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COEVOLUTION BETWEEN *LAMELLODISCUS* (MONOGENEA: DIPLECTANIDAE) AND SPARIDAE (TELEOSTEI): THE STUDY OF A COMPLEX HOST-PARASITE SYSTEM

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Abstract.—Host-parasite coevolution was studied between Sparidae (Teleostei) fishes and their parasites of the genus *Lamellodiscus* (Monogenea, Diplectanidae) in the northwestern Mediterranean Sea. Molecular phylogenies were reconstructed for both groups. The phylogenetic tree of the Sparidae was obtained from previously published 16S mitochondrial DNA (mtDNA) sequences associated with new cytochrome-*b* mtDNA sequences via a ''total evidence'' procedure. The phylogeny of *Lamellodiscus* species was reconstructed from 18S rDNA sequences that we obtained. Host-parasite coevolution was studied through different methods: TreeFitter, TreeMap, and a new method, ParaFit. If the cost of a host switch is not assumed to be high for parasites, all methods agree on the absence of widespread cospeciation processes in this host-parasite system. Host-parasite associations were interpreted to be due more to ecological factors than to coevolutionary processes. Host specificity appeared not to be related to host-parasite cospeciation.

Key words.—18S rDNA, coevolution, cytochrome-*b* mitochondrial DNA, fish, *Lamellodiscus*, monogenean, Sparidae.

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Host-parasite coevolution has been the subject of numerous studies for a long time (e.g., Kellogg 1913; Bychowsky 1961; Brooks 1979, 1981; Brooks and Glen 1982; Cressey et al. 1983; Hafner and Nadler 1988; Klassen and Beverley-Burton 1988; Page 1993a, 1994a; Paterson et al. 1993; Hafner et al. 1994; Boeger and Kritsky 1997; Desdevises et al. 2000; Paterson and Banks 2001); see Klassen (1992) for a historical perspective. From this important body of work, we now recognized that ''Farenholz' rule'' (that the parasite phylogeny mirrors the host phylogeny) does not seem to be generally true in host-parasite associations (see Paterson and Banks 2001). It was suggested early (Kellogg 1913) that host switching could be a component of host-parasite coevolution, even if priority was given to evolution through cospeciation (Bychowsky 1961; see Klassen 1992). The term ''coevolution'' is used here to describe the extent to which the host and parasite phylogenetic trees are congruent. When the trees are perfectly congruent, coevolution is the equivalent of cospeciation. This corresponds to the definition of Brooks (1979, 1988), Klassen and Beverley-Burton (1987), Brooks and McLennan (1991), and Klassen (1992), and refers to the macroevolutionary context. This should not be confused with a more restrictive meaning, used in genetic and microevolutionary studies, which defines coevolution as the influence of the host genome on the parasite genome, and vice versa (Toft and Karter 1990).

Most studies in which the host and parasite phylogenies were found to be congruent, with parasites found in similar positions on the tree as their hosts, involve very particular groups in which biological characteristics made host-switching events highly improbable (see Barker 1994). This is the case of the well-known association between pocket gophers and their chewing lice (Hafner and Nadler 1988; Hafner at al. 1994), and that of swiftlets and their parasitic lice (Page et al. 1998). In a review about coevolution of lice and their hosts, Barker (1994; but see Page et al. 1996) pointed out that every time a possibility of host switching was encountered (i.e., contact between hosts), it effectively took place (see Hafner and Nadler 1988). Cospeciation of parasites with their hosts mainly happened when the hosts were allopatric to one another.

Monogenean-fish associations were seldom studied in this context (Klassen and Beverly-Burton 1987, 1988; Boeger and Kritsky 1989, 1997; Guégan and Agnèse 1991; Desdevises et al. 2000). The high host specificity encountered in monogeneans, in terms of the number of hosts parasitized (Baer 1957; Llewellyn 1957; Kennedy 1975; Rohde 1979, 1982; Noble et al. 1989; Sasal et al. 1998), allows one to assume close interactions with their hosts, and therefore anticipate a high level of coevolution via cospeciation (Noble et al. 1989; Kearn 1994). This kind of host-parasite complex is characterized by high dispersion of the mobile larval stage (oncomiracidium) of the parasites, which finds its host through chemical cues (Kearn 1967, 1988). Chemical interaction could be assumed to be a determinant of host specificity, and therefore would support a cospeciation hypothesis. Moreover, the direct life cycle of monogeneans avoids the influence of an intermediate

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host in the phylogenetic relationship of the final host with its parasite. However, other authors have argued that the capacity of dispersion of the larval stage in monogeneans suggests important possibilities for new host colonization (Brooks and McLennan 1991). Moreover, adults of some monogenean species are supposed to be able to survive for a short time period outside the host (see Bakke et al. 1992 on *Gyrodactylus*), thus increasing the possibility of dispersion. It has been suggested that monogeneans are transmitted via host contact. It has also been suggested that no or little competition exists among ectoparasitic monogeneans (Rohde 1979, 1994; Simkova et al. 2000). So, potentially competing species would not be an obstacle to host switching in monogeneans, in contrast to lice for example (see Barker 1994). Studies on the coevolution of monogeneans with their hosts apparently supports the existence of host-switching events: Boeger and Kritsky (1997) have suggested that several dispersion events took place in the early history of monogeneans; Klassen and Beverley-Burton (1987, 1988) suggested that *Ligictaluridus* ancyrocephalid monogeneans have not strictly cospeciated with their fish hosts; Desdevises et al. (2000) suggested that the *Lamellodiscus* species associated with *Pagellus* (Sparidae) have not cospeciated with their hosts; and Sinnappah at al. (2001) proposed the existence of dispersion events in the history of Polystomatid monogeneans.

The precise reconstruction of a hypothetical coevolutionary scenario between hosts and their parasites is not always straightforward. Page (1993b, 1994b), and Page and Charleston (1998) have shown that sometimes, and at least in theory, no switching event is necessary to reconcile the host and parasite trees. A host-parasite association whose phylogenetic trees are not congruent can closely coevolve via duplication, cospeciation and lineage sorting, without any host-switching events. Likewise, the absence of congruence may not always be equated with a lack of historical association between the two components. When the host and parasite phylogenetic trees are similar or almost similar, with parasites found in similar positions on the tree as their hosts, the use of a special analytical method to study coevolution among them may not be necessary. In that case, the putative colonization events can be inferred by visual examination of the trees (e.g., Verneau et al. 1997). However, if the pattern becomes more complicated, a rigorous method should be used to choose among a number of potential scenarios. Several methods have been developed to study host-parasite coevolution. These are Brooks' parsimony analysis (BPA; Brooks 1981; Brooks and McLennan 1991), component analysis (Component; Page 1993c), trees reconciliation (TreeMap; Page 1994b), event-based methods (TreeFitter; Ronquist 1995, 1997; Jungles; Charleston 1998), the maximum-likelihood method (Huelsenbeck et al. 1997), and a method based on Bayesian inference (Huelsenbeck et al. 2000). These methods search for an optimal evolutionary scenario for the association between a set of hosts and their parasites; they either take into account, or do not, estimates of the probability of occurrence of each type of evolutionary event. To achieve that, each method has a specific way of using cospeciation, duplication, sorting, and switching events. For more explanation of the terminology, see Page (1994b), Ronquist (1997), Charleston (1998), Page and Charleston (1998), and Paterson and Banks (2001).

