Molecular phylogenetic perspective on the evolution of the deep-sea fish genus *Cyclothone* (Stomiiformes: Gonostomatidae)

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(Received *12 March 1996*; in revised form *2 September 1996*; accepted *18 September 1996)*

Ichthyological Research

Miya, M. and M. Nishida. 1996. Molecular phylogenetic perspective on the evolution of the deep-sea fish genus *Cyclothone* (Stomiiformes: Gonostomatidae). Ichthyol. Res., 43 (4): 375–398.

Abstract A portion of mitochondrially encoded 12S and 16S ribosomal RNA genes were sequenced from 13 currently recognized species of the midwater deep-sea fish genus *Cyclothone* (Stomiiformes: Gonostomatidae) and three gonostomatid outgroup taxa. Phylogenetic analyses using maximum parsimony and maximum likelihood methods were performed on unambiguously aligned, combined sequences (803 bp) of the two genes. The resultant tree topologies from the two methods were congruent, being robust and supported by various tree statistics, enabling the evolutionary history of *Cyclothone* to be described in detail. The molecular phylogeny demonstrated striking inconsistencies with previously proposed "natural groups," although the latter could be confidently refuted by the molecular data. The most significant characteristic of the evolutionary history of *Cyclothone* was the independent acquisition of an apomorphic depth habitat from the relatively ancestral, lower mesopelagic habitat, by each of three major distinct lineages that had diverged earlier in their evolution. Moreover, such macroevolutionary habitat shifts had been necessarily accompanied by morphological and ecological novelties, presumably originating from paedomorphosis. Repeated evolution of such changes strongly suggests ontogenetic plasticity in *Cyclothone* which could enable these fishes to acquire larval-like, simple organization of body structure. Such a body plan could help them subsist in food-poor surroundings and regulate reproductive variables that take advantage of increasing larval survival toward shallower depths. Recent speciation events, on the contrary, have produced contemporary sister species of allopatric (or microallopatric) distributions, but few morphological and ecological differences. Even if remarkable miniaturization has occurred, such as in the Mediterranean endemic *C. pygmaea*, it had to have been a simple truncation of ancestral species' ontogeny without attendance of any discernible paedomorphic features. On the basis of the fossil record, geological history of the Mediterranean region, and ectotherm molecular divergence rate, it was estimated that *Cyclothone* radiation had already started in the early–middle Miocene (17–20 million years ago).

Key words. — Molecular phylogeny; mitochondrial DNA; paedomorphosis; progenesis; protandry.

The midwater deep-sea fish genus *Cyclothone*, currently including 13 species (Miya, 1994a), is the most speciose of the seven genera comprising the family Gonostomatidae (sensu Weitzman, 1974). *Cyclothone* species are small (maximum size, 25–75 mm SL) and fragile, with body

coloration varying from virtually transparent to completely black. Unlike other midwater deep-sea fishes, such as lanternfish, that undertake extensive diurnal vertical migrations, they exhibit no diurnal migratory behavior, remaining at meso- and bathypelagic depths (ca. 200–2000 m) both day and night (e.g., Badcock and Merrett, 1976, 1977; Maynard, 1982; Miya and Nemoto, 1991). They occur abundantly in tropical to temperate regions, as well as being found in subarctic and antarctic latitudes.

Probably the most remarkable characteristic of *Cyclothone* is their numerical dominance over other midwater deep-sea fishes, the former comprising approximately 50–70% of the midwater fish catches across various parts of the world's oceans (Berry and Perkins, 1966; Goodyear et al., 1972; Maynard et al., 1975; Badcock and Merrett, 1976; Miya et al., 1995). Some authors have even claimed them to be the most abundant vertebrate on earth (e.g., Ahlstrom et al., 1984; Nelson, 1994). Also, it should be noted that there are no "rare" species of *Cyclothone* known to date. Even in bathypelagic species, such as *C. parapallida* and *C. obscura*, more than ten individuals have been captured in a single tow (Badcock, 1982; Miya, 1994b; Miya, unpubl. data).

Although the basic body plan is very similar among *Cyclothone* species, they exhibit remarkable diversity in size, body coloration and ecology. Miya and Nemoto (1991) have demonstrated that aspects of such biological diversity are closely correlated with the depth of occurrence, except for the Mediterranean endemic, *C. pygmaea*. The shallowest dwellers (*C. alba*, *C. signata* and *C. braueri*), often called "transparent" species, are characterized by small size at maturity, early age at first reproduction, semelparity and low fecundity. In contrast, the deeper dwellers, "black" or dark-colored species (*C. pallida, C. livida, C. microdon, C. acclinidens* and *C. atraria*), are characterized by larger size at maturity, retarded reproduction, iteroparity and higher fecundity, with two subtropical-temperate species (*C. microdon* and *C. atraria*) being protandrous. The reproductive variables of the mid-layer dwellers (*C. kobayashii* and *C. pseudopallida*) fall between the above two ecological groups. Although not mentioned by Miya and Nemoto (1991), the poorly-known deepest dwellers (*C. obscura* and *C. parapallida*), which principally occur in the equatorial bathypelagic zone (>1000 m; Badcock, 1982; Miya, 1994b; Miya, unpubl. data), should be placed in a distinct category.

Several evolutionary or adaptive hypotheses have been proposed to explain such biological diversity in *Cyclothone*. Miya and Nemoto (1985) regarded the adaptive significance of protandry in *C. atraria* as a means of boosting fecundity. Marshall (1984), who recognized that progenetic tendency was pervasive among midwater deep-sea fishes, suggested that such was well represented by the "transparent" species of *Cyclothone*. Miya and Nemoto (1986a) subsequently confirmed the adaptive significance of progenetic tendency in *Cyclothone*, through observations on the life history of *C. alba*. Miya and Nemoto (1991), who found close correlations between vertical distribution patterns and reproductive variables, stated that trade-offs among various reproductive variables have taken place during the evolution of *Cyclothone*, probably owing to decreasing larval survival and food availability along a depth gradient. On the basis of the presumed sister relationship between *C. pseudopallida* and *C. kobayashii*, Miya (1994a) speculated that their largely separate distributions had been achieved through some large scale oceanographic subdivision of their common, formerly circumglobal, ancestral population, a supposition based on independent rather than adaptive processes.

None of these evolutionary or adaptive hypotheses, however, were based on an explicit, well-corroborated phylogeny, despite the reconstruction of such a phylogeny being fundamental to an understanding of the evolution of biological diversity. It has been well recognized that many biological characteristics, formerly assumed to be adaptations to current environmental settings, are in fact inherited from ancestral populations and conditions, and should be explained in that context (e.g., Brooks and McLennan, 1991).

Morphological characters, particularly osteological, have been used extensively to reconstruct phylogenies in fish systematics. In *Cy-*

clothone, however, limited numbers of such characters are available, because of their simplified, abbreviated anatomical structures with marked reduction in skeletal elements (Marshall, 1984). Furthermore, character independence, a prerequisite for phylogenetic analyses, is seriously violated owing to presumed evolutionary processes, such as progenesis. Also, miniaturization often results in morphological homoplasy, which can complicate phylogenetic analyses (Hanken and Wake, 1993). Nucleotide sequences, on the contrary, offer tremendous advantages for phylogenetic analysis because of the large numbers of easily interpretable, unambiguous characters. Moreover, there are reasonable models of nucleotide substitutions that can be applied to distributions of character states across taxa to evaluate the likelihood of alternative hypotheses.

In this study, we determined 886 base pairs (bp) of mitochondrial DNA sequences from two different ribosomal genes (12S and 16S) for 13 species of *Cyclothone* and three outgroup taxa and subjected the data to phylogenetic analysis. The topology of the resultant tree was well resolved and thereby permitted reconstruction of their evolutionary history and tests of alternative hypotheses regarding the evolution of interesting biological traits in *Cyclothone*.

Materials and Methods

Fish samples and DNA extraction

Mitochondrial DNA (mtDNA) sequences were obtained from examples of all 13 *Cyclothone* species currently recognized, plus three gonostomatid outgroup taxa (*Gonostoma atlanticum, G. gracile* and *Margrethia obtusirostra*). In order to examine intraspecific variation, two additional specimens of a cosmopolitan species, *C. braueri*, were examined. All specimens were preserved in 70–100% ethanol immediately after collection. Voucher specimens were deposited in the Fish Collection, Natural History Museum & Institute, Chiba (CBM-ZF).

Specimens analyzed were *Cyclothone alba* (CBM-ZF 7100—off Ryukyu Islands, southern Japan), *C. signata* (CBM-ZF 7083—off southern California, USA), *C. braueri* (CBM-ZF 7084—Sargasso Sea; CBM-ZF 7085—off east coast of New Zealand; CBM-ZF 7086—Mediterranean Sea), *C. atraria* (CBM-ZF 7087—Sagami Bay, central Japan), *C. acclinidens* (CBM-ZF 7088—Sargasso Sea), *C. pseudopallida* (CBM-ZF 7089—Sargasso Sea), *C. kobayashii* (CBM-ZF 7090—off east coast of New Zealand), *C. microdon* (CBM-ZF 7091—off east coast of New Zealand), *C. pygmaea* (CBM-ZF 7092—Mediterranean Sea), *C. livida* (CBM-ZF 7093—off Mauritania), *C. pallida* (CBM-ZF 7094—off east coast of New Zealand), *C. parapallida* (CBM-ZF 7095—Coral Sea), *C. obscura* (CBM-ZF 7099—Coral Sea), *Gonostoma atlanticum* (CBM-ZF 7096—Coral Sea), *G. gracile* (CBM-ZF 7097—Sagami Bay, central Japan) and *Margrethia obtusirostra* (CBM-ZF 7098—off Mauritania).

Total genomic DNA was isolated from the epaxial musculature of each fish. Tissue was digested overnight at 37°C in 500 μl extraction buffer (10 mM Tris, pH 8.0, 2 mM EDTA and 1% SDS) with 5 μl proteinase K (10 mg/*ml*). DNA was purified by phenol, phenol : chloroform : isoamyl alcohol (25 : 24 : 1) and chloroform : isoamyl alcohol (24 : 1) extraction, and dialyzed by filtration through a Centricon-100 microconcentrator (Amicon Inc.) before amplification.

DNA amplification and sequencing

DNA amplification was performed via the polymerase chain reaction (PCR) using oligodeoxynucleotide primers specific for two regions of the mtDNA genome. The primers used for one segment approximately 400 bases long (about 470 bases long in *C. signata*), from the 12S ribosomal RNA (rRNA) gene were L1085 (5′-TAAACCAGGATTAGATACCC-3′) and H1478 (5′-GAGAGTGACGGGCGATGTGT-3′). Those used for a second segment, about 570 bases long, from the 16S rRNA gene were L2510 (5′-CGCCTGTTTAACAAAAACAT-3′) (slightly modified from Palumbi et al., 1991) and H3059 (5′-CCGGTCTGAACTCAGATCACGT-3′). L and H denote light and heavy strands, respectively.

