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# Evolutionary Relationships of Sibling Tapeworm Species (Cestoda) Parasitizing Teleost Fishes

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DNA/DNA hybridization and sequencing of rDNA (partial 18S rDNA and ITS1) were used to investigate phylogenetic relationships among seven host-specific *Bothriocephalus* parasites (Cestoda, Pseudophyllidae). The small nucleotide divergence between six of the seven bothriocephalids suggests that isolation and differentiation of *Bothriocephalus* lineages in the different host species probably occurred recently and over a short time span. Comparison of the molecular phylogeny of the parasite species to the phylogeny of their hosts (teleostean fishes) revealed little congruence between the branching patterns of hosts and parasites, suggesting that bothriocephalids have not cospeciated with their hosts.

## Introduction

Tapeworms of the genus *Bothriocephalus* (Cestoda, Pseudophyllidae) have been described as endoparasites in a large number of teleost fishes belonging to several major lineages of Pleuronectiformes, Scorpaeniformes, Gadiformes, and Anguilliformes (Protasova 1977). The first described species, *Bothriocephalus scorpii* (Mueller 1776), originally reported from the bull-rout (*Myoxocephalus scorpius*) was not considered to be host-specific (Jones 1975). Indeed, this parasite was also found in other host species, such as the turbot (*Psetta maxima*), the brill (*Scophthalmus rhombus*), and the five-bearded rockling (*Ciliata mustela*). However, studies carried out on the population genetic structure of this parasite have shown strict reproductive isolation between the parasite populations of each host species (Renaud, Gabrion, and Pasteur 1983, 1986; Renaud and Gabrion 1984). These analyses have revealed that *B. scorpii* constituted a complex of sibling biological species comprising a number of similar morphological species with host preference. Thus, whether *B. scorpii* is only parasitizing the bull-rout, *B. gregarius* is found in the turbot, *B. barbatus* is found in the brill, and *B. funiculus* is found in the five-bearded rockling. In addition, it is known that two other pleuronectiform fishes, the scaldfish (*Arnoglossus lateralis*) and the spotted flounder (*Citharus linguatula*), and one anguilliform, the eel (*Anguilla anguilla*), harbor three other *Bothriocephalus* species, *B. clavibothrium* (Ariola 1899), *B. andresi* (Porta 1911), and *B. claviceps* (Goeze 1872), respectively.

Thus, the high degree of biological specificity of the different parasite species among hosts belonging to so distantly related orders of teleost fishes (Lauder and Liem 1983) raises the question concerning the compared host-parasite phylogenesis in this heterospecific assemblage. We might ask if concomitant speciations (cospeciation) have occurred, leading to an evolutionary picture where the parasites' phylogeny mirrors the hosts'

phylogeny (Fahrenholz 1913). Several analyses of evolutionary processes have been conducted (Brooks and Glen 1982; Mitter and Brooks 1983; Bandoni and Brooks 1987; Deets 1987; Klassen and Beverley-Burton 1987; O'Connor 1988; Brooks and McLennan 1991), all of which involve the appraisal of branching similarities between host and parasite phylogenies and between their taxonomic boundaries or geographic distributions. However, relatively few studies have compared genetic divergence and speciation events in hosts and their parasites, permitting a discussion of the relative timing of speciation within and between host and parasite lineages (Baverstock, Adams, and Beveridge 1985; Hafner and Nadler 1988, 1990; Rannala 1992; Hafner et al. 1994). In the literature, the clearest evidence for cospeciation derives from isozyme electrophoretic data and from sequences of the mitochondrial cytochrome oxidase I gene in a pocket gopher and chewing louse assemblage (Hafner and Nadler 1988; Hafner et al. 1994). These data evidenced synchronous speciation events between sets of coevolving taxa.

The aim of this paper is to estimate the evolutionary relationships of seven species of teleostean parasites (*Bothriocephalus*) by using two complementary approaches. First, we will employ the nonrepeated DNA/DNA hybridization methodology which allows an estimation of the overall nucleotide divergence between the entire genomes of compared species (Werman, Springer, and Britten 1990). This is the first time that this approach has been applied to parasitic helminths to directly ascertain levels of interspecific genomic divergence. Second, we will use sequence data from nuclear rDNA (the first internal transcribed spacer, or ITS1, and partial 18S rDNA) to investigate the evolutionary relationships within bothriocephalids. Finally, we compare the phylogeny of these parasites with the evolutionary relationships of their teleostean hosts to elucidate biological and ecological factors that may have governed the evolution of this host-parasite association.