In this study, we investigate coevolutionary interactions in a Mediterranean fish-monogenean association that has been known for a long time and is well studied (Euzet and Oliver 1966, 1967; Oliver 1968, 1973, 1974, 1987; Euzet 1984; Euzet et al. 1993). For that reason, the known pattern of host specificity may be considered close to reality. The inventory of fish parasites in the Mediterranean Sea is considered one of the most exhaustive in the world (Caro et al. 1997). The host-parasite association studied here is formed by the monogenean gill ectoparasites of the genus *Lamellodiscus* Johnston and Tiegs 1922, which are parasites of teleost fishes of the Sparidae family (Oliver 1987; Euzet et al. 1993). The pattern of host-parasite relationships is described in Table 1. The observations made during the present study, in addition to the many papers cited above, support this pattern. There are 20 described *Lamellodiscus* species and 16 Sparidae in the study area. This host-parasite system is distinctive because some of its monogenean species are not as specific as those usually observed in monogeneans. Some parasites in this system can indeed be considered generalists (e.g., *L. ignoratus* uses six host species, whereas *L. elegans* uses five). However, more than half of these parasite species (11) are strict specialists (using one host species), and three species use only two host species, making it possible to study the influence of tight host-specificity on cospeciation. In addition, this system is characterized by a high number of parasite and host species living in sympatry (Whitehead et al. 1986). All potential hosts are then always ''available'' to the parasites. The observation of a tight cospeciation pattern, in this case, would be the sole result of the influence of the hosts on the parasite evolutionary history, because no ecological or geographic barrier could be invoked to explain parasite speciation. Several of the above-mentioned methods will be used here to study coevolution, in addition to a new method that makes use of the host and parasite phylogenetic distance matrices as well as a matrix describing the host-parasite association links (Legendre et al. 2002).

The objectives of this study are to reconstruct molecular phylogenies for the hosts (Sparidae) and their monogenean parasites (*Lamellodiscus*), to assess the extent of cospeciation in this association, and to propose an interpretation of the observed pattern.

Materials and Methods

Sampling

Sparid fish were caught in several locations in the northwestern Mediterranean Sea: in the Golfe du Lion near Banyuls-sur-Mer (France), near Marseille (France), and in Corsica (Scandola Natural Reserve, France). *Lamellodiscus* were dislodged from the gills of the fish under a dissecting microscope and identified using the morphology of the haptor and copulatory organ observed under an optical microscope with 400× magnification. Parasites were stored in 95% alcohol before DNA extraction.

Phylogenies

For the host and parasite phylogenetic analyses, saturation levels in the DNA sequences were estimated by plotting dis-

TABLE 1. *Lamellodiscus*-Sparidae associations and GenBank accession numbers.

Lamellodiscus and outgroup species	Sparidae species	GenBank accession numbers	
		Parasites (18S)	Hosts (16S/cytochrome-*b*)
L. baeri	*Pagrus pagrus*	AY038187*	AJ247277/AJ319815*
L. bidens	*Diplodus puntazzo*	AY038188*	AJ247291/AJ277368
L. coronatus	*Diplodus annularis*	AY038189*	AJ247286/AJ277366
	Diplodus cervinus		AJ247290/AJ277367
	Diplodus sargus		AJ247293/AJ277369
L. drummondi	*Pagellus acarne*	AJ276441	AJ247281/AJ276879
L. elegans	*Diplodus annularis*	AF294956	
	Diplodus sargus		
	Diplodus vulgaris		AJ247294/AJ277370
	Oblada melanura		AJ247296/AJ319813*
	Spondyliosoma cantharus		AJ247280/AJ319811*
L. ergensi	*Diplodus annularis*	AY038190*	
	Diplodus puntazzo		
	Diplodus sargus		
	Diplodus vulgaris		
L. erythrini	*Pagellus erythrinus*	AJ276440	AJ247284/AJ276881
L. fraternus	*Diplodus annularis*	AY038191*	
	Diplodus vulgaris		
L. furcosus	*Diplodus annularis*	AY038192*	
	Diplodus sargus		
L. gracilis	*Diplodus annularis*	AY038193*	
	Diplodus sargus		
	Oblada melanura		
L. hilii	*Diplodus puntazzo*	AY038194*	
L. ignoratus	*Diplodus annularis*	AF294957	
	Diplodus puntazzo		
	Diplodus sargus		
	Diplodus vulgaris		
	Lithognathus mormyrus		AJ247285/AJ277371
	Sarpa salpa		AJ247269/AJ319812*
L. impervius	*Diplodus puntazzo*	AY038195*	
L. knoepffleri	*Spondyliosoma cantharus*	AY038196*	
L. mirandus	*Diplodus sargus*	AY038197*	
L. mormyri	*Lithognathus mormyrus*	AF294954	
L. parisi	*Sarpa salpa*	AY038198*	
L. verberis	*Lithognathus mormyrus*	AF294955	
L. virgula	*Pagellus acarne*	AJ276442	
	Pagellus bogaraveo		AJ247283/AJ276880
Furnestinia echeneis	*Sparus aurata*	AF294953	AJ247279/AJ319809*
Not parasitised	*Boops boops*		AJ247268/AJ319810*
Not parasitised	*Diplodus dentex*		AJ247271/AF143197
Diplectanum aequans†	*Dicentrarchus labrax*†	AJ276439	
Pseudomurraytrema ardens†		AJ228793	
Dactylogyrus minor†		AF294952	
	Spicara maena†		AJ247298

* Sequence obtained during this study.
† Outgroup taxa.

tances (percent differences) calculated only for transversions against distances calculated only from transitions. Phylogenetic analyses were carried out using the maximum-parsimony (MP) and maximum-likelihood (ML) methods. Evolutionary models used in the ML analysis were chosen using the program Modeltest (Posada and Crandall 1998), which makes use of hierarchical likelihood ratio tests. All phylogenetic analyses were performed with PAUP* 4.0d8 (Swofford 2001). Maximum-parsimony trees were validated with a bootstrap procedure using 1000 replicates.