Numbers refer to the position of the 3′ end of the oligonucleotide with reference to the human mtDNA sequence (Anderson et al., 1981). PCR was done in a Perkin Elmer-Cetus Model 480 thermal cycler and reactions carried out with 35 cycles of a 25-μl reaction volume containing 12.5 μl sterile, distilled H_2O, 2.5 μl ×10 PCR buffer II (Perkin Elmer-Cetus), 2.5 μl dNTP (2 mM), 2.5 μl each primer (5 μM), 1.5 μl $MgCl_2$ (25 mM), 0.125 μl of 5 units *Thermus aquaticus* DNA polymerase (AmpliTaq, Perkin Elmer-Cetus) and 1 μl template. The thermal cycle profile was as follows: denaturation for 30 sec at 94°C, annealing for 30 sec at 50–55°C (depending on the primer specificity for different species) and extension for 1 min at 72°C. PCR products were electrophoresed on a 2% NuSieve agarose gel stained with ethidium bromide for band characterization via ultra violet transillumination.

Double-stranded DNA products from PCR, purified by filtration through a Centricon-100 microconcentrator, were used for direct cycle sequencing with dye-labeled terminators (Applied Biosystems Inc.). Primers used were the same as those for PCR. All sequencing reactions were performed according to the manufacturer's instructions. Labeled fragments were analyzed on a Model 373A DNA sequencer (Applied Biosystems Inc.). Sequences were obtained from both strands of the two gene segments for verification.

All sequences are available from DDBJ, EMBL and GenBank under accession numbers D84029–D84044 for the 12S rRNA and D84045–D84060 for the 16S rRNA.

Phylogenetic analysis

The DNA sequences were edited with the multiple sequence editor DNASIS (Hitachi Software Engineering Co. Ltd.) and preliminary alignment achieved using several parameter sets of CLUSTAL (gap penalty, 5–20; gap length penalty, 5–20; Higgins and Sharp, 1988), the output of which was later improved by eye on the sequence editor with reference to the proposed secondary structure of the 12S and 16S rRNA for teleostean fish (Alves-Gomes et al., 1995). Pairwise comparisons and statistical information from the sequences were obtained with MEGA ver. 1.0 (Kumar et al., 1993).

Phylogenetic relationships were analyzed by the following two major, character-based approaches: maximum parsimony (MP) and maximum likelihood (ML) methods.

Maximum parsimony analyses were performed with the branch-and-bound algorithm in PAUP ver. 3.1.1 (Swofford, 1993), using equal weighting for all substitutions. Transition/transversion weightings were not applied because informative transitions in conservative regions of rDNA were likely to be masked by transversions in more variable regions (Allard and Miyamoto, 1992; see Discussion). Gaps were considered as missing rather than as fifth characters, to circumvent those longer than one or two bases being considered multiple events (Swofford, 1993). All uninformative sites were neglected. To evaluate the robustness of the internal branches of the MP trees, 500 bootstrap replications (Felsenstein, 1985) were executed, with 30 heuristic, random stepwise additions being performed at each replication. A "decay index" (Donoghue et al., 1992) was also calculated using strict consensus trees of up to five steps longer than the most parsimonious tree. In addition, phylogenetic information content in the data set was evaluated by the skewness of tree distribution and the g_1 statistic (Hillis and Huelsenbeck, 1992) for 10,000 randomly generated trees. The g_1 statistic was calculated at first without topological constraints and, subsequently, by constraining the well-supported clades. Character optimization for aligned nucleotide sequence data was done using ACCTRAN (accelerated transformation). MacClade ver. 3.02 (Maddison and Maddison, 1992) was used in various phases of the phylogenetic analyses, such as preparing data matrices, exporting tree files, comparing two alternative trees site by site, and exploring alternative tree topologies.

Maximum likelihood analyses were performed using DNAML in PHYLIP ver. 3.57c (Felsenstein, 1995) with jumble option on (30 times) to determine the statistically most likely phylogeny under the estimated transition/transversion bias and rates of base pair substitution at different codon positions. The transition/trans-

Table 1. Matrix of sequence differences for the combined 12S and 16S mitochondrial ribosomal genes from *Cyclothone* and outgroup taxa

Species	Mob	Ggr	Gat	OBS	PAR	PAL	LIV	PYG	MIC	KOB	PSE	ACC	ATR	BRA	SIG	ALB
Mob	—	110/33	112/22	145/53	149/57	148/57	137/57	155/57	147/53	148/53	148/54	149/54	145/52	155/57	149/55	154/59
Ggr	13.7	—	123/43	153/58	160/60	153/58	149/62	164/62	161/58	153/58	156/59	153/57	148/57	161/62	158/62	160/64
Gat	13.9	15.3	—	149/53	154/55	151/55	150/63	159/59	155/55	150/57	152/56	155/54	149/50	164/59	166/53	171/57
OBS	18.1	19.1	18.6	—	41/16	37/12	56/22	60/20	68/22	60/16	61/17	56/19	51/17	77/24	77/30	95/34
PAR	18.6	19.9	19.2	5.1	—	20/8	54/22	67/20	71/18	67/16	65/15	65/21	60/19	84/26	77/28	98/32
PAL	18.4	19.1	18.8	4.6	2.5	—	52/22	64/16	74/18	67/16	68/15	57/15	54/13	74/22	70/26	90/32
LIV	17.1	18.6	18.7	7.0	6.7	6.5	—	57/14	56/14	45/10	51/11	59/19	53/17	74/22	76/30	84/28
PYG	19.3	20.4	19.8	7.5	8.3	8.0	7.1	—	27/6	35/6	39/5	59/13	55/11	76/14	80/22	86/26
MIC	18.3	20.0	19.3	8.5	8.8	9.2	7.0	3.4	—	35/6	39/7	61/17	60/15	83/18	87/26	92/26
KOB	18.4	19.1	18.7	7.5	8.3	8.3	5.6	4.4	4.4	—	16/3	66/15	58/13	78/16	87/24	93/26
PSE	18.4	19.4	18.9	7.6	8.1	8.5	6.4	4.9	4.9	2.0	—	63/14	61/12	81/15	84/21	88/23
ACC	18.6	19.1	19.3	7.0	8.1	7.1	7.3	7.3	7.6	8.2	7.8	—	15/4	72/17	65/25	74/29
ATR	18.1	18.4	18.6	6.4	7.5	6.7	6.6	6.8	7.5	7.2	7.6	1.9	—	67/15	61/21	71/25
BRA	19.3	20.0	20.4	9.6	10.5	9.2	9.2	9.5	10.3	9.7	10.1	9.0	8.3	—	58/20	76/26
SIG	18.6	19.7	20.7	9.6	9.6	8.7	9.5	10.0	10.8	10.8	10.5	8.1	7.6	7.2	—	49/14
ALB	19.2	19.9	21.3	11.8	12.2	11.2	10.5	10.7	11.5	11.6	11.0	9.2	8.8	9.5	6.1	—

Values above the diagonal are total number of substitutions/transversion; below diagonal are uncorrected percentage differences.
Mob—*Margrethia obtusirostra*; Ggr—*Gonostoma gracile*; Gat—*G. atlanticum*; specific names of *Cyclothone* are abbreviated to first three letters.

version ratio was set at a default value (2.0) on the basis of the observed ratios for all pairwise comparisons among ingroup+outgroup species (mean, 2.02; Table 1).

All trees were rooted with a relatively distantly-related gonostomatid, *Margrethia obtusirostra*; two other outgroup taxa, *Gonostoma atlanticum* and *G. gracile*, which have been assumed to be more closely related to *Cyclothone* than to the other five gonostomatid genera (Grey, 1964; Kobayashi, 1973; Weitzman, 1974; Ahlstrom et al., 1984), were treated as an ingroup in the analyses in a simultaneous attempt to resolve *Cyclothone* relationships and examine *Cyclothone* monophyly.

Character evolution

Evolution of various biological traits was traced on the molecular phylogenetic tree using MacClade ver. 3.02 (Maddison and Maddison, 1992) as the most parsimonious reconstruction set.

Speciation modes

Unlike terrestrial or coastal zones, oceanic environments are highly three dimensional with no definite geographic barriers except for those demarcating major ocean basins. Therefore protocols for identifying speciation modes that require accurate knowledge of distribution areas in addition to the well corroborated phylogeny, such as those advocated by Lynch (1989), cannot be applied directly to oceanic animals, including *Cyclothone* (for application of this method, see Grady and LeGrande, 1993). In the present study, inference on speciation modes was restricted to those between contemporary sister species (five pairs) on the basis of information implicit in their geographical and vertical distribution patterns, which were divided into allopatric (including peripheral and vicariant speciations according to Lynch's [1989] terminology) and sympatric speciations. Since postspeciational dispersals necessarily occur in any oceanic animals, distributional overlaps in peripheral zones were not taken as evidence for sympatry.

Tests of evolutionary hypotheses

Some evolutionary hypotheses regarding the origin of biological characteristics can be tested using statistical procedures. Bootstrapping is appropriate when the null hypothesis includes multiple origin of biological characteristics or non-monophyletic groups (DeBry, 1992). When the null hypothesis includes single-origin of biological characteristics or monophyletic groups, it can be tested by comparing differences in the distribution of character state changes per site between the constrained and unconstrained MP trees using Wilcoxon signed-ranks tests (Templeton, 1983). In the present study, tests of alternative phylogenetic or evolutionary hypotheses were accomplished using the constraint tree option in PAUP ver. 3.1.1 (Swofford, 1993). Differences in the distribution of character state changes per site were evaluated using Wilcoxon signed-ranks tests (Templeton, 1983).

Results

Sequence variation

Multiple sequence alignment of the amplified region for the 12S rRNA and 16S rRNA genes from 13 species of *Cyclothone* and three outgroup taxa resulted in a matrix consisting of 360 (Fig. 1) and 506 positions (Fig. 2), respectively, owing to numerous inferred insertion/deletion events. Fragment size from individual taxa ranged from 353 to 358 (354–358 in the ingroup) for the 12S rRNA and from 478 to 497 (488–495 in the ingroup) for the 16S rRNA genes. Of the aligned sequences, 13 sites in the 12S rRNA and 50 sites in the 16S rRNA (underlined in Figs. 1 and 2, respectively) that could not be aligned unambiguously were discarded for all the analyses, resulting in a total of 803 bp from the two genes used for the phylogenetic analyses.

