Comparing Host and Parasite Phylogenies: Gyrodactylus Flatworms Jumping from Goby to Goby

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Abstract.—The combination of exceptionally high species diversity, high host specificity, and a complex reproduction system raises many questions about the underlying mechanisms triggering speciation in the flatworm genus Gyrodactylus. The coevolutionary history with their goby hosts was investigated using both topology- and distance-based approaches; phylogenies were constructed of the V4 region of the 18S rRNA and the complete ITS rDNA region for the parasites, and 12S and 16S mtDNA fragments for the hosts. The overall fit between both trees was significant according to the topology-based programs (TreeMap 1.0, 2.0β and TreeFitter), but not according to the timed analysis in TreeMap 2.0β and the distance-based method (ParaFit). An absolute timing of speciation events in host and parasite ruled out the possibility of synchronous speciation for the gill parasites, favouring the distance-based result. Based on this information together with the biological background of host and parasite, the following TreeMap solution was selected. The group of gill parasites evolved from a host switch from G. arcuatus, parasitizing the three-spined stickleback onto the gobies, followed by several host-switching events among the respective goby hosts. The timing of these events is estimated to date back to the Late Pleistocene, suggesting a role for refugia-mediated mixing of parasite species. In contrast, it is suggested that speciation in the fin-parasites resulted in several host-associated species complexes. This illustrates that phylogenetically conserved host-switching mimics the phylogenetic signature of cospeciation, confounding topology-based programs. [Coevolution; Gobies; Gyrodactylus; host-parasite interactions; host-switching; speciation.]

One of the most significant radiations of platyhelminth fish parasites is documented in the monogenean “super-genus” Gyrodactylus (Cribb et al., 2002). The 409 Gyrodactylus species named so far (Harris et al., 2004) are expected to represent only 2% to 10% of the estimated diversity (Bakke et al., 2002). Gyrodactylus displays the widest host range of any monogenean family (19 orders of bony fish), encompassing both highly specific and generalist species. These remarkable facts can be linked with their even more remarkable reproduction mode. Gyrodactylus spp. contain a fully grown daughter in utero, which in turn encloses a developing embryo, boxed inside one another like Russian dolls; asexual, parthenogenetic and sexual reproduction alternate (Cable and Harris, 2002). In optimal conditions, a first-born daughter can be produced within 24 hours of her parent’s birth (Cable and Harris, 2002). This can result in explosive population growths, and the direct life cycle (only one host involved) allows them to invade new habitats with their hosts. All these factors may accelerate population differentiation and thus speciation.

On the one hand, the direct life cycle and the high host-specificity enforce a tight relationship of a Gyrodactylus species and its host, promoting coevolution (Poulin, 1992; Kearn, 1994). On the other hand, the ability to produce an entire population from a single individual increases the chance for sympatric speciation and speciation by host-switching (Brooks and McLennan, 1993). They may also survive for some time independently of their host and a “swimming behavior” has been observed (Cable et al., 2002). These features add to the colonizing capability of Gyrodactylus, illustrated by several switches between fish families (Harris, 1993; Žíťara and Lumme, 2002) and even fish orders (Huyse et al., 2003). However, phylogenetic radiations (diversification constrained/triggered by host phylogeny) have been described as well (Bakke et al., 2002). As such, all speciation modes appear plausible in this group.

In spite of these interesting characteristics, together with the fact that Gyrodactylus may pose serious threats to aquaculture (e.g., the highly pathogenic salmon parasite G. salaris), quantitative host-parasite cospeciation studies are lacking (Bakke et al., 2002). According to Page et al. (1996), the prerequisites for coevolutionary studies are (1) the availability of a sound alpha taxonomy of both host and parasite, (2) robust phylogenies of hosts and parasites, (3) although not essential, molecular phylogenies are preferably based on homologous characters, (4) wide taxon sampling, and (5) quantitative comparison of host and parasite trees by means of explicit statistical tests. The first four prerequisites are fulfilled in our model consisting of the Gyrodactylus parasites living on gobies of the genus Pomatoschistus. (1) The various Gyrodactylus taxa have been described by morphological, morphometric, and molecular methods (Huyse and Malmberg, 2004; Huyse et al., 2004a), whereas the host species have been extensively studied by Miller (1986). They occur in the Atlantic and Mediterranean coasts of Europe; some goby species are sympatric and hybridization has been reported (Webb, 1980). (2) The phylogenetic relationships of Gyrodactylus have been studied using the complete ITS rDNA region and the V4 region of the 18S rRNA (Huyse et al., 2003); the phylogeny of the host has been reconstructed from 12S and 16S mtDNA fragments, and in order to compare homologous characters. (3) The ITS 1 locus has been sequenced as well (Huyse et al., 2004b). (4) In order to minimize sampling bias, sampling has been extended over time and space throughout the natural geographical distribution of the fish hosts (see below).