Hosts

Phylogenetic relationships among Sparid fish species have long been controversial. Their present classification only relies upon morphological characters, particularly the types of fin rays and dentition (Whitehead et al. 1986). There are presently four recognized subfamilies: the Sparinae, Denticinae, Boopsinae, and Pagellinae (Smith and Smith 1986; Fiedler 1991). These subfamilies are differentiated by their dentition and trophic specialization. However, no clear phylogeny has been proposed until recently (Hanel and Sturmbauer 2000). Previous attempts were unsatisfactory (Cataudella et al. 1980; Basaglia 1991; Reina et al. 1994; Garrido-Ramos et al. 1995, 1998, 1999). Hanel and Sturmbauer (2000) reconstructed a phylogenetic tree of the Sparid fish species based on 16S mtDNA, for 24 species from the Atlantic Ocean and the Mediterranean Sea (among them the 16 Mediterranean species under study here; see Fig. 1a). Their tree showed considerable differences from current taxonomy, but was not fully resolved. To infer the phylogenetic relationships among

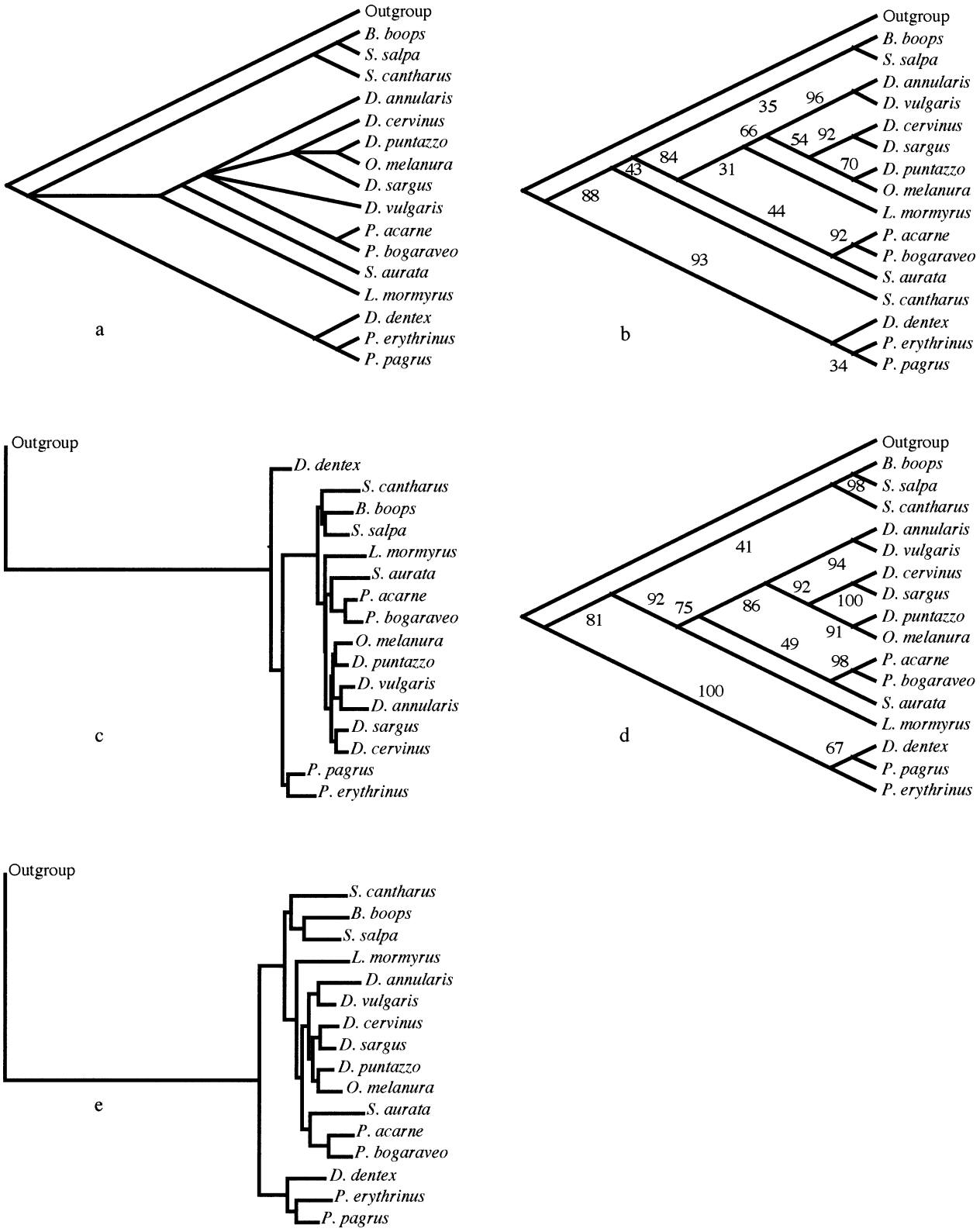

FIG. 1. Host phylogenetic trees estimated from mtDNA partial sequences; numbers are bootstrap values. (a) Consensus tree published by Hanel and Sturmbauer (2000), estimated from 16S mtDNA. (b) Maximum-parsimony (MP) tree estimated from cytochrome-*b* mtDNA sequences. (c) Maximum-likelihood (ML) tree computed from cytochrome-*b* mtDNA sequences. (d) MP tree computed from cytochrome-*b* and 16S mtDNA sequences (total evidence). (e) ML tree estimated from cytochrome-*b* and 16S mtDNA sequences (total evidence).

Sparids and perform an independent external validation of their dataset, we partially sequenced the cytochrome-*b* mitochondrial DNA (mtDNA). DNA extraction and sequencing followed the same protocol as in Desdevises et al. (2000). Some of these sequences were used by Jousson et al. (2000). A phylogenetic tree was estimated from these data, with *Dicentrachus labrax* (Moronidae) as the outgroup. Hanel and Sturmbauer (2000) had estimated the phylogeny of the same group of fish using 16S mtDNA, with *Spicara maena* (Centracanthidae) as the outgroup. We added the 16S mtDNA sequence data from Hanel and Sturmbauer (2000) to our cytochrome-*b* data table and performed a partition homogeneity test (PHT; Farris et al. 1994; the null hypothesis is the congruence between the two datasets underlying the two trees) on the pooled dataset (16S + cyt-*b* mtDNA), as well as a Mantel test (Mantel 1967; the null hypothesis is the absence of correlation between the pairwise species distances computed for each dataset), to assess whether these two datasets can be safely used together in a ''total evidence'' approach (Lapointe 1998). Because the homogeneity (or concordance) of the two datasets was not rejected by PHT (100 heuristic searches, $P = 0.18$) and was supported by the Mantel test (999 permutations, $P = 0.001$), this analysis was carried out to increase the resolution of the phylogenetic tree. We created a synthetic outgroup by merging the sequences from the two outgroups used in the previous separate analyses, *Dicentrachus labrax* and *Spicara maena*. Phylogenetic analysis of the two DNA fragments used here (16S and cyt-*b* mtDNA) requires the use of a single outgroup species, which strongly reduces potential choices. The taxonomically closest suitable outgroup is *Gadus morhua*, which is much farther from the ingroup than the two species that were used as outgroups in the separate analyses. The use of *G. morhua* as an outgroup would lead to an important increase in the number of homoplastic characters. On the other hand, using the same outgroups for the separate and combined analyses leads to more reliable comparisons between them.

Parasites

It has been suggested that 18S rDNA is a reliable marker to infer phylogenetic relationships among *Lamellodiscus* species (Desdevises et al. 2000; Desdevises 2001). The same protocol as in Desdevises et al. (2000) was used here for extraction, amplification, cloning, and sequencing of the partial 18S rDNA. These DNA fragments were independently sequenced from two individuals per species. Three clones were sequenced for each individual. Interindividual and interclone variability were always negligible (less than 0.5%), so consensus sequences were used. With the primers used (L7 and H7, designed by Verneau et al. 1997), the internal transcribed spacer 1 (ITS1) was amplified and sequenced with the partial 18S rDNA. It has been shown that ITS1 is highly variable and not useful to infer evolutionary relationships among *Lamellodiscus*, but it can be used to identify species that can exhibit different morphologies (Desdevises et al. 2000). The amplification of the sole partial rDNA 18S fragment, with L7 and an internal primer, sometimes led to irreproducible results, perhaps because pseudogenes were present. *Furnestinia echeneis* Euzet and Audoin 1959, the parasite of *Sparus aurata*, was added to the 19 *Lamellodiscus* species under study after it was suggested that it should be included in the genus *Lamellodiscus* on the basis of 18S rDNA (Desdevises 2001). Based on the molecular data, *Lamellodiscus obeliae*, which had been described as a specific parasite of *Pagellus bogaraveo* by Oliver (1973), has been considered the same species as *L. virgula* (Desdevises et al. 2000), which is a parasite of *P. acarne*. In the present study, these two species are considered *L. virgula*, which then parasitizes *P. acarne* and *P. bogaraveo*. Sequence alignment was done with ClustalX (Thompson et al. 1997) and visually checked. Gaps were treated as missing data.