Sequences from three individuals of *C. braueri*, taken from widely separated localities (western South Pacific, western North Atlantic and Mediterranean Sea), showed virtually no intraspecific variation, with the exception of a sin-

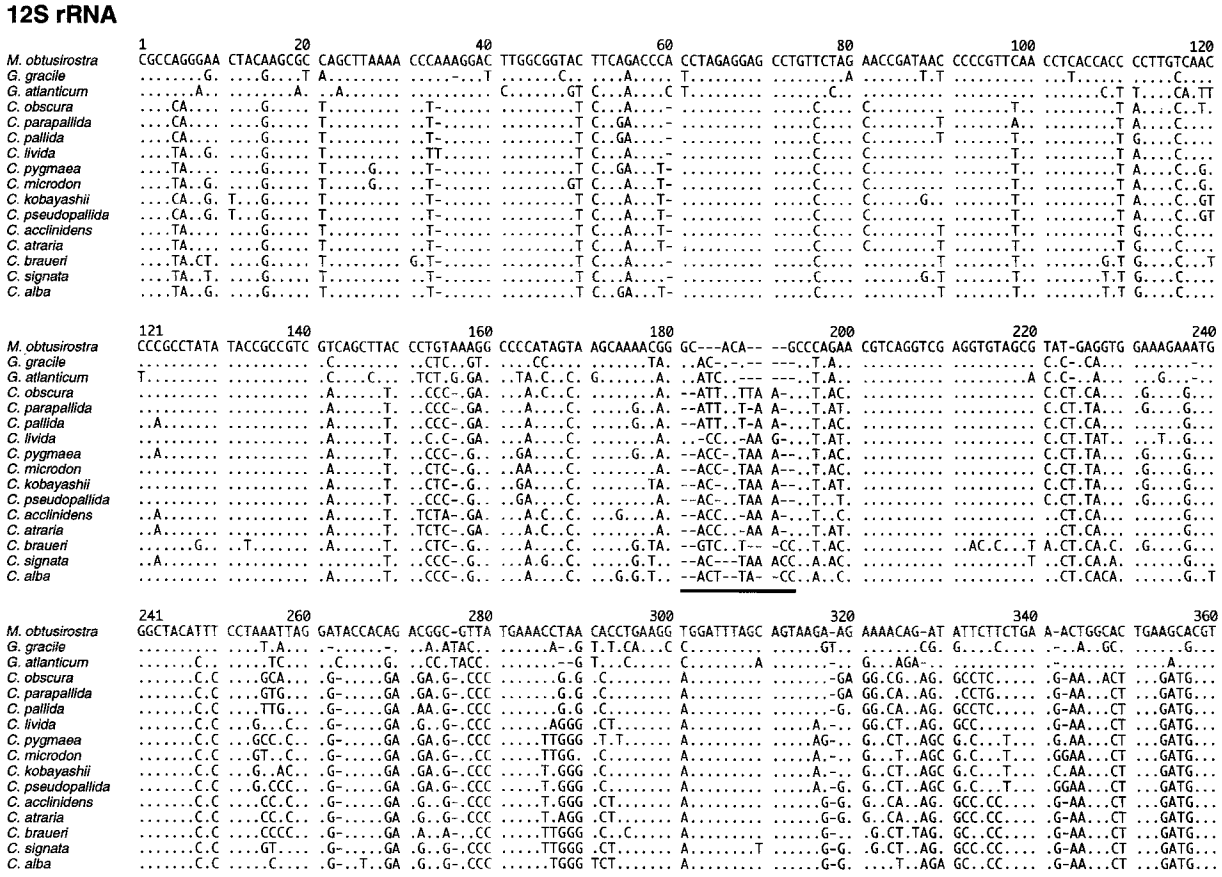

Fig. 1. Aligned DNA sequences from 360 bp of the 12S rRNA gene. Identity with first sequence denoted by dots. Insertions/deletions of specific nucleotides indicated by dashes (–). A solid line under alignment denotes sequences excluded from the phylogenetic analyses because of uncertain alignment.

gle nucleotide substitution at position 93 in the 16S rRNA (Fig. 2; "C" for the South Pacific specimen and "T" for the North Atlantic and Mediterranean specimens). Pairwise percentage differences among these sequences without correction for multiple hits (0–0.1%) were negligible when compared to the interspecific differences among *Cyclothone* species (Table 1; range, 1.9–12.2%; mean, 8.0%)

Of the 803 bp unambiguously aligned sequences, 207 (59.7%) and 283 sites (63.9%) (total, 490 sites) were invariant in the 12S rRNA and 16S rRNA segments, respectively. Of the remaining 313 variant sites, 99 (28.5%; 59 in the ingroup) and 110 (24.8%; 69 in the ingroup) (total, 209 sites; 128 in the ingroup) in the 12S rRNA and 16S rRNA segments, respectively, were phylogenetically informative (shared by at least two taxa).

Phylogenetic analyses

Maximum parsimony analyses using a branch-and-bound algorithm produced a single, fully bifurcating most parsimonious tree (Fig. 3), of length=519, consistency index (CI)=0.582, retention index (RI)=0.639 and rescaled consistency index (RCI)=0.372.

All internal branches, except for those of the three basal clades (B, D, E), were supported by high (>93%) bootstrap values (Fig. 3). The decay indices were generally correlated with bootstrap values, most of the former being >5 along those branches with >90% bootstrap values.

16S rRNA

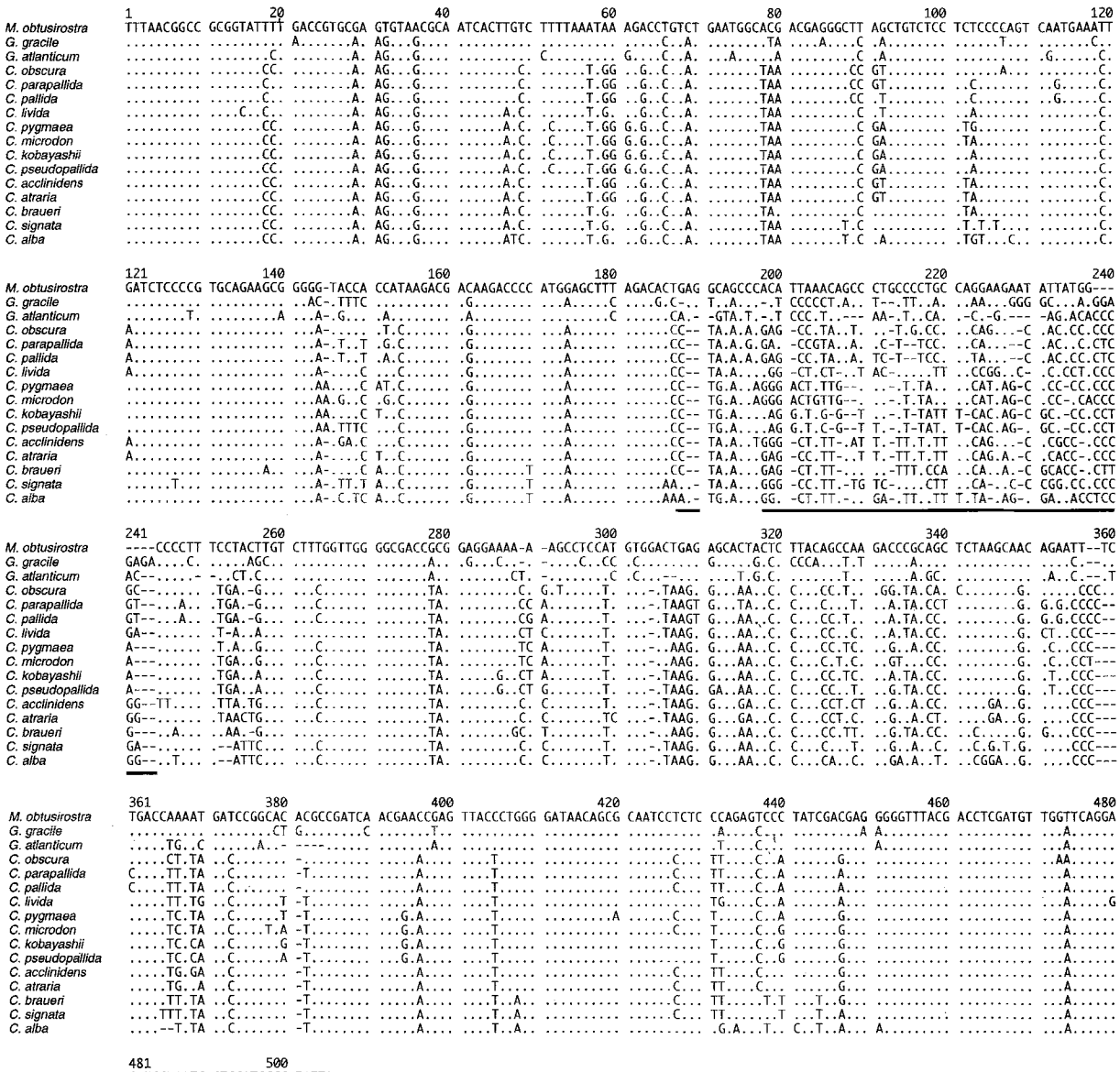

Fig. 2. Aligned DNA sequences from 506 bp of the 16S rRNA gene. Identity with first sequence denoted by dots. Insertions/deletions of specific nucleotides indicated by dashes (–). A solid line under alignment denotes sequences excluded from the phylogenetic analyses because of uncertain alignment.