All respective Gyrodactylus species display phylogenetic host specificity towards gobies of the genus...
Pomatoschistus (Huyse et al., 2003). They can be split into two groups by their genetic and ecological parameters. Group A represents a monophyletic group of closely related and host-specific species, mainly infecting gills, all belonging to the subgenus G. (Mesonephrotus). Host specificity is lower in group B, which comprises species that are dominantly found on fin and skin and belong to G. (Paranephrotus). In this article, we fulfill the last prerequisite for coevolutionary studies by comparing host and parasite phylogenies using a combination of statistical approaches. In addition, we test whether the difference in niche is reflected in the evolution and distribution on the host species: Are exposed fin parasites more subject to host-switching than the more “sheltered” gill parasites? Did the monophyletic group of gill parasites evolve through cospeciation with the host, as generally expected for host-specific parasites (Poulin, 1992; Kearns, 1994)? Alternatively, did the group of the more generalist fin parasites evolve through repeated host-switching events instead of cospeciation?

**Material and Methods**

**Host and Parasite Data**

During 1999 to 2002, 15 Gyrodactylus taxa were collected on Gobiusculus flavescens and several Pomatoschistus species (Gobiidae, Teleostei) along the northeastern Atlantic continental shelf, the Baltic, Adriatic, and Mediterranean Seas (see Huyse et al., 2003). Two Gyrodactylus parasites of the three-spined stickleback, Gasterosteus aculeatus, were collected as outgroup species (G. arcuatus and G. branchicus). Phylogenetic relationships of the Gyrodactylus parasites were inferred from the V4 region of the 18S rRNA and the complete ITS rDNA region obtained in a previous study (Huyse et al., 2002, 2003). The phylogenetic relationships of the goby hosts were derived from ITS 1 rDNA and 12S and 16S mtDNA (Huyse et al., 2004b).

**Phylogeny Reconstruction**

The V4 and ITS region of the parasites and the 12S and 16S sequences of the hosts were treated as one data set because the incongruence-length difference test (Farris et al., 1995) implemented in PAUP+ (Swofford, 2001) provided no evidence for significant difference in the phylogenetic signal of both regions (P = 0.10, P = 0.65, respectively). Analyses have been conducted on three data sets consisting of groups A and B separately and all parasite species together. Within the respective groups the 5.8S sequence was identical, so only the ITS 1, ITS 2, and V4 sequences were aligned using Clustal X v. 1.81 (Thompson et al., 1997). When pooling groups, the highly variable ITS 1 region was omitted and only the V4, 5.8S, and ITS 2 sequences were aligned using the program SOAP (Löytynoja and Milinkovitch, 2002). This program identifies unstable sites, which are easily excluded and reincluded during subsequent analyses. All data matrices are available from TreeBASE (http://www.treebase.org/). The base composition for all sequences was compared using a 5% χ² test on the average composition (TREE-PUZZLE v. 5.0, Schmidt et al., 2002). Plotting transitions and transversions against divergence of the complete data set using DAMBE v. 4.0.75 (Xia and Xie, 2001) did not show saturation. The molecular-clock hypothesis was tested assuming the HKY+Γ model with the likelihood-ratio test (LRT) for the clock hypothesis implemented in TREE-PUZZLE. ModelTest v. 3.06 (Posada and Crandall, 1998) was used to estimate the optimal model of molecular evolution in a likelihood-testing framework. These parameters were used in the maximum likelihood (ML) method using PAUP⁺; trees were statistically tested by calculating P values for the individual branches. This likelihood-ratio test assesses whether branch-lengths are significantly different from zero by optimizing all branch lengths under the constraint that one of the branches is zero, for each branch tested. For maximum parsimony (MP) the exhaustive search method was performed using the branch and bound algorithm. In these analyses gaps were treated both as missing data and fifth character because indels might be phylogenetically informative; all sites were equally weighted. The neighbor-joining (NJ) search was conducted (1000 replicates of tree-bissection reconnection branch swapping) from a matrix of ML genetic distances calculated under the optimized model. ML branch lengths have been generated under the specified model and topology, with and without a molecular clock assumption. Bayesian inference (BI) using MrBayes v. 3.0b4 (Huelsenbeck and Ronquist, 2001) was performed, using the model obtained by ModelTest. Posterior probabilities were estimated over 2.0 × 10⁶ generations, sampling the Markov chains at an interval of 100 generations. In addition, four incrementally heated Markov chains were used. Sample points generated before reaching stationary were discarded as “burn in” samples. Shimodaira-Hasegawa (SH) tests were used to compare alternative MP and ML topologies obtained during the analyses (Shimodaira and Hasegawa, 1999), as implemented in PAUP⁺. If no significant differences were found, only the ML topology was used; otherwise all topologies were investigated.

