puzzling support values and bootstrap confidence level above 50% are shown.

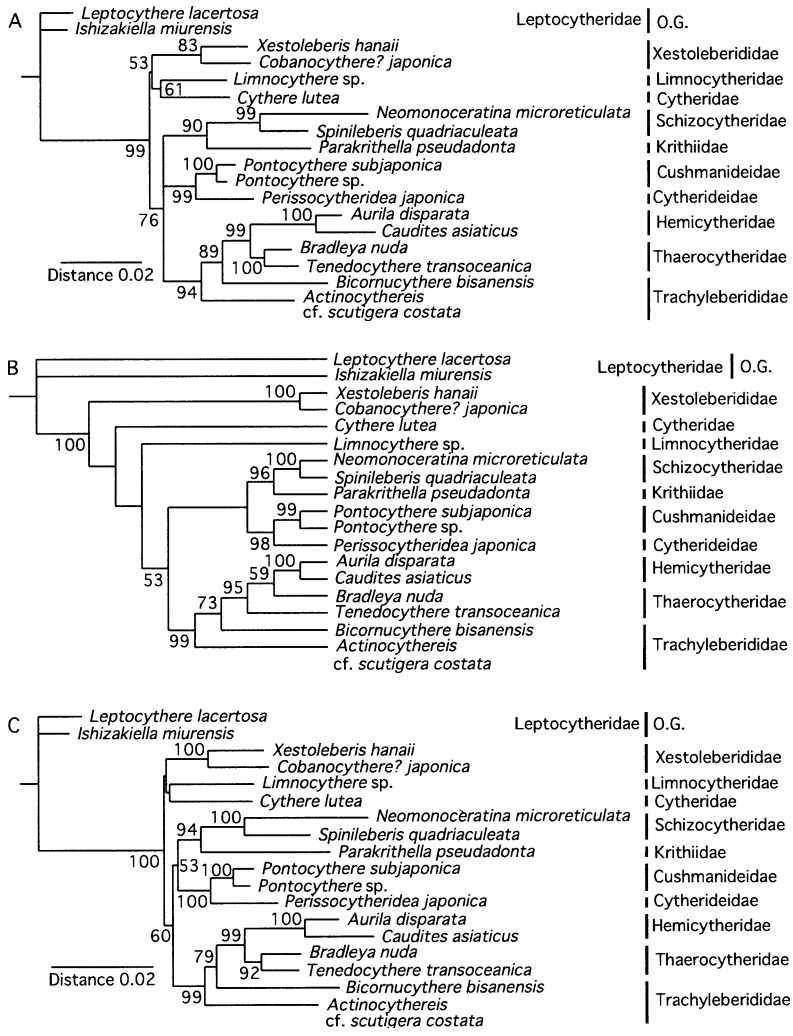

Fig. 6. Molecular phylogenetic trees based on the 18S rDNA sequences of 18 specimens from the ten cytheroidean families and outgroup (Leptocytheridae) based on the 1,797 bp of unambiguously aligned sequences (Sequence 4). A, ML tree. The quartet puzzling support value (Strimmer and von Haeseler, 1996) based on 1,000 puzzling steps is shown at each branching point. B, MP tree. One parsimonious tree found in the branch and bound search [tree length = 640 steps, CI = 0.62, RI = 0.57, g1 = –1.00]. C, NJ tree based on Kimura's two-parameter evolutionary distance. The bootstrap confidence level (Felsenstein, 1985) based on 1,000 replications is shown at each branching point of MP and NJ trees. Only quartet puzzling support values and bootstrap confidence level above 50% are shown.

type hingements are synapomorphic for loxoconchids, leptocytherids, and cushmanideids, respectively, supporting the suggestions of Hanai (1961) (Fig. 11).

The cytherocopine hingements are thought to have evolved from a simple one to a complicated one (Hartmann, 1963; Benson, 1966). Most parsimonious mapping of the hinge structures in the molecular phylogenetic tree suggests that the hinge structural type of the common ancestor of cytherurids and paradoxostomatids was either one of the merodont types or the lophodont. If so, the lophodont hingement of paradoxostomatids was evolved from one of the merodont types or retains a plesiomorphic feature in the cytheroidean ostracods (Fig. 11). On the other hand, it is thought that the Paradoxostomatidae was derived from a cytherurid in Late Cretaceous, because the first appearance of cytherurid fossils (Early Triassic) is earlier than that of paradoxostomatid ones (Late Cretaceous) (Yamaguchi, in preparation).