## Materials and Methods

### Specimens Examined

Pleuronectiform fishes were trawled in the Mediterranean Sea, along French coasts (Sete) for turbot, brills, and scaldfishes, and along Spanish coasts (Valen-

Key words: evolution, helminth, teleostean, DNA/DNA hybridization, 18S rDNA, ITS1.

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cia) for spotted flounders; scorpaeniforms (bull-rout) and gadiforms (five-bearded rockling) were obtained from the British Channel (Roscoff), and anguilliforms were caught by electrofishing in a river in southern France (Montpellier). After capture, fishes were anesthetized in the laboratory with 3-aminobenzoic acid ethyl ester (Sigma A-5040). Digestive tracts were dissected to collect tapeworms, which were rinsed several times in 0.9% NaCl.

## Methods

DNA was extracted from 5–20 individuals of each parasite species, following the procedures of Sambrook, Fritsch, and Maniatis (1989) and Verneau, Renaud, and Catzeflis (1991). Purified DNA was sheared by sonication with a Branson 250 Sonifier calibrated to yield an average fragment size of 500 bp or 2,000 bp, respectively. The size of sonicated fragments was confirmed by agarose gel electrophoresis, where DNA was stained with ethidium bromide and compared with the pBR 322-*Taq* I marker. Two thousand base pairs of DNA was heat denatured (100°C) and incubated at 55°C until all repeated DNA sequences were reassociated (Verneau, Renaud, and Catzeflis 1991). Fractionation of single-copy versus repeated DNA sequences was performed on hydroxyapatite columns (Britten, Graham, and Neufeld 1974). Twenty-five nanograms of the resulting nonrepeated DNA was radiolabeled with 5-<sup>125</sup>Iodo-2'-deoxycytidine 5'-triphosphate (Amersham, code IM.5103) by using random primer extension (Feinberg and Vogelstein 1983, 1984). Nonincorporated nucleotides were removed from radiolabeled DNA on G50 Sephadex columns (Sambrook, Fritsch, and Maniatis 1989). Duration of the enzymatic reaction was calibrated to yield radiolabeled DNA with a size of 300–500 bp, which was confirmed with sizing gels following standard procedures (Hall et al. 1980; Hunt, Hall, and Britten 1981; Caccone, Amato, and Powell 1987). Nonrepeated radiolabeled DNA, used as "tracer" in the DNA/DNA experiments, was hybridized to a thousand-fold excess of nonlabeled 500-bp DNA (driver) in homologous hybridization (i.e., DNA tracer and driver from the same species), and heterologous hybridization (i.e., DNA tracer and driver from different species). The mixtures were denatured at 100°C and then incubated at 60°C until completion of complementary strand reassociation at equivalent Cot of 16,000 (Cot = the product of DNA concentration × time of reassociation). The hybrids were passed through hydroxyapatite columns preheated in a water bath at 55°C, and single-stranded DNA was eluted with 12–15 ml of 0.12 M phosphate buffer (Kohne and Britten 1971; Werman, Springer, and Britten 1990). Temperature of the water bath was then increased by steps of 2.5°C until it reached 95°C, and the radioactivity contained in all collected samples was counted. The percentage of single-stranded DNA was estimated for each elution temperature. For each available tracer (size around 300–500 bp), hybrids were formed with two to five replicates. After reassociation, all thermal elution experiments were performed on the same day in order to minimize experimental error resulting from variation

among experimental runs of the thermal chromatography (Dickerman 1991). The parameter  $T_m$ , which represents the median temperature at which 50% of hybrid DNA has been dissociated, was deduced from the melting curves of DNA hybrids and used for the analysis of evolutionary relationships.

The complete matrix of delta- $T_m$  values was subjected to a bootstrapping procedure to assess its robustness (Krajewski and Dickerman 1990). Resampling of distance data was performed 1,000 times, and the resulting asymmetric matrices were adjusted by using the procedure of Sarich and Cronin (1976). An unrooted network was constructed by the least-squares approach of Fitch and Margoliash (1967) from each pseudoreplicate matrix. The percentage of occurrence of each node observed in the resulting networks was calculated by the program Consense in the PHYLIP package (Felsenstein 1990). Because the bootstrapping procedure does not generate normally distributed data (the variance is slightly biased; Weir 1990), a random sample of 100 replicate trees was used to calculate the modal value and 95% confidence interval for each branch length. The resulting network was unrooted.

Because the parasites studied are specific to distantly related hosts belonging to four major fish lineages, we expected to find high levels of molecular changes between bothriocephalid species if cospeciation had occurred. Therefore, partial sequences of the 18S rDNA, a slow-evolving nuclear gene, were obtained. Preliminary results obtained from the 18S rDNA sequences showed few substitutions between the species studied. So we decided to sequence one intron (ITS1) which evolves more rapidly than 18S rDNA.