**Testing for Cospeciation**

Several methods for testing cospeciation are available; most of them have been reviewed by Paterson and Banks (2001). Four of these methods were used to analyze the host-parasite interactions in the present system. The first method, TreeMap 1.0 (Page, 1994), reconciles the host and the parasite tree by introducing four types of events: cospeciation (C), host-switching (H), duplication or intrahost speciation of the parasite (D), and sorting or extinction of the parasite lineage (S). Using a parsimony argument, the program attempts to explain the differences between both phylogenies by postulating the fewest possible number of events, and maximizing the number of cospeciation events. A randomization test was performed to assess if both phylogenies are more similar to each other than expected by chance alone.
Completely resolved trees are necessary, but alternative tree topologies can be imported and evaluated. Recently, the beta version of TreeMap 2.0 was released (Charleston and Page, 2002 available at http://taxonomy.zoology.gla.ac.uk/~mac/treemap/), which computes all optimal solutions by exhaustive search, represented by jungles (Charleston, 1998). In the timed analysis, branch length information can be taken into account. It also allows an assignment of different costs to each of the four cophylogenetic events, a feature also available in the program TreeFitter v. 1.1 (Ronquist, 2001, available at http://www.ebc.uu.se/systzoo/research/treefitter/treefitter.html). The optimal reconstruction is that which minimizes the global cost. TreeFitter uses a permutational procedure to statistically test the overall cost and contribution of each type of event.

Whereas these methods are topology based, the program ParaFit (Legendre et al., 2002) makes use of raw or patristic distances, thus overcoming the need for well-resolved topologies. It statistically assesses the fit between host and parasite phylogenetic distance matrices mediated by the matrix of host-parasite links. Moreover, ParaFit can estimate and test the contribution of each individual link to this global fit. These distance matrices can be computed from any data including sequence data, DNA/DNA hybridization data, or morphological characters. In the present study both the ML genetic distances and the patristic distances were compared (i.e., the ITS 1 region, from both the host and the parasite phylogenies were used.

Host-switching and cospeciation can also be detected by analyzing genetic distances (Paterson and Banks, 2001), on the condition that hosts and parasites evolve at a constant rate (which may differ in both groups). There-}