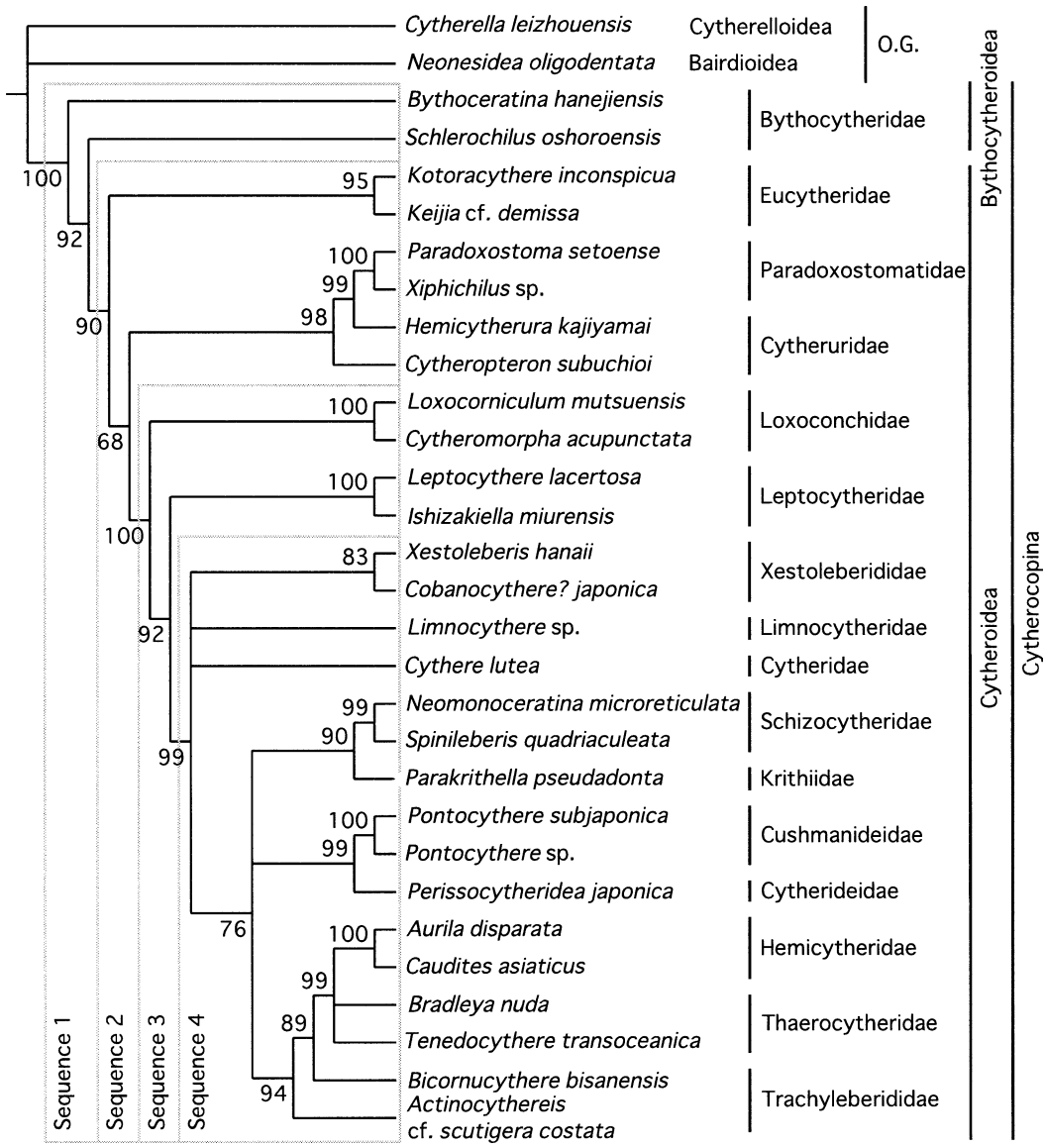

Fig. 7. Branching topology of sixteen cytherocopine families inferred from the molecular phylogenetic trees based on the 18S rDNA sequence comparisons. Gray boxes indicate the range of ingroups analyzed using sequence 1, 2, 3, and 4, respectively. This topology was based on a strict consensus of maximum likelihood, maximum parsimony, and neighbor joining trees shown in Figs. 3–6. The quartet puzzling support value for each ingroup is shown at each branching point.