On the basis of g_1 statistics of −1.36 for 10,000 random tree generations, the data set was considered to contain highly significant phylogenetic signals ($p<0.01$) when compared to a random distribution of tree lengths (Hillis and Huelsenbeck, 1992). When topological constraints were enforced as a backbone to those branches with ≥90% bootstrap values, the g_1 values changed to −0.77, which was still significant at the $p=0.01$ level. Therefore the sequence

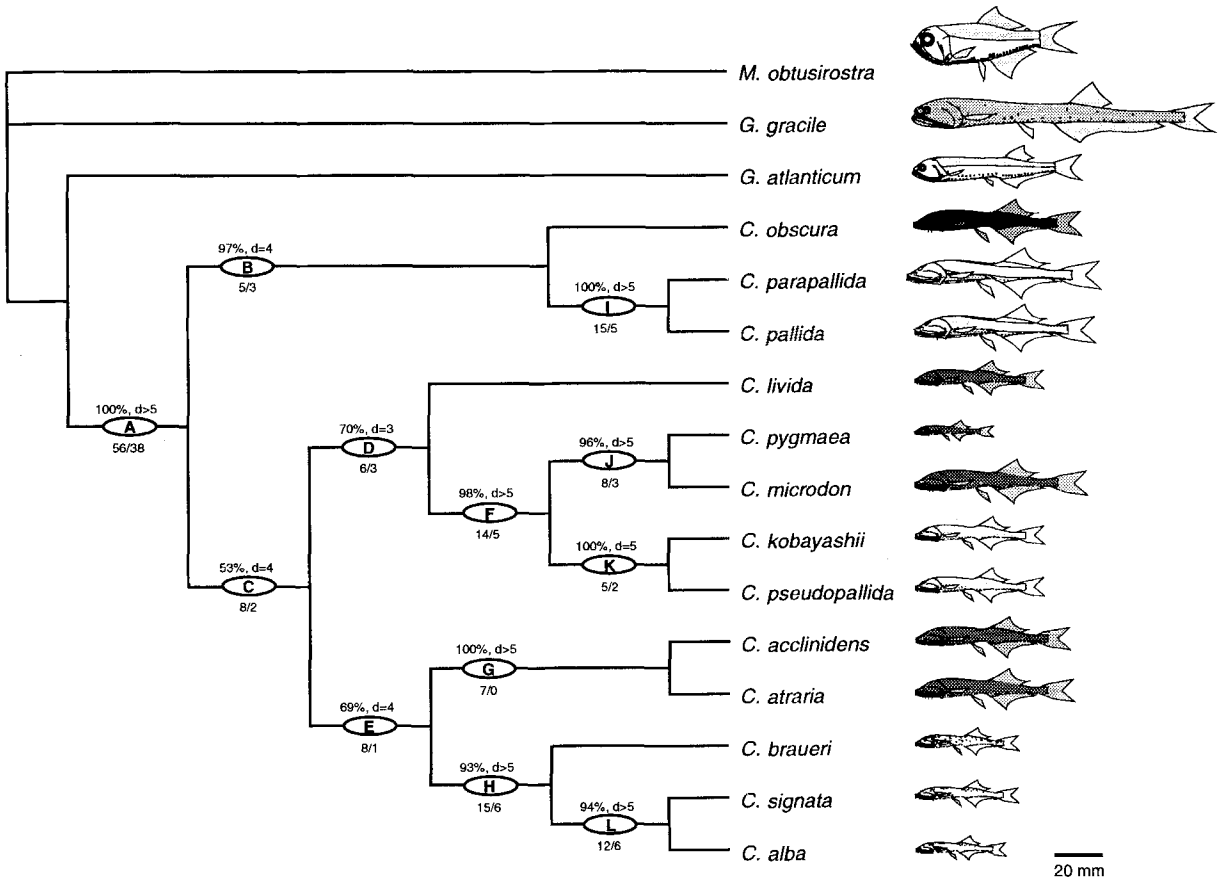

Fig. 3. The single most parsimonious tree of *Cyclothone* and outgroup taxa. Internal branches for the ingroup designated by capital letters A to L. Numbers above branches indicate bootstrap values obtained for 500 replicates and decay indices (d) up to five steps longer than the most parsimonious tree. Numbers below branches are unambiguous changes and unambiguous synapomorphies using ACCTRAN optimization. Size of fish illustrations proportional to the maximum size recorded (see Table 2).

data contained much phylogenetic information, even at the basal clades with relatively low bootstrap values.

Maximum likelihood analysis produced the same tree as that found by MP analysis (Fig. 4), all branches having lengths significantly different from zero ($p<0.01$).

Reconstruction of Evolutionary History

The robustness of the present phylogenetic tree allowed the depiction of the evolutionary history of *Cyclothone* in detail. The following description includes a consideration on the phenotypic variation, including body coloration, morphology and life history traits, as well as geographic and vertical distribution patterns, allowing several evolutionary processes to be inferred. Selected biological characteristics for each *Cyclothone* species are shown in Table 2.

In the following accounts, upper, mid- and lower mesopelagic, and bathypelagic refer to those overlapping layers approximately 300–500 m, 400–700 m, 500–1000 m and 800–2000 m, respectively, which correspond to the observed depth ranges of shallow (*C. alba, C. signata* and *C. braueri*), mid-layer (*C. pseudopallida* and *C. kobayashii*), deeper (*C. atraria,*

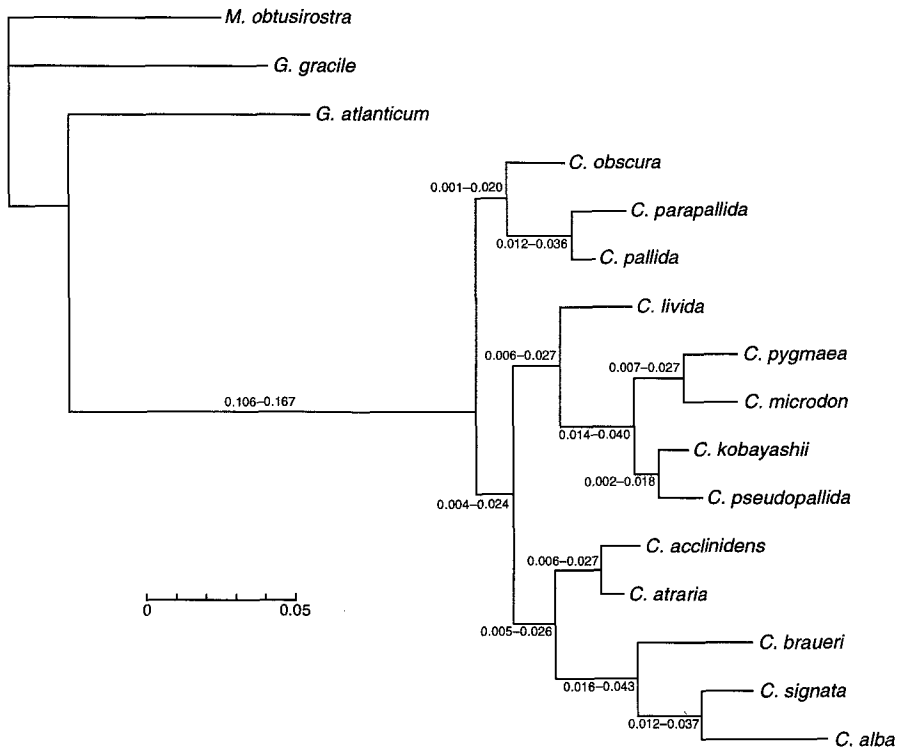

Fig. 4. Best maximum likelihood tree of *Cyclothone* and outgroup taxa (Ln=−4329.02). The tree topology was fully congruent with that obtained by MP analyses. Confidence limits of internal branch lengths are indicated along branches. All branch lengths were significantly different from zero ($p<0.01$). Scale indicates expected nucleotide substitutions per site.

C. acclinidens, *C. microdon*, *C. pygmaea*, *C. livida* and *C. pallida*) and deepest dwellers (*C. parapallida* and *C. obscura*) (Miya and Nemoto, 1991; Miya, unpubl. data). The actual depths of these arbitrarily defined layers, however, vary slightly with locality (e.g., Maynard, 1982).

It should be noted that one of the most remarkable results from the present phylogenetic analyses, as shown in the maximum likelihood tree (Fig. 4), was the exceptionally long branch length of the common ancestor of *Cyclothone* (0.136; confidence interval, 0.106–0.167) compared to those of other internal and terminal branches (range, 0.008–0.047). Furthermore, numerous, unambiguous nucleotide substitutions (56) had occurred along this lineage (Fig. 3), in addition to observed large percentage sequence differences between the outgroups and the ingroup (mean, 19.1%; range, 17.1–21.3%; Table 1). If the present outgroup taxa (*Gonostoma atlanticum* and *G. gracile*, both included in the ingroup for the analyses) were correctly chosen, these facts together suggest that the common ancestor of *Cyclothone* had undergone remarkable anagenetic evolution, which might have led to *Cyclothone* being distinct from other midwater deep-sea fishes in numerical abundance, morphology and ecology.

The early evolutionary history of *Cyclothone* is characterized by the divergence into two major basal lineages (Fig. 3), rather than by "ladder-like" evolution with single species' derivations at each node. One of the former, Clade B, comprises three species (*C. obscura*, *C. parapallida* and *C. pallida*; Fig. 5), each attaining 65–75 mm SL, the largest size among *Cyclothone* (Table 2). The first speciation event of the clade involved the derivation of *C. obscura*, a species of circumequatorial distribution and probably the deepest dweller among *Cyclothone* (>1000 m; Kobayashi, 1973; Mukhacheva, 1974; Miya, unpubl. data). It has acquired a completely black

Table 2. Comparisons of selected biological characteristics of 13 species of *Cyclothone*

Species	Dioecious/ Protandrous	Size at maturity (female, mm SL)	Maximum size (female, mm SL)	Age at maturity (female, year)	Semelparous/ iteroparous	Highest fecundity	Egg size at hydration (mm)
C. obscura[1]	dioecious	45	70	—	iteroparous?	—	—
C. parapallida[2]	dioecious	50	75	—	iteroparous?	—	—
C. pallida[3]	dioecious	45	70	≥5	iteroparous	3000	0.5
C. livida[4]	dioecious	—	50	—	iteroparous?	—	—
C. pygmaea[5]	dioecious	20	30	1–2	semelparous	300	—
C. microdon[6]	protandrous	40	65	—	iteroparous	4500	—
C. kobayashii[7]	dioecious	30	50	—	iteroparous	1000	>0.4
C. pseudopallida[8]	dioecious	30	50	3	iteroparous	1500	0.5
C. acclinidens[9]	dioecious	35	60	—	iteroparous	—	0.5
C. atraria[10]	protandrous	40	60	≥4	iteroparous	3000	0.5
C. braueri[11]	dioecious	20	35	2	semelparous	1000	0.5
C. signata[12]	dioecious	20	35	2	semelparous	500	0.5
C. alba[13]	dioecious	20	30	2	semelparous	500	0.5

Source of data: [1]Badcock (1984), Miya, unpubl. data; [2]Badcock (1982), Miya, unpubl. data; [3]Miya and Nemoto (1987); [4]Badcock (1984); [5]Jespersen and Tåning (1926), Badcock (1984), Miya, unpubl. data; [6]Badcock and Merrett (1976), McKelvie (1989); [7]Miya (1994); [8]Miya and Nemoto (1991); [9]Badcock (1984), Bailey and Robison (1986), Miya and Nemoto (1987); [10]Miya and Nemoto (1991); [11]Badcock and Merrett (1976), McKelvie (1989); [12]Aughtry (1953), Bailey and Robison (1986); [13]Miya and Nemoto (1991).