**RESULTS**

**Phylogenetic Analyses: Are the Phylogenies Resolved?**

A total of about 800 bp of 12S and 16S mtDNA were used for the construction of the host phylogeny; the base composition was homogenous and the GTR+I+\(\Gamma\) model was selected with the gamma shape parameter and the proportion of invariable sites estimated at 0.6. Because the Bayesian, ML, MP, and NJ topologies were not significantly different (\(P > 0.05\)), the ML tree was used as input file. The relationships between the closely related *Pomatostichus minutes*, *P. lozanoi*, and *P. norvegicus* were not resolved, but the three possible branching patterns imported in TreeMap did not affect the number of cospeciating nodes. Exclusion of the unstable characters in the V4 and complete ITS region of the parasites resulted in a 675-bp fragment. An alternative evaluation of the alignment by means of dot plots implemented in the GeneWorks software (Intelligenetics, Oxford, UK) resulted in a similar fragment of 690 bp. The resulting V4-5.8S-ITS 2 data set had no deviating base composition (\(P = 0.70–0.92\)) and yielded 118 parsimony informative sites. When the unstable alignment positions were re-included in the analysis or when gaps were treated as a fifth character, the number of parsimony informative sites increased to 136 and 126, respectively. This did not affect the topology; the bootstrap values varied only slightly. The complete parasite tree, including groups A (gill parasites) and B (fin parasites), was not completely resolved. Separate analyses (A and B alone) included the complete data set (a total of 1125 bp) of group A; because the 5′ ITS 1 region of group B was still too variable, only the 3′ end of ITS 1 was used, resulting in a fragment of 1125 bp. Both data sets displayed homogenous base composition and resulted in fully resolved phylogenies; GTR+\(\Gamma\) was used with gamma shape parameter estimated at 0.3. These phylogenies were used as backbone constraints in the analysis on the complete data set. Both host and parasite sequences evolved in a clock-like fashion as tested by TREE-PUZZLE.

**Comparison of ITS 1 Variation between Host and Parasite**

The ITS 1 sequences of the gobies and the *Gyrodactylus* spp. of group A behaved clock-like according to the LRT in TREE-PUZZLE, in contrast to group B. The genetic distances constructed from the ITS 1 region of the hosts *Pomatostichus minutes* and *P. microps*, and their respective parasites, are presented in Table 1. Each time, the parasite sister species were compared (i.e., comparison within group A and group B, respectively). With the exception of *G. rugiensoides*–*G. microps*, genetic distances were much higher between the host-pair than the respective parasite species-pairs.

**Is There Evidence for Coevolution?**

**TreeFitter.**—Using the default settings (\(C = 0, D = 0, S = 1, H = 2\)), the fit between the host and parasite phylogenies showed that the overall cost is significantly lower than expected by chance (\(P = 0.01; 1000 permutations\)). However, it could not be determined which cophylogenetic event contributed to this fit because none of the individual \(P\)-values were significant. Lowering the cost of host-switching from 2 to 1.5 (considering high parasite dispersal capabilities and sympathy of host species), resulted in significant values for the number of codivergence events (six to eight events; \(P = 0.034\) and the number of host-switching events (six to eight events, \(P = 0.008\)). Applying maximum codivergence
settings \((C = -1, D = 0, S = 0, \text{ and } H = 0)\), the global fit between the two trees was not significant anymore \((P = 0.1)\). By assigning a cost of 1 to sorting and host-switching events, the fit was significant \((P = 0.017)\) with host-switching as the main factor contributing to this \((four \text{ to } six \text{ events}; \ P = 0.023)\). If cospeciation and sorting were assigned a very high cost (Fitch optimization), the significant values disappeared, confirming the signal of cospeciation in the present host-parasite system.

**TreeMap 1.0.**—Without invoking any host-switching event, TreeMap had to introduce seven cospeciation events, nine duplications, and 27 sorting events to reconcile the trees. Adding host-switching events (using a heuristic search) lead to the postulation of seven cospeciation events, eight duplications, one host-switching, and 23 sorting events. By randomizing the parasite tree with the proportional-to-distinguishable option, a null frequency distribution was generated. The observed number of cospeciations appeared significantly higher \((P = 0.01)\) than expected by chance. The percentage of cospeciating nodes (i.e., the number of cospeciating nodes divided by the total number of nodes in the parasite phylogeny, multiplied by 100) amounted to 44%. Figure 1 shows the host and parasite phylograms and the respective host-parasite associations.