Thus, the lophodont hingement of paradoxostomatids appears to have evolved degeneratively from one type of the merodont hingement of cytherurids, because the lophodont hingement is conjectured plesiomorphic in the cytherocopine ostracods.

Instances of parallel evolution from an entomodont hingement to an amphidont hingement, i.e., polyphyly of the amphidont hingement, are known in several lineages such as trachyleberidids and hemicytherids (Triebel, 1954; Sylvester-Bradley, 1956; Pokorny, 1957). The hinge structures of the amphidont basic type, i.e., pentodont, gongylodont, entomodont, schizodont, and amphidont types indicate homoplastic distributions in the molecular phylogenetic tree (Fig. 11). The pentodont is sometimes considered to be one of the hinge structures of the merodont basic type (Hanai, 1957). Therefore, the amphidont basic type

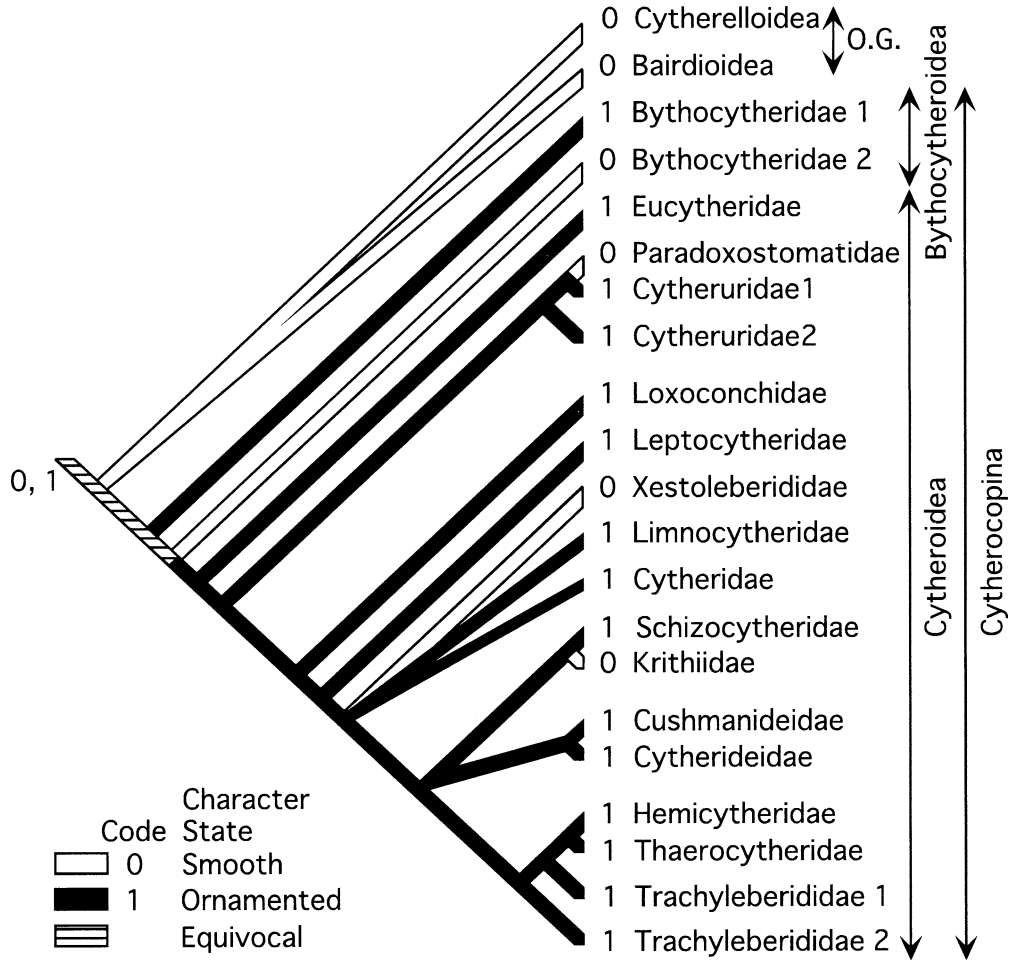

Fig. 8. Morphological evolutional hypothesis of the surface ornamentation of carapace based on the most parsimonious reconstruction using the inferred branching topology shown in Fig. 7. Smooth surface of carapace evolved at least three times independently in the lineages leading to paradoxostomatids, xestoleberidids, and krithiids. Each number on the left side of taxonomic name indicates the ornamentation character sate of taxon analyzed in this study. OG denotes outgroups used for the phylogenetic analysis.