Dactylogyrus minor, *Pseudomurraytrema ardens*, and *Diplectanum aequans* were used as outgroups, because of their phylogenetic positions relative to the genus *Lamellodiscus* (Boeger and Kritsky 1997; Desdevises et al. 2001).

Coevolution

Several methods to assess the coevolutionary interactions in a host-parasite association have been proposed in the literature. The first method dedicated to the study of such association is BPA (Brooks 1981; Brooks and McLennan 1991), which consists of building a host phylogeny from characters derived from the parasite phylogeny and comparing this tree to the known host phylogeny. BPA has been widely used in coevolutionary studies (e.g., Paterson et al. 1993; Boeger and Kritsky 1997). Another method, component analysis, popularized by Page (1993b), relies on the comparison of tree topologies, but does not allow the incorporation of host-switching events. This was made possible with TreeMap (Page 1994b), which uses reconciled trees to compute the fit between the host and parasite phylogenies. Ronquist (1995) proposed to use methods based on generalized parsimony to assess the same fit, incorporating a differential cost to the four types of potential events (see Ronquist 1995; Page and Charleston 1998; Paterson and Banks 2001) occurring in a host-parasite association: cospeciation (C), duplication (D), sorting (S), and host switching (H). The optimal reconstruction is the one that minimizes the global cost. This is implemented in the program TreeFitter 1.0 (available from the author, F. Ronquist, at http://www.ebc.uu.se/systzoo/research/treefitter/treefitter.html), which also allows statistical testing of the overall cost and occurrence of each type of event. Testing is done through a permutational procedure. By assigning different costs to the events, TreeFitter allows one to carry out analyses equivalent to BPA (but this function is not implemented in the current version of TreeFitter) and TreeMap. We used TreeFitter 1.0 with default settings ($C = 0$, $D = 0$, $S = 1$, $H = 2$) and TreeMap settings ($C = -1$, $D = 0$, $S = 0$, $H = 0$). In addition, we varied the host-switching cost (H) to study its effect on the reconstruction. TreeMap 1.0b was also used and its results were compared to those of TreeFitter. Compared to the current version of TreeFitter, TreeMap allows a graphic display of the results and therefore the identification of coevolutionary events. TreeMap also includes a testing procedure, by generating random trees and comparing the random number of cospeciation events in the association to the observed num-

ber, to assess whether it is significantly higher than chance alone.

Although they can theoretically work with various numbers of parasites per host (and hosts per parasite), these methods ideally require one host-one parasite associations; else the complexity of the problem could become too great to guarantee finding an optimal solution. They all compare the topologies of the host and parasite phylogenies and allow proposing precise evolutionary scenarios for the history of the association. This supposes, however, that the trees are well known. Since a topology is sensitive to the presence or absence of taxa, the methods assume a thorough knowledge and good sampling of the clades under study. But even when all species are known and sampled, consideration of an extinct species could alter the topology of the actual tree and change the proposed coevolutionary scenario (see Brooks and McLennan 1991); this problem unfortunately cannot be resolved. This is why, in addition to the comparison of the topologies, we found it desirable to use another method especially designed for the assessment of the coevolutionary character of the data.

In addition to TreeFitter and TreeMap, a new method was used in this study to test the null hypothesis (H_0), which states that each parasite species parasitizes hosts selected at random on the host phylogenetic tree. The alternative hypothesis (H_1) is that the positions of the individual host-parasite associations are not random but associate corresponding branches of the two evolutionary trees. This method, called ParaFit (Legendre et al. 2002), combines the information from three data matrices: the first one (matrix **A**, 0–1 data) contains a description of the host-parasite (H-P) association links observed in nature. In matrix **A**, the parasites are in rows and the hosts in columns, and 1 indicates the presence of a parasite on a host. The two other matrices contain some estimates of the phylogenetic trees or phylogenetic distances among the hosts and parasites, respectively. One may start with either matrices of patristic distance derived from phylogenetic trees, or raw distance matrices issued directly from the comparison of sequences, or distance matrices representing DNA/DNA hybridization data, or even distance matrices computed from morphological characters. ParaFit is not affected by polytomies in the tree and it can be used with any number of hosts per parasite or parasites per host. Matrix **B** used in the analysis contains principal coordinates (Gower 1966) representing the parasite phylogenetic tree or phylogenetic distances. Likewise, matrix **C** contains the transpose of the matrix of principal coordinates representing the host phylogenetic tree of phylogenetic distances. Matrices **B** and **C** may be incommensurable: the number of hosts and parasites may be different. Their relationship is mediated by the host-parasite relationships in matrix **A**; using the fourth-corner approach (Legendre et al. 1997), a matrix $\mathbf{D} = \mathbf{C}\,\mathbf{A}'\mathbf{B}$ is computed, and from it a trace statistic that is used to test the hypothesis of cospeciation through a permutational procedure. The values in matrix **A** are randomly permuted many times to obtain realizations of the null hypothesis. During each permutation, for each parasite, a set of hosts equal in size to the number that the parasite was actually observed to affect is picked at random. The trace statistic is computed after each permutation. The set of values of the trace statistic obtained from the permutations provides a reference distribution against which the true value of the trace statistic is assessed for significance. A test of significance for each host-parasite link in matrix **A** is also obtained as follows: compute matrix **D** and its trace statistic with and without a given H-P link, calculate the difference between the trace statistics, and test this difference by permutation. Numerical simulations have shown that the global test as well as the test of individual links have correct type-I error and good power under various types of error conditions; see Legendre et al. (2002). This test is thus complementary to the methods described in the previous paragraphs for studying host-parasite coevolution.

For the three methods used in this paper, all permutational tests of significance were performed using 999 permutations.