**TreeMap 2.0β.**—In the nontimed analysis (using only topology and no branch length information), 22 optimal reconstructions were found. The optimal solutions postulated 16 cospeciations, five losses, six switches, and 16 duplication events (Fig. 2). The randomization test on the complete data set suggested that the global fit between the host and parasite trees was statistically significant \((P = 0.02 \pm 0.01)\). When the data set was split in groups A and B, only the association between group A and the host tree appeared to be significant \((P = 0.01 \pm 0.01)\) with 11 optimal reconstructions; the level of congruence between group B and the host tree was not higher than expected by chance \((P = 0.11 \pm 0.03)\). In the timed analyses (using branch length information but not having the host and parasite tree set on the same scale), the number of cospeciation events in the gill parasites was not significant anymore.

**ParaFit.**—For each of the three data sets, both raw and patristic distances were used as input matrix. The global test of cospeciation on the complete data set of raw distances did not reveal a global association between hosts and parasites \((P = 0.095)\). Using patristic distances did not influence the results \((P = 0.094)\). Considering the

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**FIGURE 1.** Evolutionary patterns of host association in *Gyrodactylus* species. Comparison of the goby host (left) and *Gyrodactylus* (right) phylograms, constructed from 12S and 16S mtDNA and the V4 and ITS region, respectively. TreeMap 1.0 (Page, 1994) called upon seven cospeciation events (denoted as a black circle; \(P = 0.01\)) to reconcile both trees. Branch lengths are proportional to the amount of evolutionary change, except for the branches connecting the ingroup with the outgroup sequences. Bootstrap support from minimum evolution analysis \((n = 1000)\). Evolutionary rate for host and parasite 1% and 5.5%, respectively, per million years (Huyse et al., 2004b; Ziętara and Lumme, 2002). “B” and “V” following the species *G. ostendicus* and *G. branchialis* stand for the collection sites “Belgium” and “Venice” respectively. Groups A and B are boxed with dotted lines. (Picture from *Pomatoschistus microps* by Van Kampen; scanning electron micrograph of *Gyrodactylus ostendicus* with scale bar = 100 µm).
individual host-parasite links, only the links between the outgroup host and parasites were significant. However, when a separate matrix was constructed from the complete data set of group B (about 1300 bp), the result of the global test was highly significant ($P = 0.001$). Significant host-parasite links include: *G. cf. micropsi* and *P. minutus/P. lozanoi; G. cf. micropsi 2 and P. minutus/P. lozanoi*. In contrast, when taking the gill parasites separately, only the link between *G. gondae* and *P. minutus/P. lozanoi* turned out to be significant ($P = 0.023$), besides the *G. arcuatus* and *G. aculeatus* link ($P = 0.029$). However, the overall signal was also significant ($P = 0.029$).
DISCUSSION

So far, phylogenetic congruence is imperfect or absent in most interactions (Poulin, 1998). As also illustrated in this study, most associations represent a combination of cospeciation and host-switching (Page and Hafner, 1996; Roy et al., 2001; Weiblen and Bush, 2002; Ricklefs et al., 2004). Examples of strict cospeciation are found in systems where host-switching is prevented by the asocial lifestyle of the host and the low mobility of the parasite. Examples include the rodent-lice (Hafner and Nadler, 1988; Page and Hafner, 1996), and insect-symbiotic associations where the bacteria, needed for host reproduction, are transmitted maternally (Clark et al., 2000). The only other study on flatworm-fish associations has been conducted by Desdevises et al. (2002). No significant signal of cospeciation could be detected and they concluded that specialization is mainly influenced by ecological factors. Solitary fish species harbored a single parasite species, whereas the highest species richness was found on gregarious fish living in sympathy.

Because Gyrodactylus species can readily disperse to new hosts (see introductory section), speciation by host-switching is expected to be an important speciation mode (Brooks and McLennan, 1993; Bakke et al., 2002). The impressive colonizing capability of Gyrodactylus is demonstrated by several ecological radiations (diversification triggered by ecological factors) on distant fish species (Harris, 1993; Zieta and Lumme, 2002) and the epidemic spread of G. salaris on Atlantic salmon in Norway (Johnsen and Jensen, 1991). As such, the significant signal of cospeciation produced by the topology-based programs was rather unexpected. On the other hand, due to their direct life cycle and progenetic development, Gyrodactylus species live in close association with their host. It is shown that Gyrodactylus-host interactions are very subtle and host specificity among monogeneans is governed by a number of dynamic interactions (Buchmann and Lindenstrom, 2002).