presumably evolved at least four times independently in the lineages leading to Loxoconchidae, Leptocytheridae, Schizocytheridae and to the common ancestor of Hemicytheridae, Thaerocytheridae, and Trachyleberididae (Fig. 11). However, the amphidont (holamphidont and hemiamphidont) hingement is probably not polyphyletic but synapomorphic for the Hemicytheridae, Thaerocytheridae, and Trachyleberididae, and evolved from the merodont-type hingement.

Yet, the origins of gongylodont, entomodont, and schizodont hingements are unclear, because their ancestral character states turned out as equivocal in parsimonious mapping of the hinge structures; the ancestral character state for gongylodont and entomodont is one of merodont types or lophodont, and that for schizodont and pseudadont is one of pseudadont, merodont types, amphidont types, or schizodont (Fig. 11). However, these suggest that the gongylodont and entomodont hingements evolved at least from either one of merodont types or lophodont, and that the schizodont evolved at least from one of pseudadont, merodont types, or amphidont types. Based on molecular phylogeny and fossil record assuming budding cladogenesis (*sensu* Wagner, 2000), it is considered that the ancestor of Loxoconchidae and that of Leptocytheridae have derived from the lineage of the Limnocytheridae by Early Triassic and Middle Jurassic

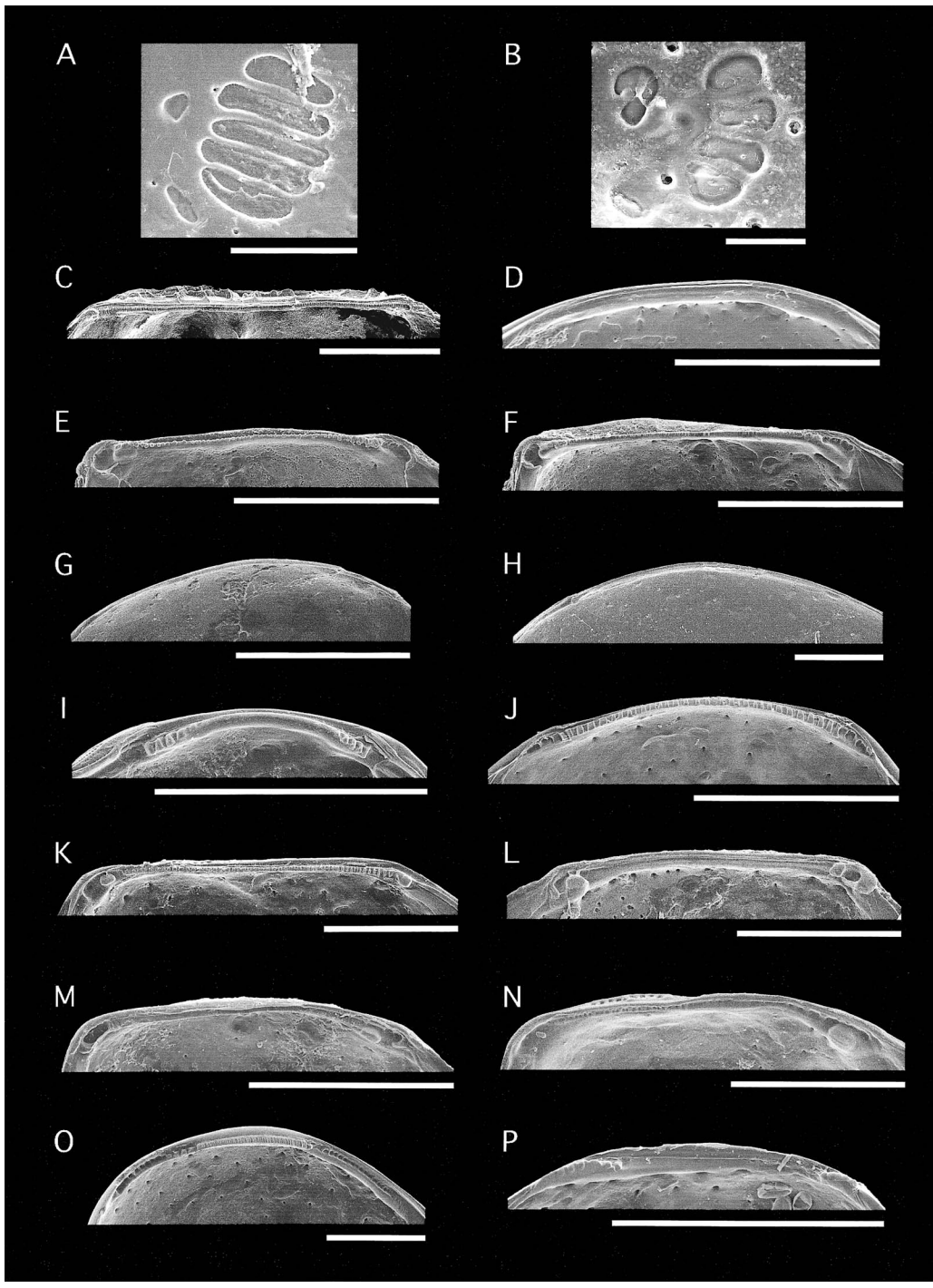

Fig. 9. Internal lateral view of carapaces of the ostracods used for phylogenetic analysis. A–B, Central muscle scars. A, RV of *Sclerochilus oshoroensis* (UMUT RA 27982). B, RV of *Cythere lutea* (UMUT RA 28026). C–P, Hingement. C, RV of *Bythoceratina hanejiensis* (UMUT RA 27981). D, RV of *Sclerochilus oshoroensis* (UMUT RA 27982). E, LV of *Kotoracythere inconspicua* (UMUT RA 27984). F, LV of *Keijia* cf. *demissa* (UMUT RA 28013). G, RV of *Paradoxostoma setoense* (UMUT RA 28007). H, RV of *Xiphichilus* sp. (UMUT RA 28008). I, LV of *Hemicytherura kajiyamai* (UMUT RA 28011). J, LV of *Cytheropteron subuchioi* (UMUT RA 28012). K, LV of *Loxocorniculum mutsuense* (UMUT RA 28014). L, RV of *Cytheromorpha acupunctata* (UMUT RA 28015). M, LV of *Leptocythere lacertosa* (UMUT RA 28016). N, LV of *Ishizakiella miurensis* (UMUT RA 28017). O, LV of *Xestoleberis hanaii* (UMUT RA 28018). P, RV of *Cobanocythere*? *japonica* (UMUT RA 28019). Scale bar indicates 50 μm for A–B, and 200 μm for C–P.