Partial 18S rDNA (about 650 bases) and the entire ITS1 region (about 570 bases) were amplified from genomic DNA by using the polymerase chain reaction (PCR). This was done for the seven species of bothriocephalids studied and another parasite *Triaenophorus nodulosus* hosted by *Esox lucius*, which belongs to the pseudophyllidean family Ancistrocephalidae. Oligonucleotide primers used for the PCR reactions were designed from regions of 18S rDNA and 5.8S rDNA that are highly conserved in a wide range of organisms: *Caenorhabditis elegans* (EMBL accession number: X03680), *Opisthorchis viverrini* (X55357), *Drosophila melanogaster* (X70692 and X15707), *Alligator mississippiensis* (M59383 and M36342), and *Mus musculus* (X56974). The first primer (L7) hybridized in the 18S rDNA (5'-TGATTTGTCTGGTTTATTCCGAT-3') and is homologous to the sequence of the nematode *C. elegans* at positions 1143–1165 (Ellis, Sulston, and Coulson 1986). The second primer (H7) hybridized in the 5.8S rDNA (5'-GCTGCGTTCTTCATCGATACTCG-3') and is the complementary sequence of the 5.8S rDNA gene of *C. elegans* at positions 2132–2154 (Ellis, Sulston, and Coulson 1986). PCR products were immediately cloned in the pGEM-T vector (Promega) and transfected in competent bacteria (JM101 strain of *Escherichia coli*). Recombinant clones were identified by color screening on indicator plates and were sequenced directly by the method of Sanger, Nicklen, and Coulson

**Table 1**  
**Delta-Tm and Tamura-Nei Distances Between Bothriocephalid Parasites**

	<i>B. gregarius</i>	<i>B. barbatus</i>	<i>B. clavibothrium</i>	<i>B. andresi</i>	<i>B. funiculus</i>	<i>B. scorpii</i>	<i>B. claviceps</i>	<i>T. nodulosus</i>
<i>B. gregarius</i> . . . . .	0.0	2.05 ± 0.23 <i>n</i> = 8	2.90 ± 0.14 <i>n</i> = 9	2.91 ± 0.08 <i>n</i> = 7	2.89 ± 0.35 <i>n</i> = 6	3.50 ± 0.51 <i>n</i> = 8	—	—
<i>B. barbatus</i> . . . . .	0.0097	0.0	3.15 ± 0.36 <i>n</i> = 7	3.19 ± 0.28 <i>n</i> = 8	3.29 ± 0.11 <i>n</i> = 6	3.62 ± 0.33 <i>n</i> = 7	—	—
<i>B. clavibothrium</i> . . . . .	0.0167	0.0123	0.0	0.31 ± 0.15 <i>n</i> = 9	3.09 ± 0.52 <i>n</i> = 7	3.12 ± 0.54 <i>n</i> = 8	—	—
<i>B. andresi</i> . . . . .	0.0203	0.0158	0.0052	0.0	3.40 ± 0.21 <i>n</i> = 8	3.56 ± 0.15 <i>n</i> = 7	—	—
<i>B. funiculus</i> . . . . .	0.0132	0.0880	0.0141	0.0176	0.0	3.12 ± 0.05 <i>n</i> = 8	—	—
<i>B. scorpii</i> . . . . .	0.0105	0.0079	0.0096	0.0132	0.0097	0.0	—	—
<i>B. claviceps</i> . . . . .	0.1592	0.1549	0.1583	0.1614	0.1561	0.1562	0.0	—
<i>T. nodulosus</i> . . . . .	0.2167	0.2153	0.2122	0.2130	0.2182	0.2110	0.2489	0.0

NOTE.—Upper right matrix: average delta-Tm values (in °C) with standard deviation estimated from DNA/DNA experiments. *n* = number of replicate experiments for each pair of hybrids. Dashes (—) indicate nondetermined values. Lower left matrix: Tamura-Nei distances estimated from sequence analysis.

(1978). For sequencing, we used the Reverse and Forward plasmid primers and two other primers designed for 18S rDNA and ITS1 regions. Sequences of these oligonucleotides were as follows: 18S-1 (5'-ATTGAGCAATAACAGGTCTGT-3') and ITS1-1 (5'-AA-CAATCATGATGGGGCGTAG-3').