Results

Phylogenies

Hosts

The length of the cytochrome-*b* mtDNA sequences was 1102 bp. The saturation plot suggested a high level of saturation for the cytochrome-*b* sequences (Fig. 2a). We decided to weight the transversions at the first and third codon positions. Since the Ti/Tv ratio was estimated by ML to 14 for these positions (and to one for the second position), a weight of 14 was applied to transversions at the first and third codon positions. This value is very close to the highest Ti/Tv value observed between the closest species (*Diplodus puntazzo* and *Oblada melanura*), which is 13.4. The number of parsimony-informative characters was 355. Using a heuristic search algorithm, the tree-bisection-reconnection branch swapping option, and a random addition sequence (10 replicates), the MP analysis led to a single most-parsimonious tree (MPT) (CI = 0.515), shown in Fig. 1b. A Tamura-Nei 93 model taking into account a proportion of invariable sites and a gamma distribution for substitution rate heterogeneity (TrN 93 + I + Γ) was chosen for the ML analysis (see parameters in Table 2). The tree obtained via ML (through a heuristic search using the same settings as MP) is shown in Fig. 1c. These trees are roughly similar and differ only by the positions of *Dentex dentex* and *Spondyliosoma cantharus*. We then combined the 16S (435 bp) and cytochrome-*b* sequences before a ''total evidence'' analysis (430 parsimony-informative characters). The highly linear saturation plot observed for 16S sequences (Fig. 2b) led us not to weight transversions versus transitions for this fragment. In the MP analysis, all 16S positions were given a weight of 14, the same weight as cytochrome-*b* transversions for first and third codon positions. This analysis led to a single MPT (CI = 0.530) shown in Figure 1d. The ML analysis (heuristic search with the previous settings) was also carried out with a TrN 93 + I + Γ model (see parameters in Table 2). It led to a very similar tree, shown in Figure 1e. The only difference between these two trees is the presence of the clade (*Pagellus erythrinus* (*D. dentex*, *P. pagrus*)) in the MP tree, whereas it is (*D. dentex* (*P. pagrus*, *P. erythrinus*)) in the ML tree. We decided to keep the ML tree for the subsequent coevolution analysis, because the clade (*D. dentex* (*P. pagrus*, *P. erythrinus*)) is also found in our cytochrome-*b* tree and in the Hanel and Sturmbauer (2000) tree (Fig. 1a).

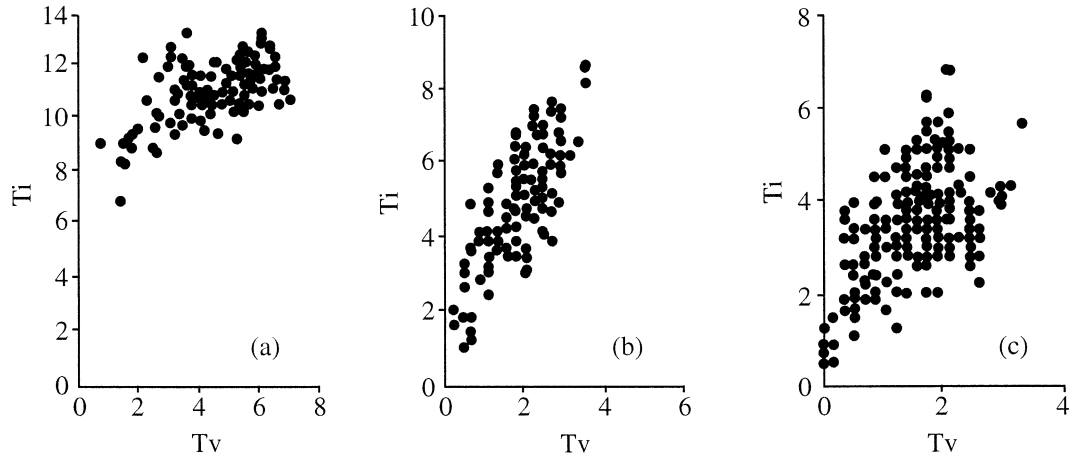

FIG. 2. Saturation plots showing distances (% differences) computed from transitions (Ti) versus distances computed from transversions (Tv). (a) Cytochrome-*b* mtDNA sequences from Sparidae. (b) 16S mtDNA sequences from Sparidae. (c) 18S rDNA sequences from *Lamellodiscus*.

Parasites

The sequence length of the partial 18S rDNA fragment varied between 524 and 525 bp, with an alignment length of 525 bp. Note that it was not possible to align unambiguously the ITS1 sequences across species. The roughly linear saturation curve observed (Fig. 2c) led us not to use any weighting scheme. The trees were first rooted using the three outgroup taxa. Since the ingroup, formed by *Lamellodiscus* spp. and *Furnestinia echeneis* (hereafter considered a *Lamellodiscus*-like species), was always clearly monophyletic in all trees, with the pattern (*Dactylogyrus minor*, *Pseudomurraytrema ardens*, *Diplectanum aequans* (*Lamellodiscus* spp.)) always present, only *D. aequans* was kept as the outgroup. The number of parsimony-informative characters was 66. The MP analysis (using the branch-and-bound algorithm) led to five MPTs (CI = 0.632). All MPTs contain three main clades, hereafter labeled after one of their representative members: the *elegans* clade, the *ignoratus* clade, and the *gracilis* clade. They differ in the placement of *L. ignoratus*, *L. knoepffleri*, and the monophyletic clade formed by *L. baeri* and *L. erythrini*. These taxa are inverted in the five MPTs, but remain together in the *ignoratus* clade. A strict consensus of these five trees is presented in Figure 3a. This consensus tree is fully compatible with a tree generated by neighbor-joining (NJ; not shown). A TrN 93 + I + Γ model with equal base frequencies was used in the ML analysis (parameters are shown in Table 2). The ML analysis, performed using a heuristic search procedure with the previous settings, produced the tree presented in Fig. 3b. This tree is roughly similar to the trees generated by MP. They differ by the basal relationship between the three main clades and the placement of *F. echeneis*, which is a sister taxon to *L. drummondi* in all MPTs, whereas it is basal to the (*L. furcosus* (*L. coronatus*, *L. elegans*)) group in the ML tree.

All these phylogenetic hypotheses will be used in the ParaFit coevolution study of the host-parasite association. TreeFitter and TreeMap require a fully resolved tree; for these analyses, we used a modified ML tree in which the polytomies were resolved following the NJ tree. This resolution of the *ignoratus* clade is compatible with what was observed in one of the MPTs, and the *gracilis* clade was never fully resolved in any of the MPTs.

Coevolution

The fully resolved host and parasite phylogenies, with the pattern of observed host and parasite associations, are presented in Figure 4.

TreeFitter

The analysis performed with TreeFitter 1.0, using default settings, suggests that there is a phylogenetic structure in this association. The fit between the host and parasite phylogenies, tested by permutation, shows that the overall cost is significantly lower than expected by chance alone (P = 0.006) and that the main factor contributing to this is a rel-

TABLE 2. Parameters used in each evolutionary model in the maximum-likelihood analyses for hosts and parasites phylogenetic reconstructions. Cyt-*b*, phylogenetic reconstruction using cytochrome-*b* mtDNA of hosts only; Comb, combined analysis using cytochrome-*b* and 16S mtDNA of hosts; 18S, analysis using 18S rDNA of parasites. Inv denotes the proportion of invariant sites, and α refers to the shape of the Γ distribution accounting for substitution rate heterogeneity.