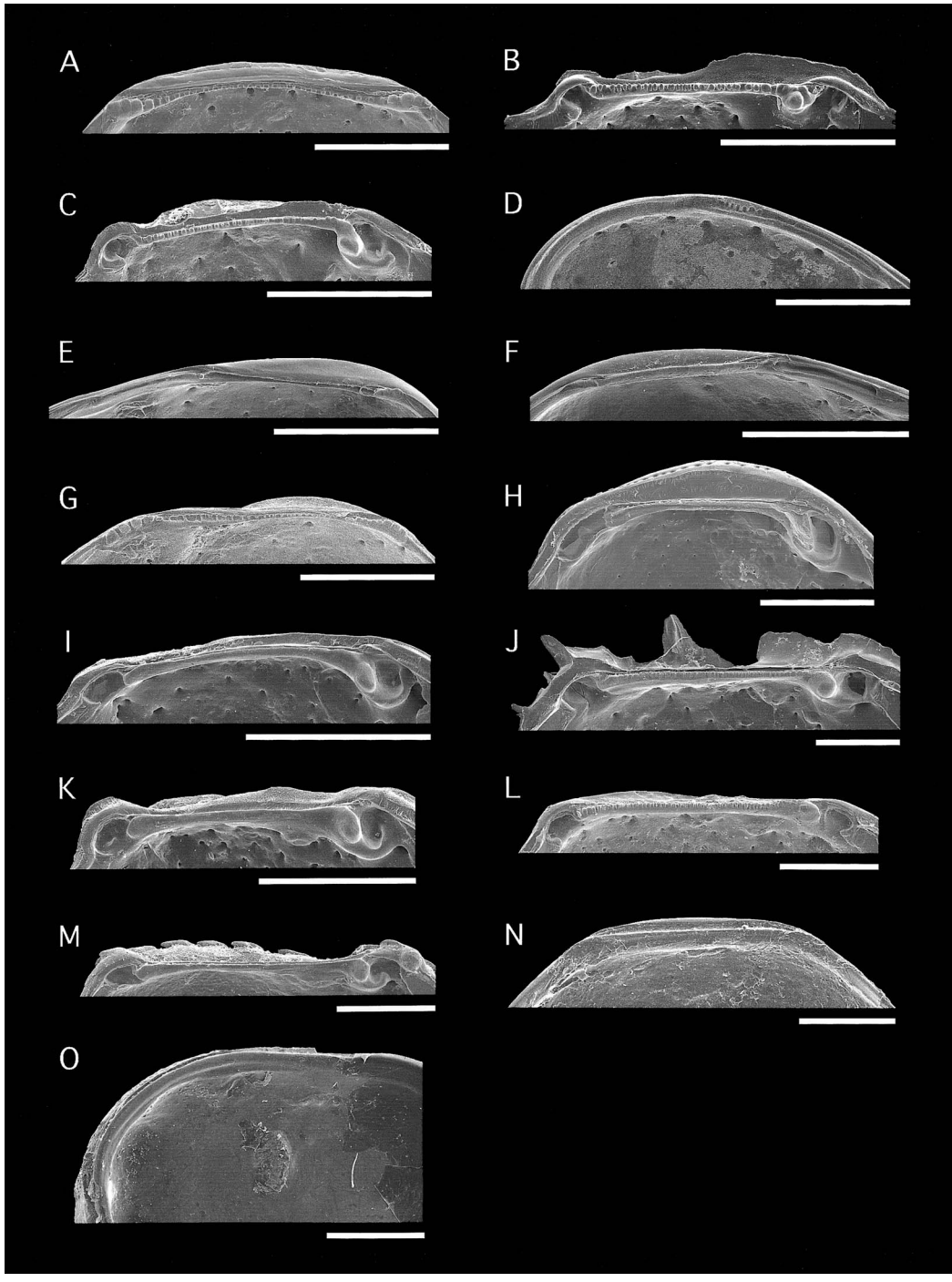

Fig. 10. Internal lateral view of carapaces of the ostracods used for phylogenetic analysis. A–O, Hingement. A, RV of *Cythere lutea* (UMUT RA 27983). B, LV of *Neomonoceratina microreticulata* (UMUT RA 28020). C, LV of *Spinileberis quadriaculeata* (UMUT RA 28021). D, LV of *Parakrithella pseudadonta* (UMUT RA 28022). E, RV of *Pontocythere subjaponica* (UMUT RA 28023). F, LV of *Pontocythere* sp. (UMUT RA 28024). G, RV of *Perissocytheridea japonica* (UMUT RA 28025). H, LV of *Aurila disparata* (UMUT RA 27996). I, LV of *Caudites asiaticus* (UMUT RA 27999). J, LV of *Bradleya nuda* (UMUT RA 28000). K, LV of *Tenedocythere transoceanica* (UMUT RA 28001). L, LV of *Bicornucythere bisanensis* (UMUT RA 28002). M, LV of *Actinocythereis* cf. *scutigera costata* (UMUT RA 28005). N, RV of *Neonesidea oligodentata* (UMUT RA 27989). O, LV of *Cytherella leizhouensis* (UMUT RA 27990). Scale bar indicates 200 μm.