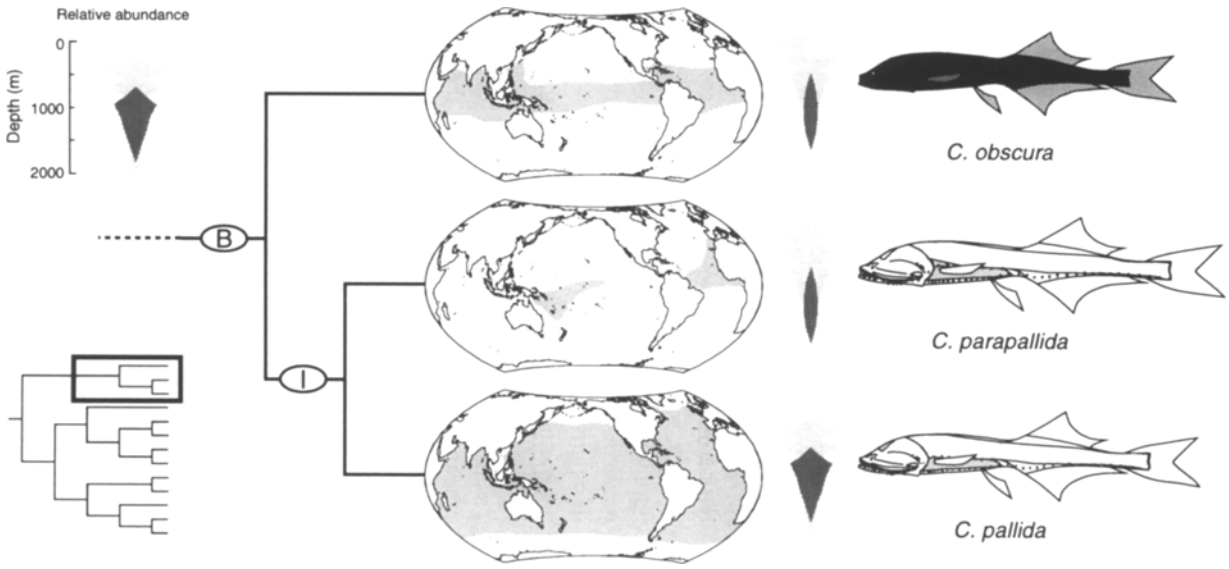

Fig. 5. Relationships between phylogeny and distribution patterns of Clade B (*Cyclothone obscura, C. parapallida* and *C. pallida*). Shaded portions of maps represent distributions based on literature (Mukhacheva, 1974; Badcock, 1982; Miya, 1994b) and unpublished records (Miya, unpubl. data). Vertical profiles of relative abundance indicated between maps and fish illustrations. Fish sizes proportional to largest size recorded.

body with conspicuous scales, having lost all photophores, and reduced its eye size (3–5% in head length vs >6% in other species; Kobayashi, 1973).

Clade B subsequently bifurcated into *C. pallida* and *C. parapallida* (Clade I), darkly-pigmented but semitransparent species of *Cyclothone* (Fig. 5). As the specific name implies, *C. parapallida* had been a cryptic species, included in *C. pallida* until Badcock (1982) separated it from the latter on the basis of subtle differences in meningeal and body pigmentation. Although the occurrence of *C. parapallida* is restricted to Atlantic and western Pacific equatorial waters (Badcock, 1982; Miya, 1994b), *C. pallida* exhibits a circumglobal, tropical to subtropical distribution, which thoroughly overlaps that of the former (Fig. 5). Depth segregation, however, has been achieved between the two species, with *C. pallida* and *C. parapallida* occupying shallower (500–1000 m) and deeper layers (900–1500 m), respectively (Badcock, 1982; Miya, unpubl. data). Despite such a microallopatric distribution (i.e., two species found in the same geographic range but in different habitats), at least 20 nucleotide substitutions (sequence divergence, 2.5%) have occurred after the lineage split. Another noteworthy evolutionary event within the lineage (Clade I) is that, despite their deeper habitats, both species have tended to lose body pigmentation, especially the virtually transparent *C. parapallida*, which has dark, dispersed pigments restricted to the lateral surface of the body. Therefore the deeper/darker trend is not always the case for *Cyclothone*.

Another basal lineage, Clade C, subsequently diverged into two major lineages (Clades D and E), each containing five, seemingly heterogeneous species of various size, body coloration and ecology (Fig. 3). In one of the two lineages, Clade D, the first speciation event involved the derivation of *C. livida*, a black species endemic to the tropical eastern Atlantic (Fig. 6). This species is unique in being the only pseudoceanic species among *Cyclothone*, showing a land mass (or Mauritanian upwelling) affinity along the western coast of North Africa (Badcock, 1982, 1984).

Clade F, comprising four heterogeneous species in terms of body coloration and ecology,

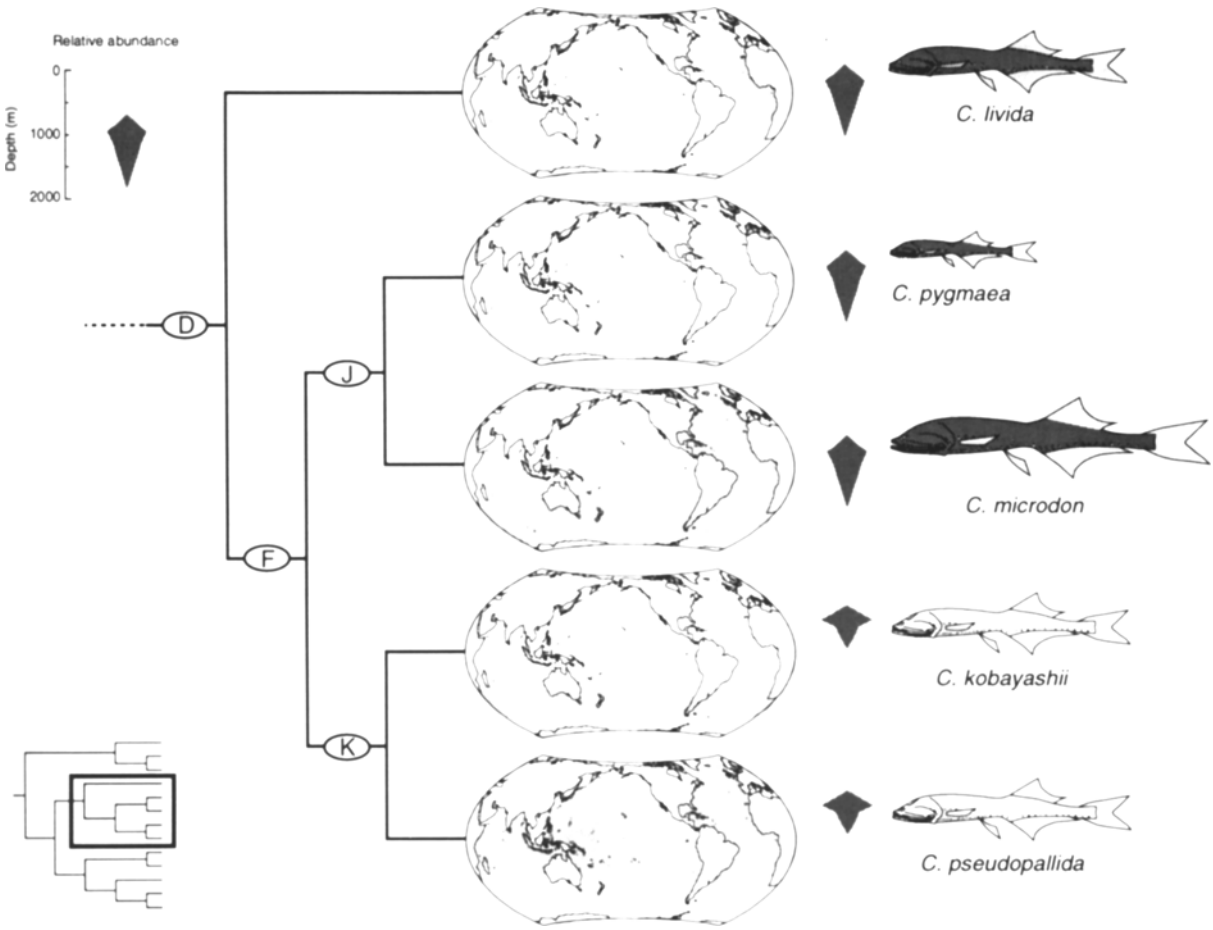

Fig. 6. Relationships between phylogeny and distribution patterns of Clade D (*Cyclothone livida*, *C. pygmaea*, *C. microdon*, *C. kobayashii* and *C. pseudopallida*). Shaded portions of maps represent distributions based on literature (Mukhacheva, 1974; Miya, 1994a) and unpublished records (Hartel, pers. comm.; Miya, unpubl. data). Vertical profiles of relative abundance indicated between maps and fish illustrations. Fish sizes proportional to largest size recorded.

subsequently bifurcated into Clades J and K (Fig. 3). The former includes two black species, the protandrous *C. microdon*, a species with Atlantic and Southern Ocean distribution, and the dwarf *C. pygmaea*, endemic to the Mediterranean (Fig. 6). The latter had long been considered a geographic variant [*C. microdon pygmaea* (Jespersen & Tåning, 1926)], until Mukhacheva (1974) elevated it to species' level on the basis of small meristic differences. The present sequence data supported the action of Mukhacheva (1974), since at least 27 nucleotide substitutions have occurred in the two gene segments after the lineage split. Autapomorphies include protandry in *C. microdon* and features associated with dwarfism in *C. pygmaea*. Female *C. pygmaea* attains sexual maturity at about 20 mm SL, having only 200–300 eggs in its ovary pair (Jespersen and Tåning, 1926). The largest female recorded was only 27 mm SL, the smallest size among *Cyclothone* (Jespersen and Tåning, 1926). The emergence of dwarfism in *C. pygmaea* can be ascribed to the unique environment in the Mediterranean lower mesopelagic depths, because its upper mesopelagic counterpart, the Mediterranean *C. braueri*, is itself a little smaller than conspecific individuals found elsewhere (maximum size, 30 mm vs. 38 mm SL; Jespersen and Tåning, 1926; Badcock, 1984), although *C. braueri* is not so dwarfed nor genetically diver-