In contrast to the topology-based programs, the distance-based approaches suggested that there was no significant fit between both data sets. These contrasting results reflect the differences between the underlying methods (topology versus distance) and possibly the genes under study. Therefore, we used a complementary approach by absolute and relative timing of speciation in both host and parasite (see below).

Is Cospeciation More Frequent in the Host-Specific and Monophyletic Gill Parasites?

TreeMap 1.0 and nontimed 2.0β suggested a significant fit between the host and parasite trees, in contrast to the timed analysis and ParaFit. According to the topology-based programs the number of cospeciation events was higher than expected by chance, although host-switching events were suggested as well. The 11 proposed solutions by TreeMap 2.0β could be divided in either predominantly cospeciating or predominantly host-switching scenarios. To discriminate between both scenarios, relative or absolute calibration of both host and parasite trees is necessary (see below).

Is Host-Switching More Frequent in the Fin Parasites?

In contrast to the previous case, the number of cospeciation events was not significant according to TreeMap whereas the fit between host and parasite trees was highly significant according to ParaFit. Page (1993) pointed out that the presence of one or more parasite lineages on the same host results in incongruent host and parasite phylogenies. As seen on Figure 1, up to four Gyrodactylus species were present on a single host species. However, TreeMap reduces the multihost–parasite associations to a single association as can be seen in Figure 2. The low host specificity of some species further complicates the comparison of the host and parasite phylogenies in TreeMap. This might partly explain the incongruent results of both programs.

As is shown here, host specificity is not necessarily a prerequisite for cospeciation. Although G. rugiensoides is found on Pomatoschistus minutus, P. lozanoi, and P. pictus, it might have coevolved with one of these hosts and colonized the other hosts without speciating (Brooks and McLennan, 1993). Generalism, like specificity, is sometimes a pure transitional state rather than a fixed trait (Nosil, 2002). A specialist colonizing a new host might first become a generalist (increases its host range), and eventually speciates on its new host(s). Laboratory experiments are required to test such hypothesis. For example, P. minutus and P. lozanoi occur in sympathy and share Gyrodactylus gondae, but this parasite species is not found on the closely related P. norvegicus. By experimentally transferring G. gondae populations grown on P. lozanoi to P. minutus and P. norvegicus (and analogous for populations grown on P. minutus), the reproductive success in the different cross-infections can be compared. If each population performs best on its original host, this might indicate local adaptation. This can be regarded as an onset to specialisation. If each population performs equally well on all hosts, G. gondae should be regarded as a generalist species. Information on the mtDNA variation between G. gondae populations collected from the two host species might already give a clue on possible population differentiation. According to Futuyma and Moreno (1988), the ultimate cause for specificity is linked to behavior. However, an experiment by Clayton et al. (2003) highlighted the importance of host defence in reinforcing host specificity of feather lice infecting birds. To test whether parasite behavior rather than host defence is the underlying factor in this system, all host species can be mixed in a subsequent experiment and monitored for infection levels. If the result is different from the previous experiment, this is likely due to preferential host colonization by the parasite.

The P. minutus complex consists of the closely related gobies P. minutus, P. lozanoi, and P. norvegicus. Sympathy of P. minutus and P. lozanoi might explain why they share so many Gyrodactylus species (e.g., G. gondae, G. rugiensoides, G. cf. micropsi). However, the fact that P. lozanoi also
harbors a unique parasite *G. longidactylus* (Geets et al., 1998) proves that host-switching does not always occur whenever possible. This might also be seen as cases of inertia (Paterson and Banks, 2001), where a parasite species fails to speciate despite speciation of its host (Johnson et al., 2003).

*Pomatoschistus norvegicus*, which is ecologically more isolated by occupying the deeper sections of the continental shelf down to 200 m (Miller, 1986), is caught infected with a similar species here referred to as *G. cf. longidactylus*. Morphological analysis showed distinct differences between both species (personal data); a molecular comparison is in progress. This indicates the possibility of another host-associated species complex as previously described for the species pairs *G. rugiensis*–*G. rugiensoides* and *G. micropsi–G. cf. micropsi* (Huyse and Volckaert, 2002).