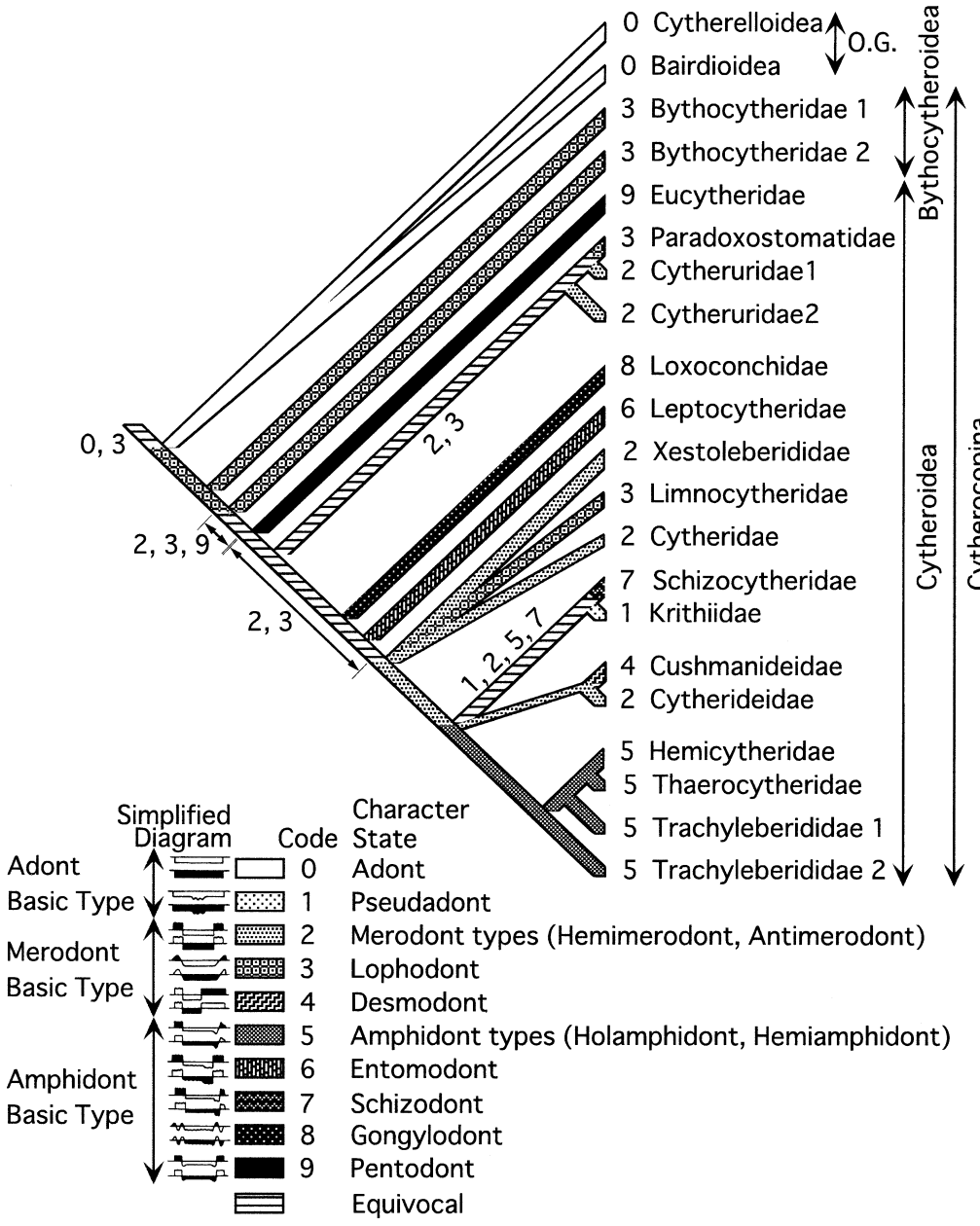

Fig. 11. Morphological evolutional hypothesis of hinge structures characterizing cytherocopine families based on the most parsimonious reconstruction using the inferred branching topology shown in Fig. 7. Amphidont hingement evolved at least four times independently in the lineages leading to loxoconchids (Gongylodont), leptocytherids (Entomodont), schizocytherids (Schizodont), and to the common ancestor of trachyleberidid, thaerocytherid, and hemicytherid ostracods (Amphidont). The classification of hinge structures is based on Hinz-Schallreuter and Schallreuter (1999). Each number on the left side of taxonomic name indicates the hingement character sate of taxon analyzed in this study. OG denotes outgroups used for the phylogenetic analysis. Simplified diagrams illustrate the dorsal views of hinge structures.

respectively, and that the ancestor of Schizocytheridae and that of Trachyleberididae have derived from the lineage of Cytherideidae by Early Cretaceous (Yamaguchi, in preparation). The gongylodont and entomodont hingements presumably evolved from the lophodont hingement, and the schizodont and the amphidont types from the hingement of merodont