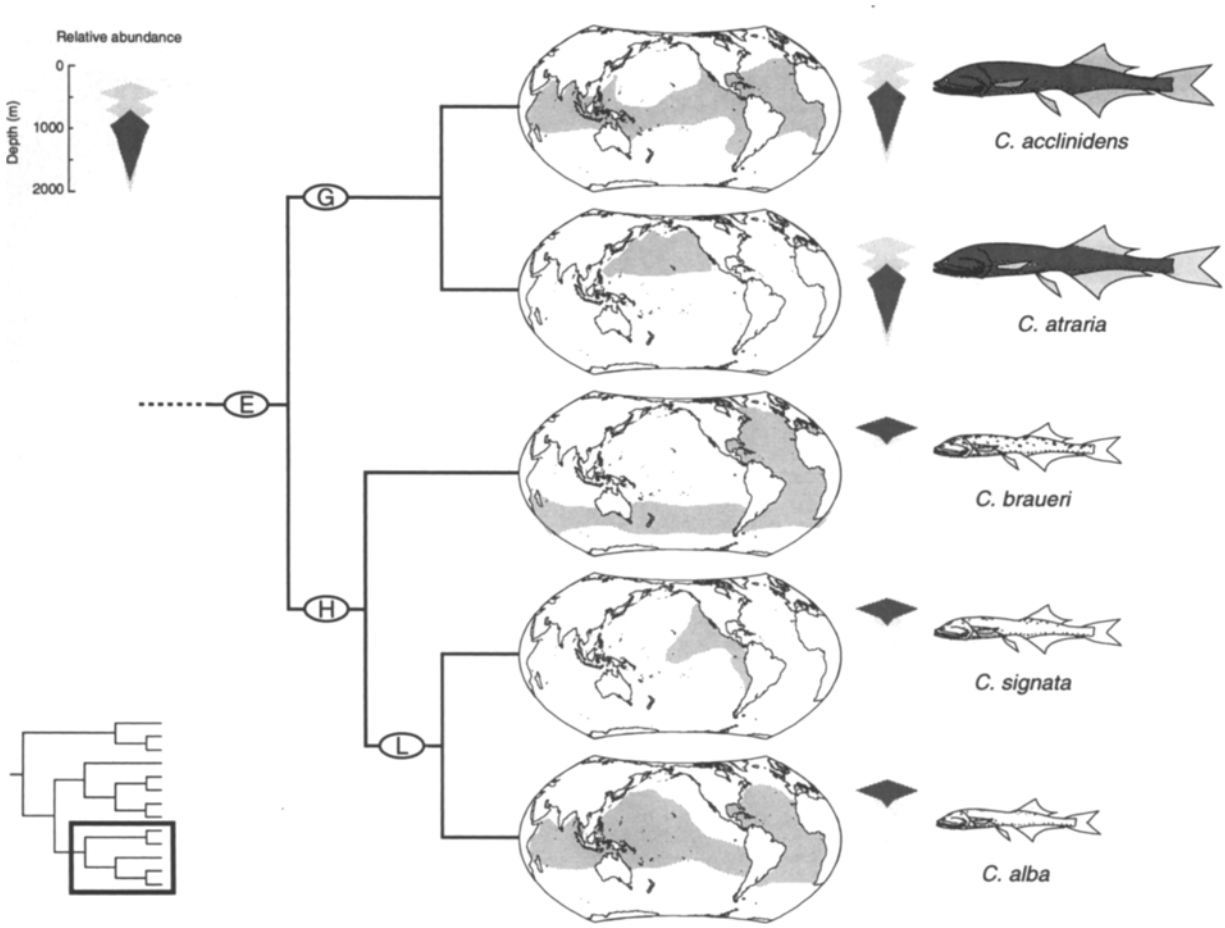

Fig. 7. Relationships between phylogeny and distribution patterns of Clade E (*Cyclothone acclinidens*, *C. atraria*, *C. braueri*, *C. signata* and *C. alba*). Shaded portions of maps represent distributions based on literature (Mukhacheva, 1974; Miya, 1994a) and unpublished records (Miya, unpubl. data). Vertical profiles of relative abundance indicated between maps and fish illustrations. Fish sizes proportional to largest size recorded.

gent so as to warrant separate species' recognition (see Results).

Clade K comprises two transparent species, *C. pseudopallida* and *C. kobayashii*, the latter having been a cryptic species until Kobayashi (1973) distinguished between the two in his unpublished thesis. *C. kobayashii* was subsequently formally described by Miya (1994a). The common ancestor of the two species have acquired many morphological and ecological novelties, including the dorsal half of the body pigmented (ventral half transparent), distinct internal sagittal pigmentation, mid-mesopelagic habitat and all reproductive variables falling between those of upper and lower mesopelagic species (Miya and Nemoto, 1991). The former two characteristics can be regarded as progenetic, as suggested by Marshall (1984). The speciation mode between the two species appears to have been allopatry, since they are presently geographically separated except for a distribution overlap along 30°S (Miya, 1994a; Fig. 6).

The third major lineage, Clade E, also comprising five, heterogeneous species, subsequently bifurcated into Clades G and H. Clade G further speciated into two large, black species, the widespread tropical-subtropical *C. acclinidens* and the protandrous subtropical-temperate *C. atraria*, endemic to the North Pacific (Fig. 7). *C. acclinidens* has acquired many apomorphies, including unique maxillary tooth morphology, a distinct supracaudal gland and well-developed

gill filaments, apparently associated with its affinity to areas of low dissolved oxygen concentration (Kobayashi, 1973). The evolution of protandry in *C. atraria* appears to have been independent of that in *C. microdon* (Clade J). Both *C. acclinidens* and *C. atraria* occupy lower mesopelagic depths, where their geographically disjunct distributions (Fig. 7) suggest allopatric speciation.

In contrast to the Clade G species, remarkable progenetic tendencies (sensu Gould, 1977) have occurred in those in Clade H, including the acquisitions of upper mesopelagic habitats worldwide (Fig. 7) and many progenesis-associated life-history characteristics, such as small size at maturity, early age at first reproduction, semelparity and low fecundity (Table 2; Miya and Nemoto, 1991). Although the Clade H species have lost most body pigmentation, with the exception of a few punctate/stellate melanophores, distinct pigmentation has developed along the internal sagittal section of the body. The first speciation event involved the derivation of *C. braueri*, a species of subtropical-temperate Atlantic and Southern Ocean distribution (Fig. 7), similar to that of *C. microdon* (Fig. 6). The sister group of *C. braueri* (Clade L) speciated into the widespread tropical-subtropical *C. alba* and the eastern Pacific endemic *C. signata*, the latter inhabiting regions of well-developed, low-dissolved-oxygen concentration layers. The low number of metameric characters in these two species, such as gill raker counts, number of vertebrae, and dorsal and anal fin ray counts (Kobayashi, 1973) can be caused by progenesis, because the latter is known to constrain the differentiation of metameric characters (Alberch and Gale, 1983, 1985). The speciation mode between the two species appears to have been allopatry because of their largely separate distribution ranges (Fig. 6).

Discussion

Phylogeny of *Cyclothone*

Phylogenetic analyses at species' level in fishes often involve great difficulties in detecting variable osteological (or qualitative) characters that have been used in conventional fish systematics (e.g., see Boughton et al., 1991). Species of *Cyclothone* are no exception. Like many other fishes, they exhibit very similar body form, being diagnosed by an array of subtle and statistically-treated characters (e.g., Miya, 1994a) and characterized by developmentally abbreviated, simple organization of anatomical structures, with marked reduction in skeletal elements (Marshall, 1984). Some authors, however, have attempted to analyze species' relationships using meristic (or other quantitative) characters, simply because to reject such characters as "bad data" is to reject phylogenetic analyses at low taxonomic levels (e.g., Boughton et al., 1991). Even so, meristic characters associated with myomere number are known to be environmentally influenced during development (Lindsey and Arnason, 1981), and the use of such quantitative data in phylogenetic analyses remains controversial (Pimentel and Riggins, 1987), as do the gap coding procedures that must be adopted when using quantitative data (Chappill, 1989). Furthermore, progenesis, which has undoubtedly played an important role in *Cyclothone* evolution, is known to constrain the differentiation of metameric characters (Alberch and Gale, 1983, 1985), thus seriously violating character independence, a prerequisite for phylogenetic analyses.

Nucleotide sequence data from mitochondrial DNA, on the other hand, are a potentially large source of phylogenetic characters that are independent of morphology, including problematic meristic characters, and the presumed evolutionary processes. The robustness of the present tree as exemplified by the generally high bootstrap values and decay indices for the internal branches, the length of which are significantly different from zero ($p<0.01$), congruence between MP and ML trees and the existence of a significant phylogenetic information content ($p<0.01$), as evidenced by g_1 statistics even for the deeper branches, all evidently corroborate the utility of the nucleotide sequence data from the 12S and 16S rRNA genes in the phylogenetic analyses of *Cyclothone*.

Transition/transversion weightings are com-

mon practices in current molecular phylogenetic systematics. An implicit assumption of the weighting is that transversion substitutions are generally more informative than transition substitutions, since transitions can accumulate more rapidly than transversions (Mindell, 1991). This does not seem to be the case for the present data set because unambiguous synapomorphies supporting *Cyclothone* monophyly, which are clearly validated by the present molecular data as well as by morphology (Grey, 1964; Weitzman, 1974; Ahlstrom et al., 1984), consist of 25 transitions and 13 transversions, the ratio (1.92) merely reflecting the observed average ratio (2.02) calculated from all pairwise sequence comparisons among the ingroup+outgroup. This supports Allard and Miyamoto (1992) and Titus and Larson (1995), who argued that differential weighting of transversions over transitions downweights informative transitions in conservative regions of rDNA, while giving higher weight to transversions in more variable regions of the molecule.

As stated above, the monophyly of *Cyclothone* was best supported by various indices, including bootstrap value (100%), decay index (>5) and numerous, unambiguous synapomorphies (38), while some basal clades (C, D and E) were less so, shown by relatively low bootstrap values (53, 70 and 69%, respectively) (Fig. 3). However, decay indices, unambiguous changes and synapomorphies for these less supported branches were not so small (4/8/2 for Clade C, 3/6/3 for Clade D and 4/8/1 for Clade E, respectively), indicating that the tree topology was sufficiently robust as to be reasonable for utilization as a template for the depiction of *Cyclothone* evolutionary history. Except for the above three basal clades, all clades were supported by high bootstrap values (≥93%) and decay indices (≥4).

Cyclothone taxonomy is now accepted with reasonable confidence, owing to the revisionary studies on a regional basis for the Pacific by Mukhacheva (1964) and Kobayashi (1973), the North Atlantic by Badcock (1982), the Southern Ocean by Miya (1994a), and on a world-wide basis by Mukhacheva (1974). However, there has been no explicit phylogenetic hypothesis published, with the exception of the three "natural groups" proposed for the Pacific *Cyclothone* by Mukhacheva (1964, 1967). In her review of Pacific *Cyclothone* systematics, Mukhacheva (1964) explicitly stated that the nine Pacific *Cyclothone* species formed three natural groups: 1) the "*signata*" group (comprising *C. alba* and *C. signata*); 2) the "*pallida*" group (*C. braueri*, *C. pseudopallida* [including *C. kobayashii* subsequently described by Miya, 1994a] and *C. pallida* [including *C. parapallida*, subsequently described by Badcock, 1982]); and 3) the "*microdon*" group (*C. microdon*, *C. pacifica* [=*C. atraria*, synonymized by Mukhacheva, 1967], *C. acclinidens* and *C. obscura*). Mukhacheva (1964) further stated that these groups were well differentiated from one another by a number of characteristic features, although she gave no substantiating evidence. Because Mukhacheva (1974) subsequently recognized the close affinities of the remaining two Atlantic species (*C. livida* and *C. pygmaea*) to *C. microdon*, it is reasonable to consider that the former two species would have been included in her "*microdon*" group.