	Base frequencies				Substitution rates							
	A	C	G	T	A–C	A–G	A–T	C–G	C–T	G–T	Inv	α
Cyt-*b*	0.259	0.329	0.123	0.289	1	13.21	1	1	12.22	1	0.544	0.968
Comb	0.278	0.303	0.148	0.271	1	9.48	1	1	12.91	1	0.567	0.819
18S	0.25	0.26	0.25	0.25	1	3.94	1	1	8.78	1	0.513	0.542

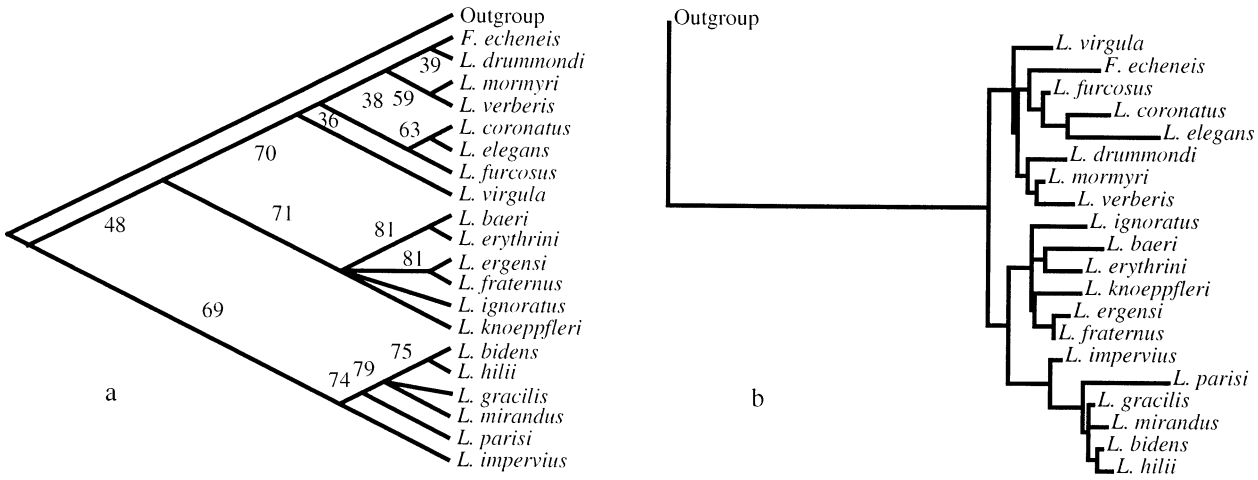

FIG. 3. Parasites phylogenetic trees estimated from 18S rDNA partial sequences; numbers are bootstrap values. (a) Strict consensus of five maximum-parsimony trees. (b) Maximum-likelihood tree.

atively small number (five to six) of switching events ($P = 0.0014$). No other type of event is common, as evidenced by the nonsignificant *P*-values for these events. If cospeciation and sorting are made almost impossible via a very high cost (2000), those significant values disappear. This implies that the significant number of host-switching events observed took place over a cospeciation-sorting background. Thus, this result suggests that some parallel cladogenesis is occurring in the system. However, these results depend highly on the costs associated with each event. If the switching cost is lowered (to one or zero, keeping default values for the other events), the global fit between the two trees is no longer significant ($P = 0.165$ for $H = 1$, and $P = 1.0$ for $H = 0$). A similar result is observed with the TreeMap settings implemented in TreeFitter ($P = 0.450$ for the global fit between the two trees).

TreeMap

As expected from the previous results, the TreeMap analysis suggests the absence of cospeciation in this association. Without invoking any host switching, reconciliation needs to

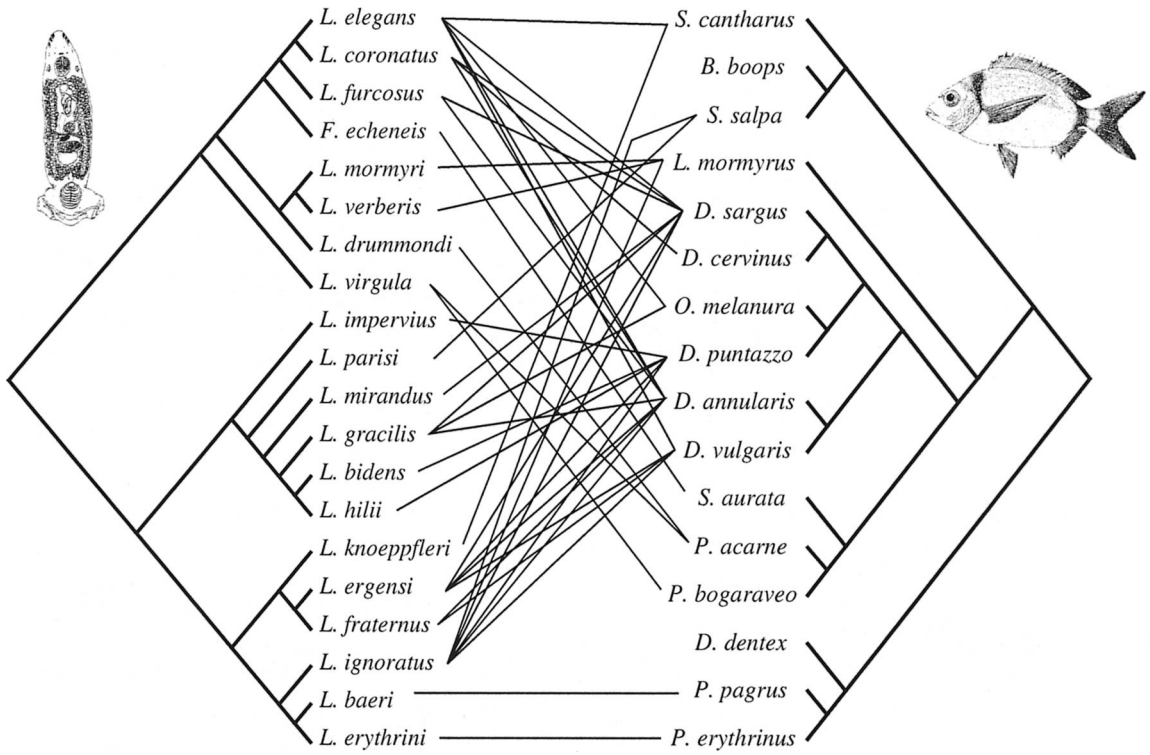

FIG. 4. Pattern of host and parasite associations, with maximum-likelihood trees estimated for the hosts and parasites. Lines depict the observed host-parasite associations. A host (*Diplodus vulgaris*, 450 mm) and a parasite (*Lamellodiscus ignoratus*, 640 μm) are also shown.