### Are There Instances of Sympatric Speciation?

The dominant mode of speciation in the present system appeared to be allopatric. However, one instance of recent intrahost speciation might have led to *Gyrodactylus cf. micropsi* and *G. cf. micropsi 1*, who are each other’s closest relative found on the same host. The programs TreeMap and TreeFitter also pointed to the importance of historical duplication or intrahost speciation events. This can be expected from the biology and population structure of *Gyrodactylus* characterized by high host specificity and autoinfection of their hosts. It has been stated that if sympatric speciation occurs, it is most likely in parasite groups like monogeneans (Brooks and McLennan, 1993; Gusev, 1995; Poulin, 1998, 2002). A recent molecular phylogenetic study showed that the main process of *Dactylogyrus* (Monogenea) diversification on cyprinid hosts was intrahost speciation (Šimková et al., 2004). The authors found that sister taxa on the same host tend to occupy niches differing at least in one niche parameter. Because monogeneans infect long-lived hosts, they may generate several generations on the same host individual, ensuring a deme continuous in time and space and reinforced by inbreeding to such an extent that they generate incipient species. Also, gene flow might initially be severed by asexual or parthenogenetic reproduction (Brooks and McLennan, 1993) or niche differentiation and specialization (de Meûs et al., 1998).

### Tentative Timing of Events

It is always difficult to estimate the evolutionary rate for a given gene fragment, especially when no fossil data are at hand. In case of the tiny, soft bodied *Gyrodactylus* flatworms, only well-documented (recent) vicariance events can provide critical information. Recently such an attempt has been made by Ziétara and Lumme (2002). Their estimate was based on the divergence of *G. aphyae* living on the minnow *Phoxinus phoxinus* on opposite sides of the Baltic–White Sea watershed. Connecting this divergence with the well-documented divergence time of the host, the authors obtained a rate of 5.5% per million years (My). If this rate is applied, most of the (sister) speciation events in groups A and B fall within the Late Pleistocene period. In case of the hosts, speciation is thought to have occurred in the early Pliocene, with exception of the *P. minutus* complex that originated in the Pleistocene (Huyse et al., 2004b). In this case, all recent speciation events in *Gyrodactylus* should be the result of duplication or host-switching. This is in agreement with ParaFit and the comparison of genetic distances based on homologous gene fragments in host and parasite (ITS 1). The divergence in the hosts was much greater than the distance between the respective parasites (Table 1). If ITS 1 in *Gyrodactylus* does not evolve at a slower rate than in fish, this would indicate a more recent speciation of the parasites then of the hosts. Due to the rapid generation time (24 to 72 hours) and a mean body size of 350 μm, we might actually expect a relatively faster rate for *Gyrodactylus* compared to the host (as illustrated for COI mtDNA, see below).

According to the clock calibration, the split between *G. arciatus* and the *Gyrodactylus* species, found on the gobies occurred 1.82 to 2.05 Mya. Such timing would support the scenario of a host-switching from three-spined stickleback onto *Pomatoschistus* gobies (see Fig. 2A and C), possibly when sharing the same refugium, e.g., the Bay of Biscay during the Pleistocene ice ages (Garcia-Marin, 2001). This event might have been followed by a combination of host-switching and cospeciation with the respective gobies.

The speciation between *G. gondae* and *G. flavescens* is estimated at 0.18 Mya. However, because the speciation between their hosts *Gobiobius flavescens* and *P. minutus* is much older (Huyse et al., 2004b), cospeciation, as suggested by Figure 2B, is rejected. Rather, the scenario described in Figure 2C, characterized by successive host-switching events between the goby species, is favored. Such phylogenetically constrained host-switching produce nonrandom patterns of phylogenetic congruence, which has also been described in plant-insect interactions (Percy et al., 2004). Information on the absolute timing