Sequences from ITS1 and partial 18S rDNA were aligned using the MUST package (Philippe 1993). When alignments were ambiguous, in particular for regions with insertions or deletions, all sites including a gap (insertion, deletion) were removed from the phylogenetic analyses which were conducted using combined sequences obtained from ITS1 and the partial 18S rDNA. The parsimony analysis was done using the PAUP program version 3.1.1., with the branch-and-bound option (Swofford 1993). All informative sites were retained and equally weighted. For the phenetic analysis, the Tamura-Nei distance (Tamura and Nei 1993) was computed between all pairs of sequences. This distance was chosen because the transition/transversion ratio was slightly biased and the four nucleotides

deviate substantially from equality. Trees were then constructed using the neighbor-joining method (Saitou and Nei 1987). Robustness of resulting trees was assessed using a bootstrapping procedure on all sites (1,000 replications), and trees were rooted using *T. nodulosus* as the outgroup. Indeed, preliminary studies based on parsimony and distance analyses on partial 18SrDNA sequences including all bothriocephalids, *T. nodulosus* and another species of helminth belonging to the order Proteocephalida (*Proteocephalus anguillae*) supported bothriocephalid parasites as a monophyletic group (data not shown). Sequences have been deposited in EMBL Data Bank under accession numbers Y09670–Y09685.

## Results

### DNA/DNA Hybridization

Table 1 depicts divergence estimates (average delta-Tm values with standard deviation) between each pair of parasite taxa. It was not possible to measure delta-Tm values between *B. claviceps* and any other species because the level of genomic divergence between this taxon and the others exceeds the resolving power of the methodology. The tree (unrooted) is represented in figure 1. A single branching pattern was observed between the parasite species where all resulting nodes are supported at the 100% level.

### Nucleotide Sequences

The alignments of 18SrDNA and ITS1 sequences for the seven bothriocephalid species and *T. nodulosus* have been deposited in EMBL under accession numbers DS28072 and DS28073. The length of the 18SrDNA region was 647 bp for *B. claviceps* and 644 bp for all other bothriocephalids and *T. nodulosus*. When alignments were obtained and compared to the sequence of *C. elegans*, bothriocephalids and *T. nodulosus* revealed an insertion of about 150 bp within the 18S rDNA. The first internal transcribed spacer was sequenced completely for all parasites studied. Length of this intron

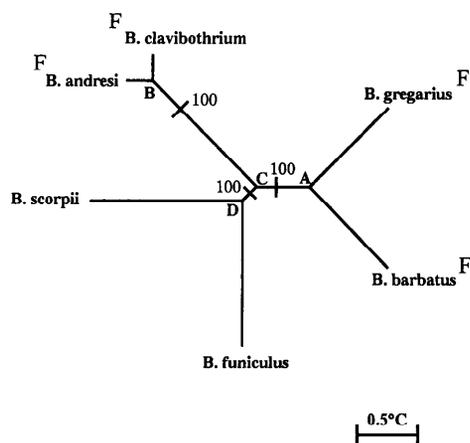


FIG. 1.—Evolutionary relationships among six species of *Bothriocephalus* constructed by phenetic analysis (FITCH) of the delta-Tm parameter from DNA/DNA hybridization. Numbers correspond to frequencies of occurrence of the different nodes in 1,000 bootstrap replicates. The letter F indicates parasites of flatfishes (Pleuronectiformes).

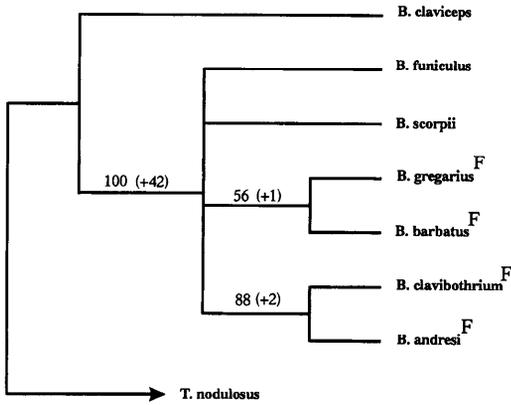


FIG. 2.—Strict consensus tree of bothriocephalids derived from PAUP analysis on all informative characters among ITS1 and partial 18S rDNA. Bootstrap percentages (1,000 replicates) are given above the corresponding branches and the decay index values are represented in brackets. The letter F indicates parasites of flatfishes (Pleuronectiformes). Abbreviations: B. = *Bothriocephalus*; T. = *Triaenophorus*.

was similar in all bothriocephalids (from 570 to 574 bp) with *T. nodulosus* having a 546-bp fragment.