Considering their body coloration and some morphological and ecological characteristics, Mukhacheva's groupings are not counterintuitive, because two small, extremely transparent species are grouped together, in addition to the remaining species being divided into darkly-pigmented "semi-transparent" and "black" species of various sizes. It appears, however, that her "natural groups" do not reflect the common ancestry relationships inferred here from molecular data, except for the "*signata*" group (Fig. 8). Five species of the "*pallida*" group are represented by three major clades (Clades B, D and E), the common ancestor of which is the common ancestor of *Cyclothone* itself. Similarly, six species of the "*microdon*" group are represented by the same set of clades as the "*pallida*" group. When monophyly of Mukhacheva's (1964) "natural groups" was enforced using the constraint option in PAUP, one minimum length tree that required an additional 60 steps was found, the difference being highly significant ($z=-6.169$, $p<0.0001$). Thus, the analysis of distribution of character state changes per site strongly indi-

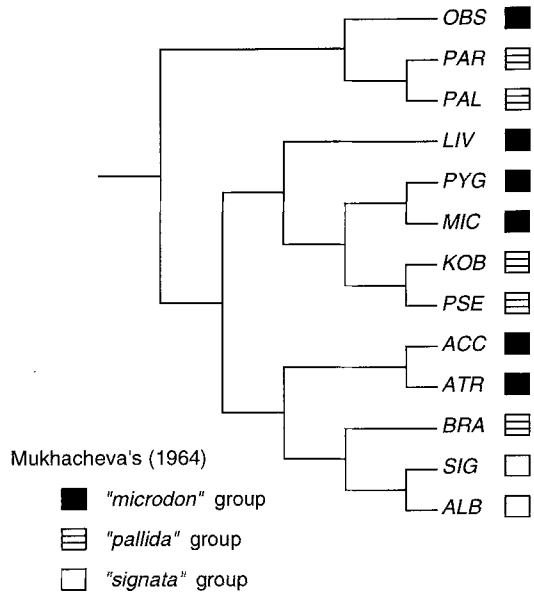

Fig. 8. Relationships between the present molecular phylogeny of *Cyclothone* and Mukhacheva's (1964) three "natural groups." Specific names abbreviated to first three letters.

cated that two of the three of Mukhacheva's (1964) "natural groups" were unnatural in that they did not share the most recent common ancestors.

Finally, it should be noted that the two species of *Gonostoma* were paraphyletic in both the MP and ML trees. When their monophyly was enforced, the most parsimonious tree required an additional three steps. Although there was no significant difference between the constrained tree and the most parsimonious tree without constraints ($z=-0.574$, $p=0.566$), paraphyly of *Gonostoma* is likely since there appear to be no uniquely derived characters diagnosable for the entire genus. Other phylogenetic studies of gonostomatids, including all species of *Gonostoma*, have shown the genus to be paraphyletic (Harold, in press; Harold and Weitzman, in press).

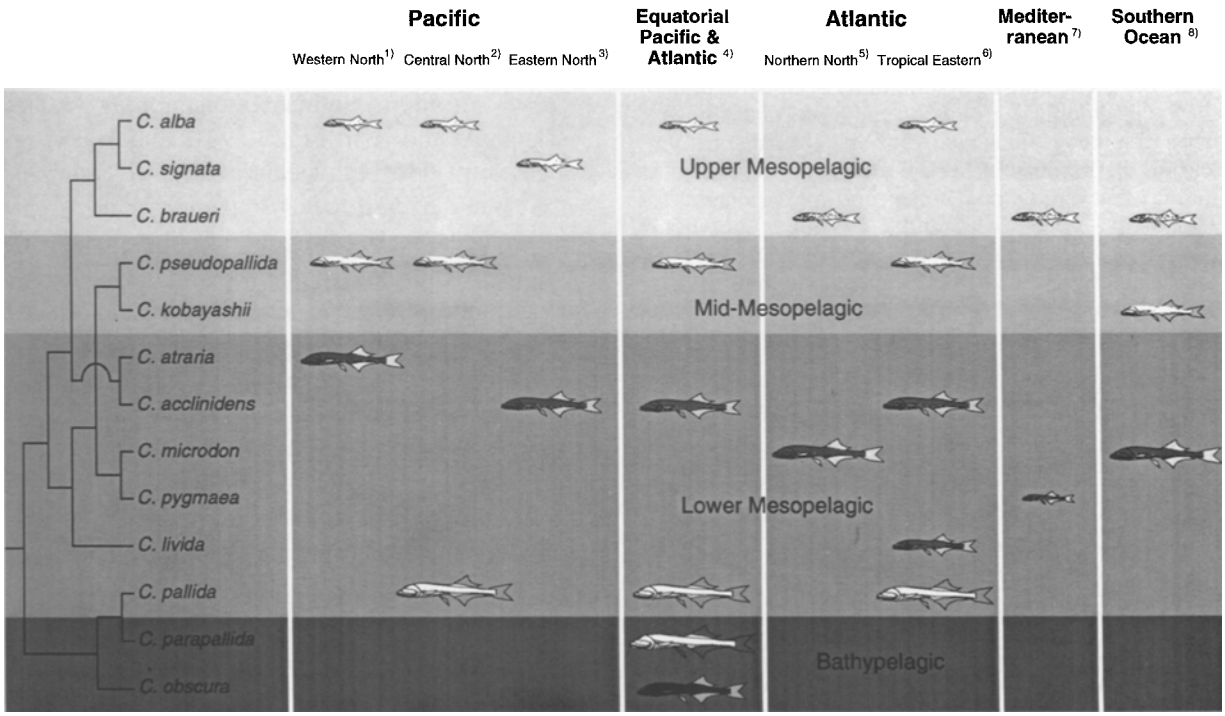

Fig. 9. Combinations of co-dominant species in selected localities from the Pacific, Atlantic and Southern oceans and the Mediterranean Sea. No Indian Ocean data available. Vertical sequences of fishes within each depth category do not infer actual depth stratification. Fish sizes proportional to largest size recorded. Sources of information: [1)]Miya and Nemoto (1991); [2)]Maynard (1982); [3)]DeWitt (1972); [4)]Badcock (1982), Miya, unpubl. data; [5)]Badcock and Merrett (1976); [6)]Badcock and Merrett (1977); [7)]Goodyear et al. (1972); [8)]Miya (1994a).

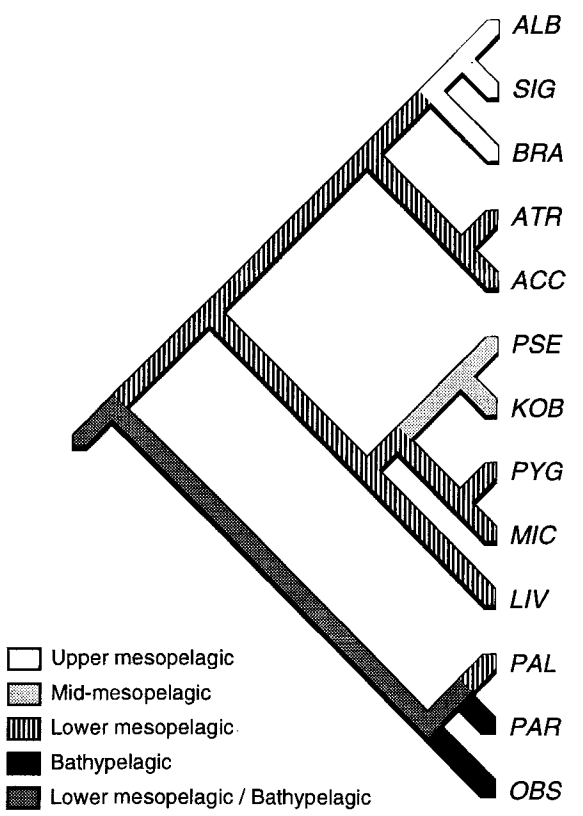

Fig. 10. The most parsimonious reconstruction of the four ecological groups on the molecular phylogenetic tree using MacClade ver. 3.02 (Maddison, W. P. and D. R. Maddison, 1992). Outgroup taxa used in the present study are upper–mid mesopelagic for *Margrethia obtusirostra* and *Gonostoma atlanticum* (Badcock, 1984) and lower mesopelagic for *G. gracile* (Kawaguchi, 1973). Specific names of *Cyclothone* abbreviated to first three letters.

Evolution of the *Cyclothone* community structure

It is has been demonstrated that different combinations of two to five co-dominant species of *Cyclothone* (these constituting >90% of the overall *Cyclothone* populations) are segregated by depth in different localities (Fig. 9; see Miya and Nemoto, 1991). The number of co-dominant species reaches a maximum around equatorial waters, and a minimum at higher latitudes (Fig. 9). Most parsimonious reconstruction of the four ecological groups, on the basis of depth range, onto the molecular phylogeny (Fig. 10) revealed the independent acquisition of relatively shallow habitats (upper and mid-mesopelagic) by two monophyletic groups (*C. alba*+*C. signata*+*C. braueri* and *C. kobayashii*+*C. pseudopallida*) from two major basal clades (Fig. 10). Although there remained ambiguity on the ancestral habitat of Clade B in the most parsimonious reconstruction using MacClade, the DELTRAN optimization provided a more likely evolutionary scenario (independent acquisitions of bathypelagic habitats by *C. obscura* and *C. parapallida*), because the latter two species have no discernible symplesiomorphies. Nevertheless, it appears that the lower mesopelagic habitat is a symplesiomorphic feature for *Cyclothone* (Fig. 10), being occupied by all representative species from the major basal clades. Therefore, *Cyclothone* communities in different localities are polyphyletic assemblages, with the exception of a portion of the equatorial community (Clade B).

Evolution of biological traits

Polyphyly of the lower mesopelagic species. — Two ecological groups (upper mesopelagic and mid-mesopelagic) appear to be monophyletic because of their higher bootstrapping values (Clades L and K in Fig. 3; 94% and 100%, respectively). The bathypelagic group (*C. parapallida* and *C. obscura*) is a paraphyletic assemblage, because one member of their clade, *C. pallida*, is not bathypelagic (Clade B in Fig. 3; bootstrap value, 97%). On this basis, the lower mesopelagic group is a polyphyletic assemblage. When topological constraints enforced the monophyly of the latter group, the resulting most parsimonious tree required 51 additional steps compared with the unconstrained MP tree, a highly significant difference ($z=-5.875$, $p>0.0001$). Therefore the lower mesopelagic group, rather than being monophyletic, is probably a polyphyletic assemblage represented by members of three distinct clades.