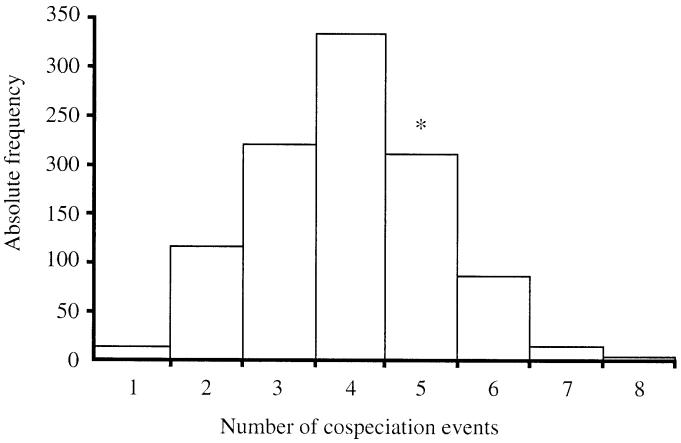

FIG. 5. Histogram generated by TreeMap: distribution of the number of cospeciation events in random associations. Asterisk indicates number of cospeciation events inferred by TreeMap for the *Lamellodiscus*-Sparidae association.

call upon five cospeciation events, 14 duplication events, and 62 sorting events to reconcile the two trees. This number of cospeciation events remains the same even if we add host-switching events in the reconstruction (using a heuristic search). This number was statistically tested using TreeMap, by repeatedly permuting the parasite tree and recalculating the number of cospeciation events, thereby generating many realizations of the null hypothesis of no association between the two trees. A thousand random parasite trees were generated using the proportional-to-distinguishable option of the program. A distribution of the number of cospeciation events was generated, and the observed number was tested against this distribution. The resulting histogram (Fig. 5), suggests that the observed number of cospeciation events is not significant ($P = 0.317$). Thus, according to this criterion, this association does not show extensive cospeciation. However, all reconstructions generated by TreeMap (not shown because they are highly complicated) suggest that cospeciation took place between (*L. baeri*, *L. erythrini*) and (*Pagrus pagrus*, *Pagellus erythrinus*).

ParaFit

The ParaFit results are the same for all the parasite phylogenetic trees, so only the results obtained for the ML tree are described. Patristic distances were computed from the ML parasite tree and compared with the patristic distances computed from the ML total evidence host tree. The global test performed by ParaFit (Table 3) indicates that there is no global relationship between the host and parasite phylogenies, mediated by the table of host-parasite association links ($P = 0.260$). This test confirms the TreeFitter and TreeMap result that the phylogenies are not generally congruent. The test computed by ParaFit for individual host-parasite links shows, however, a statistically significant structure brought by the associations *Lamellodiscus baeri-Pagrus pagrus* and *Lamellodiscus erythrini-Pagellus erythrinus* (bottom of Fig. 4), with respective probabilities of 0.028 and 0.018. When the global test is not significant but the test of an individual link is significant, simulations reported in Legendre et al. (2002) show that we may be dealing with a mixed structure containing a coevolutionary and a random portion. Therefore, Table 3 suggests that this four-species association has coevolved, confirming the result obtained from TreeMap. To investigate the link between cospeciation and host specificity, we repeated the same analyses by considering only specialist parasite species and their hosts. The same results were obtained: there is no significant global relationship between the trees ($P = 0.595$) and the only significant association is formed by *L. baeri-L. erythrini* and their hosts ($P = 0.036$ and $P = 0.015$, respectively). These results are confirmed by the TreeMap and TreeFitter analyses.

TABLE 3. Results from ParaFit. Probabilities are computed after 999 random permutations. The null hypothesis (H_0) of the global test (bottom of the table) is that parasites use hosts selected as random in the host phylogenetic tree. In the tests of individual host-parasite association links, the null hypothesis is that the link under test is random.

Parasite	Host	*P*
Furnestinia echeneis	*Sparus aurata*	0.161
Lamellodiscus baeri	*Pagrus pagrus*	0.028*
L. bidens	*Diplodus puntazzo*	0.803
L. coronatus	*D. annularis*	0.089
L. coronatus	*D. cervinus*	0.223
L. coronatus	*D. sargus*	0.210
L. drummondi	*Pagellus acarne*	0.136
L. elegans	*D. annularis*	0.065
L. elegans	*D. sargus*	0.220
L. elegans	*D. vulgaris*	0.182
L. elegans	*Oblada melanura*	0.230
L. elegans	*Spondyliosoma cantharus*	0.900
L. ergensi	*D. annularis*	0.571
L. ergensi	*D. puntazzo*	0.684
L. ergensi	*D. sargus*	0.760
L. ergensi	*D. vulgaris*	0.581
L. erythrini	*Pagellus erythrinus*	0.018*
L. fraternus	*D. annularis*	0.717
L. fraternus	*D. vulgaris*	0.666
L. furcosus	*D. annularis*	0.099
L. furcosus	*D. sargus*	0.253
L. gracilis	*D. annularis*	0.969
L. gracilis	*D. sargus*	0.800
L. gracilis	*O. melanura*	0.761
L. gracilis	*S. cantharus*	0.401
L. hilii	*D. puntazzo*	0.823
L. ignoratus	*D. annularis*	0.569
L. ignoratus	*D. puntazzo*	0.666
L. ignoratus	*D. sargus*	0.723
L. ignoratus	*D. vulgaris*	0.555
L. ignoratus	*Lithognathus mormyrus*	0.627
L. ignoratus	*Sarpa salpa*	0.620
L. impervius	*D. puntazzo*	0.828
L. knoeppfleri	*S. cantharus*	0.692
L. mirandus	*D. sargus*	0.897
L. mormyri	*L. mormyrus*	0.267
L. parisi	*S. salpa*	0.249
L. verberis	*L. mormyrus*	0.279
L. virgula	*P. acarne*	0.090
L. virgula	*Pagellus bogaraveo*	0.089
Global test		0.260

* Significant association ($P \leq 0.05$).

DISCUSSION

Phylogenies

The phylogeny for the Sparidae confirms the results of Hanel and Sturmbauer (2000) obtained using 16S mtDNA

only. However, the total evidence analysis increased the resolution of the tree: the basal nodes are better supported and the *Diplodus* clade, containing *Oblada melanura*, is now fully resolved. It also suggests that the taxonomy of the Sparidae family should be revised, as the subfamilies Boopsinae, Pagellinae, and Sparinae defined by Fiedler (1991) on the basis of dentition and diet only are not monophyletic in our tree; this cannot be assessed for the Denticinae, since they are only represented by *Dentex dentex* in our dataset. The three lineages mentioned by Hanel and Sturmbauer (2000) are found in our phylogeny. The first one (the *Boops* clade) comprises *Spondyliosoma cantharus*, *Boops boops* and *Sarpa salpa*; the second one (the *Diplodus* clade) contains all *Diplodus* species, *Oblada melanura*, *Sparus aurata*, *Pagellus acarne*, *P. bogaraveo*, and *Lithognathus mormyrus*; the third lineage (the *Pagrus* clade) is composed of *Dentex dentex*, *Pagrus pagrus*, and *Pagellus erythrinus*. Several genera appear to be polyphyletic (*Pagellus*) or paraphyletic (*Diplodus*). Our phylogenetic hypothesis suggests that the *Pagrus* clade is the most primitive one, whereas *Diplodus* is a derived genus.

The phylogenetic tree obtained for the *Lamellodiscus* species is supported by the observation of morphology, which was not included in the phylogenetic analysis. The grouping of the species is compatible with the type of their lamellodisc (attachment organ): all species in the *ignoratus* clade possess a nonsplit lamellodisc, whereas in the other two clades, species harbor a split-type. Similarly, the copulatory organ morphology in the *ignoratus* clade (type *en lyre*; see Oliver 1987) is the same for all species; the types of copulatory organs are also similar in the other two clades. These observations will be detailed in a further study. The topology is roughly the same, whatever the method employed, showing that there is a strong phylogenetic structure in the molecular dataset.