The parsimony analysis is based on 61 informative characters, and the strict consensus tree resulting from five equally parsimonious trees (356 steps; consistency index [CI] = 0.97) is shown in figure 2. The Tamura-Nei distances were computed from 307 variable sites on the 1,152 conserved sites and are summarized in table 1. The tree resulting from the neighbor-joining procedure on the pairwise distances is shown in figure 3A. Both analyses revealed an early divergence of *B. claviceps* (parasite of the eel) relative to the remaining bothriocephalids. The analyses also revealed a close relationship between *B. clavibothrium* and *B. andresi*. Branching patterns among the other taxa remain poorly resolved. Although the distance analysis (fig. 3A) shows

that the clade composed of *B. barbatus*, *B. gregarius*, *B. funiculus*, and *B. scorpii* may be monophyletic (bootstrap value around 80%), no other cluster is supported at a bootstrap level higher than 70% (figs. 2 and 3A).

## Discussion

### Evolutionary Relationships Within Bothriocephalid Parasites

Genetic distances estimated from DNA/DNA hybridization experiments revealed low levels of divergence among most bothriocephalid species (table 1 and fig. 1). The single exception was *B. claviceps*, with delta-Tm distances higher than 15°C when compared with the six other species (data not shown). Phylogenetic trees obtained from both analyses of the sequence data (i.e., parsimony and distance analyses, figs. 2 and 3A) were generally congruent. In both trees *B. claviceps* was widely divergent from all remaining bothriocephalids studied. This latter group was clearly monophyletic (100% bootstrap percentage in both trees). Thus, all these findings demonstrate that speciation of the *B. claviceps* lineage predated speciation of the other bothriocephalid species and experienced a faster rate of DNA evolution (fig. 3A). Furthermore, these data appear to support a recent origin for the six remaining parasites, which are found to be closely related (table 1 and figs. 1, 2, and 3A). Delta-Tm distances of ca. 3.0°C have now been observed between closely related genera, such as within the hominid primates (Sibley, Comstock, and Ahlquist 1990; Caccone and Powell 1989), whose radiation timing is probably not much older than 7–10 Myr. For example, Caccone and Powell (1989) report a delta-Tm of 1.60°C between *Homo* and *Pan* and a delta-Tm of 2.50 between *Homo* and *Gorilla* and between *Pan* and *Gorilla*. For organisms characterized by

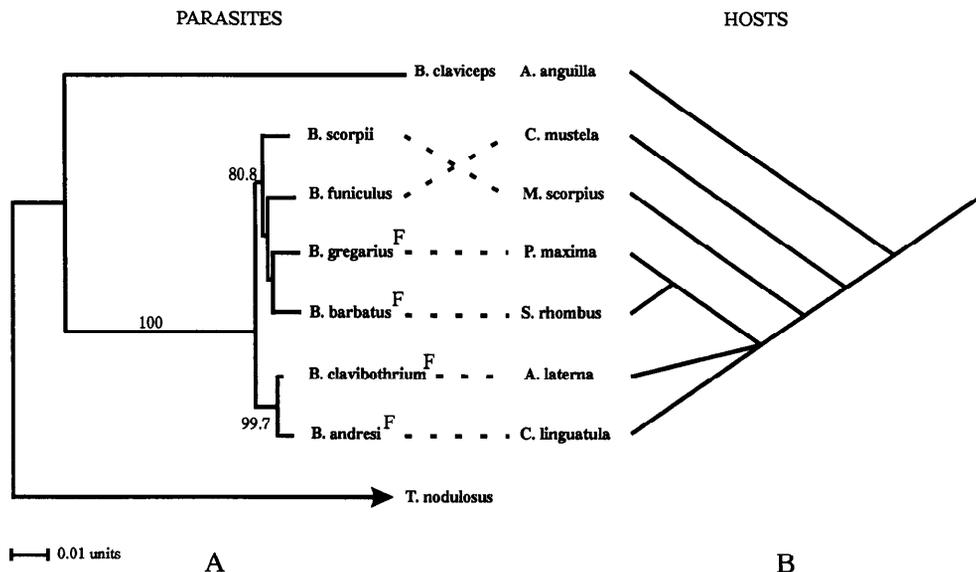


FIG. 3.—A, Phylogeny of bothriocephalids derived from the neighbor-joining procedure on pairwise distances calculated among ITS1 and partial 18S rDNA. Numbers correspond to bootstrap values (1,000 replicates). The letter F indicates parasites of flatfishes (Pleuronectiformes). B, Combined phylogeny of the seven teleostean hosts (i.e., pleuronectiformes, scorpaeniform, gadiform, and anguilliform) derived from molecular data (Le, Perasso, and Billard 1989; Le, Lecointre, and Perasso 1993) and electrophoretic data (Verneau et al. 1994). See text for abbreviations of generic names.

a higher rate of nonrepeated DNA change, such as mice and rats, delta-Tm values of ca. 3.0°C correspond to a divergence time of ca. 2–3 Myr (Catzefflis et al. 1993).