Parallel evolution of protandry. — Protandry in *Cyclothone* has been confirmed for the two lower mesopelagic species, *C. microdon* and *C. atraria* (Badcock and Merrett, 1976; Miya and Nemoto, 1985), whereas all other species are dioecious (Maynard, 1982; Badcock, 1984; Miya and Nemoto, 1986a, b, 1987). The two protan-

drous species exhibit disjunct distributions, with the former being distributed in the Atlantic and Southern Ocean (Fig. 6) and the latter being endemic to the western North Pacific (Fig. 7). Although they are genealogically distant (Fig. 3), they are so similar in external appearance and ecology that Mukhacheva (1964) placed them together in the "*microdon*" group. A single origin of protandry (monophyly of *C. microdon* and *C. atraria*), however, could be confidently rejected ($z=-5.648$, $p<0.0001$), since it required an additional 44 steps compared with the unconstrained most parsimonious tree. Since the two species are the only subtropical-temperate representatives among the six lower mesopelagic species, protandry has adaptive significance, such as boosting fecundity (Miya and Nemoto, 1985), in such environmental settings. It should be noted that protandry has been confirmed for three of the seven species of *Gonostoma* (*G. gracile*, *G. elongatum* and *G. bathyphilum*) (Kawaguchi and Marumo, 1967; Fisher, 1983; Badcock, 1986), a presumed sister group of *Cyclothone*. Also, among deep-sea fishes, protandry is known only within the family Gonostomatidae. Given that some of the species in the sister group to *Cyclothone* are protandrous, the independent expression of protandry in two derived species of *Cyclothone* suggests that this trait is labile within *Cyclothone* and its expression is favored under certain environmental conditions.

Independent loss of pigmentation. — A remarkable loss of pigmentation has occurred in seven species, representing three different clades (Clades I, K and H; see Fig. 3), although the degree of loss varies greatly. Mukhacheva (1964) classified the seven species into either the "*signata*" or "*pallida*" groups. When monophyly of the seven species was enforced, a single minimum length tree was found, which required an additional 37 steps compared with the unconstrained most parsimonious tree, a highly significant difference ($z=-4.858$, $p<0.0001$). Therefore, loss of pigmentation has occurred independently and the transparent body should not be regarded as a character implying common ancestry relationships.

Multiple origins of progenetic tendencies. — Although degrees of progenetic tendencies differ between species in mid-mesopelagic Clade K (*C. kobayashii* and *C. pseudopallida*) and upper mesopelagic Clade H (*C. braueri*, *C. signata* and *C. alba*) (see Fig. 3), they have numerous larval features (Marshall, 1984) that their sister groups (Clades J and G, respectively) do not possess. When their monophyly was enforced, a single minimum length tree was found, which required an additional 25 steps compared with the unconstrained most parsimonious tree, a highly significant difference ($z=-3.797$, $p=0.0001$). Thus, progenetic tendencies in these two clades have multiple origins, close correlations between such tendencies and precocious reproductive traits (Marshall, 1984; Miya and Nemoto, 1991) suggesting an ecological significance of progenesis (increasing intrinsic rate of natural increase [r]) as predicted by Gould (1977).

Sequence variation and the age of *Cyclothone* radiation

Sequence variations in fish mitochondrial 12S and 16S rRNA genes have been reported for two higher taxonomic categories, antarctic fishes of the suborder Notothenioidei (Bargelloni et al., 1994) and South American electric fishes of the order Gymnotiformes (Alves-Gomes et al., 1995). In an attempt to establish a notothenioid molecular phylogenetic hypothesis, Bargelloni et al. (1994) determined sequences from the two genes (total, 928 bp) for 18 species, representing 15 genera and five families of the suborder. With the exception of the Bovichthyidae, the average interfamilial percentage sequence differences ranged from 3.7 to 5.3% (15.2% in Bovichthyidae) (mean, 4.5%), while those at the intergeneric level ranged from 0.5 to 3.3% with a mean of 1.9% (all calculated from table 2 in Bargelloni et al., 1994). Similar interfamilial percentage sequence differences, for the same gene segments (total, 718 bp), were also reported from seven families of South American electric fishes in the order Gymnotiformes (Alves-Gomes et al., 1995). Although they did not present a matrix of the percentage differences, interfamilial values ranged approximately from 5 to 18%, judging from their figure (fig. 4; ratio of

transition/transversion as a function of percentage divergence) and statements in the text (Alves-Gomes et al., 1995: 304). Interspecific percentage sequence differences for *Cyclothone* were remarkably high when compared to the above interfamilial values, ranging from 1.9% between *C. acclinidens* and *C. atraria* to 12.2% between *C. alba* and *C. parapallida* (Table 1), with a mean of 8.0%, which exceed the interfamilial values of the antarctic notothenioid fishes and are comparable to those of the South American electric fishes.

It has been recently demonstrated that the rate of mtDNA evolution in sharks is slower than in mammals, presumably owing to slower ectotherm metabolic rates (Martin et al., 1992). A typical ectotherm divergence rate for mtDNA evolution ranges from 0.3 to 0.7%/million years (Myr) (Martin and Palumbi, 1993), which is considerably slower than previous estimations for terrestrial animals (1–2%/Myr; DeSalle, 1987; Moritz et al., 1987). Fishes of the genus *Cyclothone* inhabit cold temperatures (ca. 3–10°C; Miya and Nemoto, 1991) and are known to have lethargic life styles, remaining motionless for most of the time (Barham, 1971). Their metabolic rates are so slow that three individuals of the lower mesopelagic species, *C. acclinidens*, were estimated as being able to survive for up to 70 days without food on the basis of *in situ* measurements of oxygen consumption at a depth of 1300 m and energy stored as neutral lipid and glycogen (Smith and Laver, 1981). If the rate of mtDNA evolution in *Cyclothone* is taken as comparable to that of other ectotherms, the age estimate for the *Cyclothone* radiation on the basis of the largest interspecific sequence difference (12.2%; Table 1) would be 17–40 Myr. This age seems more reasonable than that estimated using a more rapid divergence rate (1–2%; 6–12 Myr old), because the oldest fossils of *Cyclothone*, which closely resemble modern species, are abundant in California Lower Miocene shales (20 Myr ago; David, 1943) as well as in Morozaki Middle Miocene Group, central Japan (17 Myr ago; Ohe, 1993). Mediterranean geological history, coupled with information on sequence divergence of the Mediterranean endemic, *C. pygmaea* (3.4%; Table 1), provides further evidence for determining the age of the *Cyclothone* radiation. If the ectotherm divergence rate is assumed for *C. pygmaea*, the estimated age of the split between *C. microdon* and *C. pygmaea* would be 5–11 Myr. The minimum age (5 Myr on the basis of 0.7%/Myr) is most likely because the Mediterranean Sea was desiccated 5.5 Myr ago (Hsü et al., 1977; Cita, 1982; Aharon et al., 1993). The divergence rates of 12S and 16S mitochondrial rRNA genes do not level off until about 100 Myr and are virtually constant over several tens of millions of years (see fig. 2A, B in Mindell and Honeycutt, 1990). If so, on the basis of a divergence rate of 0.7%/Myr, the *Cyclothone* radiation had already started in early–middle Miocene (17–20 Myr ago) and thus has a history comparable to that of antarctic notothenioid familial radiation (10–15 Myr old; Bargelloni et al., 1994), which was based on the ectotherm divergence rate because of the subzero temperature adaptations of the latter.

Concluding Remarks

The most notable characteristic of the evolutionary history of *Cyclothone* was early divergence into three major lineages (Clades B, D and E in Fig. 3) and their subsequent, independent acquisitions of the three different, apomorphic depth habitats (upper mesopelagic, mid-mesopelagic and bathypelagic) from the relatively ancestral lower mesopelagic depths (Figs. 9 and 10). Moreover, it should be noted that such macroevolutionary habitat shifts had been necessarily accompanied by morphological and ecological novelties, as seen in numerous larval-like features (see Marshall, 1984) and various life-history traits (see Miya and Nemoto, 1991). It appeared that such novelties had resulted from paedomorphic changes, degrees of which correspond to their depth ranges. Repeated evolution of such paedomorphic novelties strongly suggested ontogenetic plasticity in *Cyclothone* which could enable these fishes to acquire larval-like, simple organization of body structure. Such a body plan could help them subsist in food-poor surroundings (Marshall, 1984) and

regulate reproductive variables that take advantage of increasing larval survival toward shallower depths (Miya and Nemoto, 1991). Although it is uncertain if these major ancestral speciation events have been concurrent or sporadic, local or universal, their descendants have pervaded worldwide and attained the present multi-layer assemblages (Fig. 9). On the other hand, recent speciation events have produced contemporary sister species of allopatric (or microallopatric) distributions, but few morphological and ecological differences. Even if remarkable miniaturization has occurred, such as in the Mediterranean endemic *C. pygmaea*, its close resemblance to sister species' subadults strongly indicates that *C. pygmaea* has evolved through a simple truncation of ancestral species' ontogeny with no attendance of paedomorphic features.

In order to calibrate the molecular clock along various lineages and to identify factors responsible for their biological diversification and evolution of adaptations, more paleooceanographic information is required in addition to knowledge of these macroevolutionary sequences in biological traits from the molecular phylogeny.

Acknowledgments. — This study could not have been possible without the generous donations of tissue materials, for which we sincerely thank the following colleagues: J. E. Craddock, R. Fukai, K. E. Hartel, J. Mauchline, N. R. Merrett, M. P. Olivar, S. Ohtsuka, L. O. Relini, J. Rivaton and H. J. Walker, Jr. Also, the DNA experiments would not have been completed within a short period of time without the kind guidance and support from the graduate and undergraduate students of the junior author's laboratory to MM; above all, the contributions from M. Yamaguchi, A. Kawaguchi, T. Ohkawa, K. Iguchi, H. Morita and J. Kitaura were greatly appreciated. This paper greatly benefited from critical comments and suggestions on an early draft manuscript by A. S. Harold, A. P. Martin, A. M. Shedlock, N. Minaka and N. R. Merrett. We thank K. Kawaguchi for his generous organization of the sampling program during the KH-94-4 cruise of the R/V *Hakuho Maru*, University of Tokyo. Thanks are also due to the captain, officers, crew, scientists and students on board the cruise for their assistance in collecting samples. N. Murakami, N. Minaka, K. Mochizuki, Y. Yabumoto and H. Katoh assisted in providing relevant literature. MM also deeply thanks N. V. Parin and other staff of his laboratory for their kind cooperation, enabling the formers' examination of *Cyclothone* specimens that V. A. Mukhacheva examined some 20 years ago. A portion of this study was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan (No. 07740668) to MM. This paper is dedicated to M. Okiyama and the late N. B. Marshall, whose deep insights into fish evolution have been a source of inspiration.

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