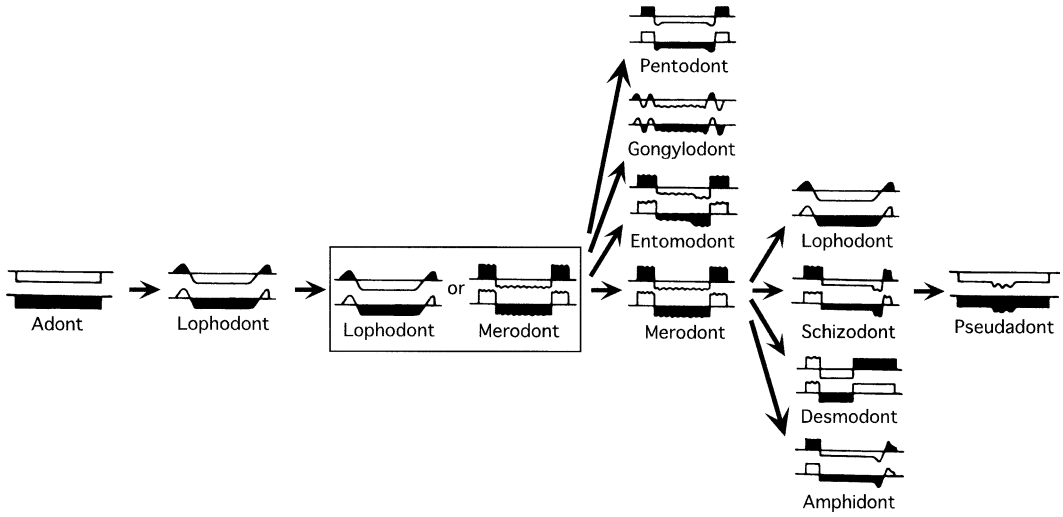

Fig. 12. Evolutionary trend of hinge structures inferred from Fig. 11. The lophodont is plesiomorphic in the cytherocopine ostracods and evolved from the adont. The origins of pentodont, gongylodont, and entomodont hingements are unclear; their ancestral character state is one of the merodont or lophodont. Some lophodont (for example, that of paradoxostomatids) evolved degeneratively from the merodont. The schizodont, desmodont, and amphidont evolved from the merodont. The pseudadont evolved from the schizodont. Simplified diagrams illustrate the dorsal views of hinge structures.

types (hemimerodont and antimerodont) (Fig. 11). The presumable evolutionary trend of hinge structures in the cytherocopine ostracods, as mentioned above, are summarized in Figure 12.

Based on the facts that several Cambrian archaeocopids often lack hinge structures and that they tend to indicate clearly separated valves due to their extensive mineralization of the carapace, Hinz (1993) suggested that an ostracod carapace with a real hingement is a consequence of increased mineralization of the carapace. Hinz-Schallreuter and Schallreuter (1999) and Pokorny (1957) pointed out a correlation between the complicated hingement of ostracods and the increase of calcification and thickness of the carapace. The extant loxoconchids, leptocytherids, schizocytherids, hemicytherids, thaerocytherids, and trachyleberidids, all with the amphidont basic type hingement, generally have a strongly calcified carapace, whereas the bythocytherids, paradoxostomatids, and limnocytherids, with the lophodont hingement, generally have a weakly calcified carapace (e.g., Hartmann and Puri, 1974; Cohen, 1982). Complication of the hinge structure seems to closely correlate with the increase of calcification of carapace. This correlation is presumed to have no connection with the phylogenetic relationships, because the hingements of the amphidont basic type indicated homoplasy. In addition, simplification of the hinge structure is also presumed to have no connection with the phylogenetic relationships, because the lophodont hingement of paradoxostomatids is shown to have evolved independently in this lineage. Thus, not only complication but also simplification of hinge structure presumably correlates, irrespective of phylogenetic relationships, with the extent of calcification and thickness of carapace. Parallel evolution of hingements must have occurred several times in the lineage of cytherocopine ostracods.

Generally, cypridoideans, darwinuloideans, and most myodocopidan ostracods have a weakly calcified carapace with a simple hinge structure, i.e., the adont basic type. On the other hand, macrocypridoideans have a moderately calcified carapace with the merodont basic type hinge structure, which is not considered to be homologous to the merodont basic type observed in cytherocopine ostracods (Maddocks, 1977). Bairdioideans have a strongly calcified carapace with the adont basic type, but the hingement has a pair of developed ridge and groove. Therefore, the hingements of the ostracod carapace must have evolved in concert with the calcification, and probably do not always show a correlation with their phylogenetic relationships, though the complicated

hingements of some ostracods have sometimes been considered to reflect their phylogenetic relationships.

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