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<th>Host species</th>
<th>Probability</th>
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<td><em>Gasterosteus aculeatus</em></td>
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</tr>
<tr>
<td><em>G. rugiensis</em></td>
<td><em>P. microps</em></td>
<td>0.069</td>
</tr>
<tr>
<td><em>G. rugiensoides</em></td>
<td><em>P. minutus</em></td>
<td>0.013</td>
</tr>
<tr>
<td><em>G. rugiensoides</em></td>
<td><em>P. lozanoi</em></td>
<td>0.023</td>
</tr>
<tr>
<td><em>G. rugiensoides</em></td>
<td><em>Pomatoschistus pictus</em></td>
<td>0.030</td>
</tr>
<tr>
<td>Global test</td>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>
of host and parasite speciations may help to distinguish this pattern from genuine cospeciation.

The occurrence of very closely related taxa on the sister species *P. microps* and *P. marnoratus* might falsely appear as two cospeciating nodes, heavily influencing the topology-based programs. *G. branchialis* V, collected in Venice from *P. marnoratus*, differs only in 0.2% (complete ITS region) from *G. branchialis* B, collected in Belgium from *P. microps*, whereas the hosts differ in 3% based on 16S mtDNA. In a study by Huyse et al. (2003), it is estimated that the two hosts speciated about 3 Mya. If the parasites speciated at the same time, the ITS region should evolve at a rate of 0.07% per My. Because the internal transcribed spacers are generally thought to be rapidly evolving because they are noncoding (Hillis et al., 1996), and the rate in *Gyrodactylus* is estimated to be much higher (5.5% per My, see above), this association cannot be the result of a cospeciation event. Excluding one taxon from a single association (*G. branchialis* V), the amount of cospeciation events indicated by TreeMap 1.0 is not significant anymore. This suggests that the signal of cospeciation was, at least partly, generated by phylogenetically constrained host-switching events.

**Future Perspectives**

Attempts have been made to find another homologous marker. Currently, the 16S mtDNA is being sequenced according to expectations because the COI mtDNA has been estimated to evolve at a rate of 28% per million years in *G. salaris* (Meinilä et al., 2004). Therefore, the COI mtDNA gene in parasites might be too variable for reliable phylogeny reconstruction. Although homologous genes are a requirement for meaningful comparisons of the rate of evolution in hosts and parasites, it is not, by itself, enough. Any statement about overall difference in rate requires that the same gene is evolving in a correlated fashion in both host and parasite, such that branch lengths in the host are a constant proportion of those in the parasite. The life history features of *Gyrodactylus* (mean body size of 350 µm and generation time in optimal conditions of 24 hours) are likely to have a higher impact on the evolutionary rate of a gene than its function. Therefore we argue that the present data set, consisting of nonhomologous genes but with a clock-like rate, should be suitable to address questions on cospeciation between *Gyrodactylus* and its fish hosts.

In conclusion, the evolutionary history of *Gyrodactylus* species and their goby hosts has gone through periods of cospeciation, followed by (and intermingled with) periods of successive host-switching events. These switches have most likely been triggered by alternating Pleistocene ice ages. Eventually a new period of coevolution may appear, but considering the colonizing capacities of *Gyrodactylus*, future host-switches are bound to happen.

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**REFERENCES**


Huyse, T., and F. A. M. Volckaert. 2002. Identification of a host-

Huyse, T., J. Van Houdt, and F. A. M. Volckaert. 2004b. Paleoclimatic

Meinilä, M, J. Kuusela, M. S. Ziara, and J. Lumme. 2004. Initial steps


Johnsen, B. O., and A. J. Jensen. 1991. The


Page, R. D. M. 1994. Parallel phylogenies: Reconstructing the history

Page, R. D. M. 1993. Parasites, phylogeny and cospeciation. Int. J. Par-

Nosil, P. 2002. Transition rates between specialization and generaliza-


Ricklefs, R. E., S. M. Fallon, and E. Bermingham. 2004. Evolutionary relationships, co-speciation and host switching in avian malaria par-

Roy, B. A. 2001. Patterns of association between crucifers and their flower-mimic pathogens: Host jumps are more common than coevo-


Šimková, A., S. Morand, E. Jobet, M. Gelinar, and O. Verneau. 2004. Molecular phylogeny of congeneric monogenean parasites (Dactyl-


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