#### Comparison of Host and Parasite Phylogenies

Comparison of the branching patterns among hosts and their associated parasites can provide information on cospeciation and host-switching events that have occurred during the evolutionary history of the association. Few molecular studies have been conducted to discern relationships between orders of teleost fishes. Le, Perasso, and Billard (1989) and Le, Lecointre, and Perasso (1993) showed, from 28S rRNA sequence analysis, that pleuronectiforms (in our survey, four species are examined: *P. maxima*, *S. rhombus*, *A. laterna*, and *C. linguatula*) are more closely related to scorpaeniforms (for example *M. scorpius*) than they are to gadiforms (for example, *C. mustela*) and, finally, to anguilliforms (for example, *A. anguilla*) which are the most divergent. However, relationships between the major orders examined in this study are moderately supported with low bootstrap values (Le, Lecointre, and Perasso 1993). Concerning relationships within pleuronectiform fishes, Verneau et al. (1994) showed from DNA/DNA hybridization and electrophoretic analyses that the two scophthalmids (i.e., *P. maxima* and *S. rhombus*) are more closely related to each other than either is to a citharid (i.e., *C. linguatula*) or a bothid (i.e., *A. laterna*). Moreover, the two latter families (Citharidae and Bothidae) are as divergent from Scophthalmidae as they are from each other (Verneau et al. 1994). So, it seems that speciation events leading to the lineages of *C. linguatula* and *A. laterna* occurred before the split between *P. maxima* and *S. rhombus*. In the light of a tree based on the combination of these sets of data (Le, Perasso, and Billard 1989; Le, Lecointre, and Perasso 1993; Verneau et al. 1994), we can discuss the evolution of this host-parasite association when the combined host tree (fig. 3B) is compared to the parasite phylogeny (fig. 3A).

Besides the early separation of *B. claviceps*, all remaining bothriocephalid parasites are grouped in just two clades: (1) *B. gregarius*, *B. barbatus*, *B. funiculus*, and *B. scorpii*; and (2) *B. clavibothrium* and *B. andresi* (fig. 3A). Thus, flatfish parasites (i.e., tapeworms of Pleuronectiformes labeled F on figs. 1, 2, and 3A) do not appear to be monophyletic. Consequently, the discrepancies observed in the branching patterns between the two biological assemblages (i.e., hosts and parasites) suggest that pleuronectiform fishes and their associated parasites have not cospeciated. The branch lengths of the parasites trees (figs. 1 and 3A) suggest that the speciation between *B. andresi* and *B. clavibothrium* is more recent than the one between *B. gregarius* and *B. barbatus*, whereas the branching pattern of the fishes indicates that *P. maxima* and *S. rhombus* are more related to each other than either is to *C. linguatula* and *A. laterna* (fig. 3B). As bothriocephalids are subdivided into two clades on the phylogenetic tree of figure 3A, the unrooted network (fig. 1) shows that *B. andresi* and *B. clavibothrium* are more closely related to each other than is *B. gregarius* to *B. barbatus*. This contrasting

pattern in evolutionary relationships between hosts and parasites suggests that a host-switching event has occurred during the evolutionary history of *C. linguatula* and *A. laterna* and their parasites.

Two different—but nonexclusive—evolutionary processes can be proposed for explaining the results of this study: (1) sequential colonizations of different host species followed by speciation of parasites, as proposed elsewhere in helminth faunas by Hoberg (1986, 1992) and Hoberg and Adams (1992); or (2) a rapid radiation of a ubiquitous ancestral parasite species, leading to isolation and differentiation of *Bothriocephalus* lineages in the different host species. As judged from the small amounts of genomic divergence measured between most pairs of tapeworms, these processes have occurred rather recently and over a short time span.