Coevolution

The phylogenetic structure found by TreeFitter for this host-parasite association is statistically significant only if we consider host switching to be a ''difficult'' event by assigning to it a cost higher than for the other three events. In the present situation, in which hosts are all sympatric and monogenean larvae are highly mobile, this cost may be overestimated in the situation corresponding to the TreeFitter default settings. Assigning an optimal cost to the various types of events is very difficult, because it may differ in every host-parasite association considered and it may depend on ecological factors. Switching weight is particularly critical (Ronquist 1995), as it greatly influences the inferred reconstruction pattern; also observed in this study. Since every host-switching event added to the reconstruction decreases the number of required cospeciation events to obtain a fit between the trees, Ronquist (1995) proposed finding the optimal switch cost by finding the cost that leads to the largest reduction in the number of cospeciation events. It could be argued, however, that this cost should be defined from biological instead of statistical data, as it is likely to be different in different types of associations. For example, this cost should be lower for parasites with dispersal stages (like monogeneans or copepods) than for parasites that are more closely dependent on their hosts for transmission (such as lice or mites). The high biological difficulty for host switching (which corresponds to a higher cost for this event) has been used to explain the high level of cospeciation in some host-parasite associations (Page and Hafner 1996).

If we consider host switching to have an equal or lower cost than sorting, all methods used—ParaFit, TreeMap, and TreeFitter—suggest that there is no cospeciation in this association. According to three different criteria (i.e., the overall cost, the number of cospeciation events, and the fit between phylogenetic distance matrices), the level of congruence in this association is not higher than expected by chance. These results confirm what had previously been suggested about fish-monogenean coevolution: that host-switching events are common (Klassen and Beverley-Burton 1987, 1988; Boeger and Kritsky 1997). Cospeciation events reported in the literature seem to be mainly found at high taxonomic levels (i.e., family or above; Boeger and Kritsky 1997). This suggests that a close phylogenetic association between the hosts and their monogenean parasites is driven by broad historical constraints (e.g., immunological or morphological) acting at large scale (i.e., high taxonomic levels). In the present case, this is consistent with the observation that *Lamellodiscus* are only found on Sparid fishes, whereas several other potential hosts can be found (Whitehead et al. 1986). This could prevent dispersal to distantly related taxa. This may also be due to an ancestral geographic separation of Sparidae leading to important parasite divergence (vicariance), impeding subsequent host-switching events across host families. This is not an absolute rule, since some switching has been hypothesized to occur in monogeneans between distant taxa (see Boeger and Kritsky 1997). At finer scale, as between species, it seems that historical constraints are less important and that dispersal is widespread. However, monogeneans are known to be highly host-specific (Baer 1957; Llewellyn 1957; Rohde 1979; Noble et al. 1989; Sasal et al. 1998), which supposes a close interaction with their hosts. Hosts may have an influence on the genetic and morphological differentiation in monogeneans, but this does not prevent subsequent host switching. This association could be seen as temporary in evolutionary time. Monogeneans seem to have an important potential for polymorphism and may be able to adapt rapidly to various conditions, as suggested by Desdevises et al. (2000), in which a single *Lamellodiscus* species was shown to exhibit two slightly different morphologies on two distinct host species.

Specialization in these monogeneans seems to be mainly under the influence of ecological factors, as supported by the study of Desdevises et al. (2002); this has also been suggested in other studies (Klassen and Beverley-Burton 1988; Bentz et al. 2001). This is supported by the observation that *D. sargus* and *D. vulgaris*, which are not sister taxa (see Fig. 3), harbor many parasites in common (see Table 1). However, these two species are ecologically close, living together in the same schools (Whitehead 1986). The putative cospeciation event between the clades (*Lamellodiscus baeri*, *L. erythrini*)-(*Pagrus pagrus*, *Pagellus erythrinus*) could be explained by the solitary behavior of *P. pagrus* (Whitehead et al. 1986). It is noteworthy that all solitary species among Sparidae studied here (data from Whitehead et al. 1986; Hanel and Sturmbauer 2000), *Sparus aurata*, *Diplodus cervinus*,

and *P. pagrus*, possess only one *Lamellodiscus* parasite species. In contrast, the host species with the highest species richness in *Lamellodiscus* are the members of the clade containing all *Diplodus* species (except *D. cervinus*), and they are all gregarious species living closely together, especially *D. sargus*, *D. vulgaris*, *D. puntazzo*, and *D. annularis* (Whitehead et al. 1986). Therefore, the social behavior of the hosts could promote host switching in *Lamellodiscus* monogeneans. The present study strongly suggests that their shared parasites were not acquired via cospeciation.

Pocket gophers and chewing lice, the ''model system'' to study host-parasite coevolution (Page and Hafner 1996), represent a very special case of biological association, because there is almost no opportunity for contact between hosts of the different species (Nadler and Hafner 1989; Nadler et al. 1990). Parasites are then separated by a strong ecological barrier. Nevertheless, some cases of incongruence interpreted as host-switching events have been observed (Hafner et al. 1994; Page 1993a; Ronquist 1995). In the case studied here, this type of ecological barrier is absent because the hosts live in sympatry. Parasites have many opportunities to switch hosts, and this seems to happen in nature. This suggests that host choices by parasites and subsequent specialization are not driven by historical factors, at least not in an important proportion. Cospeciation may be a by-product of host separation, either geographical or behavioral (see Bentz et al. 2001), and it seems to be controlled by allopatric speciation. This was also suggested by Reed and Hafner (1997) for the pocket gopher-chewing lice association.

It has been argued that host specificity is highly linked with cospeciation processes (Poulin 1992; Kearn 1994). In the association studied here, however, the specialist parasites considered as a clade do not exhibit any cospeciation pattern with their hosts. This suggests that the processes leading to specificity do not necessary lead to host-parasite cospeciation. The presence of no cospeciation associated with the presence of specialist species is interpreted by Brooks (1979) to represent a strong ecological host-parasite association for these species (see Klassen and Beverley-Burton 1988), and suggests adaptive processes. This was emphasized by Hoberg (1986), who proposed that pronounced host specificity and host-parasite cospeciation may not always be associated, especially in groups of great evolutionary age. In the present case, the observation that the ITS1 sequences are not alignable among species, and that rDNA 18S, a relatively slow-evolving molecule (Hillis and Dixon 1991), contains sufficient phylogenetic signal to infer the phylogeny of the *Lamellodiscus* species, is in favor of an old age for this genus. However, these observations may also indicate a fast evolutionary rate. The *Lamellodiscus* species seem to be able to speciate rapidly, even in sympatric conditions; the host and parasite phylogenies support the hypothesis of sympatric speciation for some species, like *L. bidens* and *L. hilii* on *Diplodus puntazzo*, and *L. mormyri* and *L. verberis* on *Lithognathus mormyrus*. This ability to speciate, and the many opportunities for host switching, could explain the absence of cospeciation pattern observed here, even for specialist species. Secord and Kareiva (1996) emphasized that the colonization ability of a parasite is a function of its morphological variability (polymorphism): some morphs would allow the use of different hosts and can lead to subsequent speciation. Such intraspecific morphological variability has been observed in monogeneans (e.g., Mo 1991a,b), among them the *Lamellodiscus* species (Desdevises et al. 2000). This supports the hypothesis of an important potential for adaptability in monogeneans, leading to their opportunistic colonization behavior.

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