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#### LITERATURE CITED

- BANDONI, S. M., and D. R. BROOKS. 1987. Revision and phylogenetic analysis of the Amphilinidea Poche, 1922 (Platyhelminthes: Cercomeria: Cercomeromorpha). *Can. J. Zool.* **65**:1110–1128.
- BAVERSTOCK, P. R., M. ADAMS, and I. BEVERIDGE. 1985. Biochemical differentiation in bile duct cestodes and their marsupial hosts. *Mol. Biol. Evol.* **2**:321–337.
- BRITTEN, R. J., D. E. GRAHAM, and B. R. NEUFELD. 1974. Analysis of repeating DNA sequences by reassociation. Pp. 363–406 in L. GROSSMAN and K. MOLDAVE, eds. *Methods of enzymology*. IRL Press, Oxford.
- BROOKS, D. R., and D. R. GLEN. 1982. Pinworms and primates: a case study in coevolution. *Proc. Helminthol. Soc. Wash.* **49**:76–85.
- BROOKS, D. R., and D. A. McLENNAN. 1991. *Phylogeny, ecology, and behavior*. University of Chicago Press, Chicago and London.
- CACCONE, A., G. D. AMATO, and J. R. POWELL. 1987. Intra-specific DNA divergence in *Drosophila*: a study on parthenogenetic *D. mercatorum*. *Mol. Biol. Evol.* **4**:343–350.
- CACCONE, A., and J. R. POWELL. 1989. DNA divergence among hominoids. *Evolution* **43**:925–942.
- CATZEFLIS, F. M., A. W. DICKERMAN, J. MICHAUX, and A. W. KIRSCH. 1993. DNA hybridization and rodent phylogeny. Pp. 159–172 in F. SZALAY, M. NOVACEK, and M. MCKENNA, eds. *Mammal phylogeny*. Springer-Verlag, New York, N.Y.
- DEETS, G. B. 1987. Phylogenetic analysis and revision of *Kroeyerina* Wilson, 1932 (Siphonostomatoida: Kroeyeri-

- idae), copepods parasitic on Chondrichthyans, with descriptions of four new species and the erection of a new genus, *Prokroyeria*. *Can. J. Zool.* **65**:2121–2148.
- DICKERMAN, A. W. 1991. Among-run artifacts in DNA hybridization. *Syst. Zool.* **40**:494–499.
- ELLIS, R. E., J. E. SULSTON, and A. R. COULSON. 1986. The rDNA of *C. elegans*: sequence and structure. *Nucleic Acids Res.* **14**:2345–2364.
- FAHRENHOLZ, H. 1913. Ectoparasiten Abstammungslehre. *Zool. Anz.* **41**:371–374.
- FEINBERG, A. P., and B. VOGELSTEIN. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**:6.
- . 1984. Addendum. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **137**:266.
- FELSENSTEIN, J. 1990. PHYLIP: phylogeny inference package. Version 3.3. Department of Genetics, University of Washington, Seattle.
- FITCH, W. M., and E. MARGOLIASH. 1967. Construction of phylogenetic trees. *Science* **155**:279–284.
- HAFNER, M. S., and S. A. NADLER. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. *Nature* **332**:258–259.
- . 1990. Cospeciation in host-parasite assemblages: comparative analysis of rates of evolution and timing of cospeciation events. *Syst. Zool.* **39**:205–226.
- HAFNER, M. S., P. D. SUDMAN, F. X. VILLABLANCA, T. A. SPRADLING, J. W. DEMASTES, and S. A. NADLER. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science* **265**:1087–1090.
- HALL, T. J., J. W. GRULA, E. H. DAVIDSON, and R. J. BRITTEN. 1980. Evolution of sea urchin non-repetitive DNA. *J. Mol. Evol.* **16**:95–110.
- HOBERG, E. P. 1986. Evolution and historical biogeography of a parasite-host assemblage: *Alcataenia* spp. (Cyclophyllidae: Dilepididae) in Alcidae (Charadriiformes). *Can. J. Zool.* **64**:2576–2589.
- . 1992. Congruent and synchronic patterns in biogeography and speciation among seabirds, pinnipeds, and cetodes. *J. Parasitol.* **78**:601–615.
- HOBERG, E. P., and A. M. ADAMS. 1992. Phylogeny, historical biogeography, and ecology of *Anophrycephalus* spp. (Eucestoda: Tetrabothriidae) among pinnipeds of the Holarctic during the late Tertiary and Pleistocene. *Can. J. Zool.* **70**:703–719.
- HUNT, J. A., T. J. HALL, and R. J. BRITTEN. 1981. Evolutionary distances in Hawaiian *Drosophila* measured by DNA reassociation. *J. Mol. Evol.* **17**:361–367.
- JONES, A. 1975. The morphology of *Bothriocephalus scorpii* (Mueller) (Pseudophyllidae, Bothriocephalidae) from littoral fishes in Britain. *J. Helminthol.* **49**:251–261.
- KLASSEN, G. J., and M. BEVERLEY-BURTON. 1987. Phylogenetic relationships of *Ligictaluridus* spp. (Monogenea: Ancyrocephalidae) and their ictalurid (Siluriformes) hosts: an hypothesis. *Proc. Helminthol. Soc. Wash.* **54**:84–90.
- KOHNE, D. E., and R. J. BRITTEN. 1971. Hydroxyapatite techniques for nucleic acid reassociation. Pp. 500–512 in G. CANTONI and D. DAVIES, eds. *Procedures in nucleic acid research*. Harper and Row, New York, N.Y.
- KRAJEWSKI, C., and A. W. DICKERMAN. 1990. Bootstrap analysis of phylogenetic trees derived from DNA hybridization distances. *Syst. Zool.* **39**:383–390.
- LAUDER, G. V., and K. F. LIEM. 1983. The evolution and interrelationships of the actinopterygian fishes. *Bull. Mus. Comp. Zool.* **150**:95–197.
- LE, H. L. V., G. LECOINTRE, and R. PERASSO. 1993. A 28S rRNA-based phylogeny of the Gnathostomes: first steps in the analysis of conflict and congruence with morphologically based cladograms. *Mol. Phylogenet. Evol.* **2**:31–51.
- LE, H. L. V., R. PERASSO, and R. BILLARD. 1989. Phylogénie moléculaire préliminaire des "poissons" basée sur l'analyse de séquences d'ARN ribosomique 28S. *C. R. Acad. Sci. Paris* **309**:493–498.
- MITTER, C., and D. R. BROOKS. 1983. Phylogenetic aspects of coevolution. Pp. 65–98 in D. FUTUYMA and M. SLATKIN, eds. *Coevolution*. Sinauer, Sunderland, Mass.
- O'CONNOR, M. 1988. Host associations and coevolutionary relationships of astigmatid mite parasites of new world primates. I. Families Psoroptidae and Audycoptidae. *Fieldiana* **39**:245–260.
- PHILIPPE, H. 1993. MUST, a computer package for management utilitarian for sequences and trees. *Nucleic Acids Res.* **21**:5264–5272.
- PROTASOVA, E. N. 1977. Bothriocephali—Taenia helminths from fishes. HAYKA editions, Moscow.
- RANNALA, B. H. 1992. Comparative evolutionary genetics of trematode parasites (Plagiorchiidae) and their anuran hosts. *Can. J. Zool.* **70**:993–1000.
- RENAUD, F., and C. GABRION. 1984. Polymorphisme enzymatique de populations du groupe *Bothriocephalus scorpii* (Mueller, 1776) (Cestoda, Pseudophyllidae). Etude des parasites de divers Téléostéens des côtes du Finistère. *Bull. Soc. Fr. Parasitol.* **2**:95–98.
- RENAUD, F., C. GABRION, and N. PASTEUR. 1983. Le complexe *Bothriocephalus scorpii* (Mueller, 1776): différenciation par électrophorèse enzymatique des espèces parasites du turbot (*Psetta maxima*) et de la barbut (*Scophthalmus rhombus*). *C. R. Acad. Sci. Paris* **296**:127–129.
- . 1986. Geographical divergence in *Bothriocephalus* (Cestoda) of fishes demonstrated by enzyme electrophoresis. *Int. J. Parasitol.* **16**:553–558.
- SAITOU, N., and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**:406–425.
- SAMBROOK, J., E. F. FRITSCH, and T. MANIATIS. 1989. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- SANGER, F., S. NICKLEN, and A. R. COULSON. 1978. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
- SARICH, V. M., and J. E. CRONIN. 1976. Molecular systematics of the Primates. Pp. 141–170 in M. GOODMAN and R. TASHIRIAN, eds. *Molecular anthropology*. Plenum Press, New York and London.
- SIBLEY, C. G., J. A. COMSTOCK, and J. E. AHLQUIST. 1990. DNA hybridization evidence of Hominoid phylogeny: reanalysis of the data. *J. Mol. Evol.* **30**:202–236.
- SWOFFORD, D. L. 1993. PAUP: Phylogenetic analysis using parsimony. Version 3.1.1. Computer program distributed by Illinois Natural History Survey, Champaign.
- TAMURA, K., and M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **10**:512–526.
- VERNEAU, O., C. MOREAU, F. M. CATZEFLIS, and F. RENAUD. 1994. Phylogeny of flatfishes (Pleuronectiformes): comparisons and contradictions of molecular and morpho-anatomical data. *J. Fish Biol.* **45**:685–696.
- VERNEAU, O., F. RENAUD, and F. M. CATZEFLIS. 1991. DNA reassociation kinetics and genome complexity of a fish (*Psetta maxima*: Teleostei) and its gut parasite (*Bothriocephalus* sp.). *J. Parasitol.* **61**:101–108.

*phalus gregarius*: Cestoda). *Comp. Biochem. Physiol.* **99B**: 883–886.

WEIR, B. S. 1990. *Genetic data analysis*. Sinauer, Sunderland, Mass.

WERMAN, S. D., M. S. SPRINGER, and R. J. BRITTEN. 1990. Nucleic acids: DNA-DNA hybridization. Pp. 204–249 in D.

HILLIS and C. MORITZ, eds. *Molecular systematics*. Sinauer, Sunderland, Mass.

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