



Putative speciation events in *Lamellodiscus* (Monogenea: Diplectanidae) assessed by a morphometric approach

TIMOTHÉE POISOT^{1,2*} and YVES DESDEVISES^{1,2}

¹UPMC Univ Paris 06, UMR 7628, Modèles en biologie cellulaire et évolutive, Observatoire Océanologique, 6665, Banyuls/Mer, France

²CNRS, UMR 7628, Modèles en biologie cellulaire et évolutive, Observatoire Océanologique, 6665, Banyuls/Mer, France

Received 21 July 2009; accepted for publication 18 September 2009

In the present study, we used morphometry as a proxy to study the microevolution of generalist *Lamellodiscus* (Monogenea, Diplectanidae) species, comprising gill parasites of sparid fish. We investigated 147 individuals, belonging to nine described species, regrouped in four morphotypes. Morphometric measurements were taken on sclerotized parts of the attachment organ. The formation of groups on the basis of the global morphometry within a host species, or between several host species, was assessed using both exploratory analyses (principal component analysis and clustering analysis) and statistical tests. We showed that: (1) for three out of four morphotypes, the global morphometry was significantly different according to host species used, and (2) the coexistence of two populations of *Lamellodiscus elegans* on *Diplodus sargus* could reflect an ongoing intra-host speciation event. We suggest that generalist *Lamellodiscus* are undergoing specialization on their different hosts, which may lead to speciation. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **99**, 559–569.

ADDITIONAL KEYWORDS: generalist – intra-host – monogeneans – morphometry – sparids.

INTRODUCTION

Monogeneans are gill parasites of aquatic organisms, known to be highly host specific in the wild (Bychowsky, 1961; Rohde, 1979; Bakke *et al.*, 2007). However, some genera include generalist species, parasitizing several hosts species. One example is the genus *Lamellodiscus* Johnston & Tiegs (1922), in which some species can infect up to six hosts from the fish family Sparidae (Euzet *et al.*, 1993). Desdevises *et al.* (2002a) proposed that apparent generalist *Lamellodiscus* species could be the result of frequent host switches, followed by fast speciation events that are required to maintain a high level of specificity.

This pattern of evolutionary radiation, previously observed and well documented in *Gyrodactylus* spp. (Zietara & Lumme, 2002; Boeger *et al.*, 2003; Meinila *et al.*, 2004; Huyse & Volckaert, 2005), was suggested to account for the lack of cospeciation pattern in the *Lamellodiscus*–sparid association (Desdevises *et al.*, 2002a). It was observed that *Lamellodiscus* are able to switch hosts in nature (Mladineo & Marsic-Lucic, 2007).

Šimková *et al.* (2004) suggested that intra-host speciation is an important mechanism of evolutionary radiation in *Dactylogyrus* spp. In this mode of speciation, conspecific populations tend to exploit different parts of host gills, establishing reproductive isolation that leads to intra-host speciation (Šimková *et al.*, 2002, 2006). These events have not received much consideration in monogeneans because only a few cases have been investigated to date (Euzet & Combes, 1980). However, monogenean life-traits (i.e.

*Corresponding author. Current address: Université Montpellier II, Institut des Sciences de l'Évolution, UMR 5554, Place Eugène Bataillon, 34095 Montpellier CEDEX 05, France. E-mail: tpoisot@um2.fr

direct life cycle, short generation time, high fecundity) make them prone to intra-host speciation (McCoy, 2003). One method of indicating that such events are ongoing in a population is to show that infra-populations (*sensu* Margolis *et al.*, 1982; Bush *et al.*, 1997) of a generalist species are different between host species (if such species are generalist, morphological difference between groups is expected to be weak), or that several different populations (e.g. with different morphometric features) of the same parasite species coexist in a single host species (intra-host speciation).

The identification of monogeneans is generally based on morphological criteria (i.e. qualitative and discrete characters). Morphometric analysis allows a quantitative approach in the analysis of several body parts of monogeneans. The hypothesis in the present study is that a morphometric analysis of several *Lamellodiscus* species could help to assess the existence of distinct populations of generalist species on their different hosts, as well as the coexistence of several conspecific populations in the same host species, corresponding to intra-host speciation. We focused on the opisthaptor (an organ composed of several sclerotized parts, situated at the posterior end of the monogenean body) of *Lamellodiscus*, which is used by parasites to attach to host gills. Variations in the length of the different parts of this organ could be considered as a by-product of adaptation to the host (Rohde & Hobbs, 1986; Rohde, 1994; Kaci-Chaouch *et al.*, 2008). We did not a priori assume that differences between groups would be reflected in a difference in the length of given haptor parts (comprising criteria that should be used for identification), but rather would be reflected by the existence of distinct morphometric groups, stressing the need for a multivariate approach.

Lamellodiscus is a convenient model for this type of study. Recent studies, including one by Amine *et al.* (2007b), focused on the description of new species as morphological variants of previously described species, thus providing an accurate description of many morphotypes in the North-Western Mediterranean Sea. However, because these descriptions were based on the observation of very small changes in the morphology, the species status of most of these new *Lamellodiscus* is questionable. Moreover, these differences are not consistent, nor discrete, and it is possible to find individuals forming a continuum of shapes between two alleged species. Because of the existence of such a continuum, it is sometimes difficult to determine to which species a given individual belongs, and we considered it more correct and more conservative to group all species sharing a high level of morphological similarity into a reduced number of morphotypes. Moreover, as previously noted, some *Lamellodiscus* species have substantially larger host

ranges compared to other monogeneans, thus allowing the investigation of putative morphometrical differences between populations on different hosts species. The analysis of 147 *Lamellodiscus* individuals from nine described species allowed us to demonstrate significant morphometric differences within generalist parasites between their different hosts for three morphotypes (named with respect to the group's most characteristic species): *Lamellodiscus elegans*, *Lamellodiscus kechemirae*, and *Lamellodiscus ergensi*. We propose that these morphometric differences indicate radiation via a host switch followed by speciation. In addition, we show the existence of sub-populations on some hosts within a parasite species, potentially supporting a sympatric speciation event.

MATERIAL AND METHODS

FISH AND PARASITE SAMPLING

Fish were captured in the Golfe du Lion, near Banyuls-sur-Mer (42°28'47"N, 3°08'10"E), by free-diving. To ensure that hosts were sampled in the same community, the sampling area was kept inferior to 1 km², at a depth less than 15 m. Sampling was conducted under constant environmental conditions ($\theta \approx 20$ °C, salinity ≈ 37.5 PSU) because the environment has been suggested to affect the development of monogeneans (Mo, 1991, 1993; Dmitrieva & Dimitrov, 2002). Immediately after capture, each fish was killed by a sharp shock on the top of the head. Gills were removed and kept in cooled sea water until parasite sampling (maximum of 30 min after capture). Twenty fish, belonging to seven species (*Diplodus sargus*, *Diplodus vulgaris*, *Diplodus annularis*, *Diplodus puntazzo*, *Oblada melanura*, *Salpa salpa*, and *Lithognathus mormyrus*) were examined (Table 1). Gills were screened under an Olympus SZ61 stereomicroscope, and *Lamellodiscus* individuals were collected alive. Identification was carried out with an Olympus CX41 light microscope. Gill screening occurred no more than 2 h after fishing.

MORPHOMETRIC ANALYSIS

Lamellodiscus individuals were mounted on a slide with 2.5% sodium dodecyl sulphate, after a modification of the protocol described by Wong *et al.* (2007), to enable a clear visualization of the sclerotized parts, thus allowing greater precision during measurements.

A picture of the haptor of each parasite was taken using a Sony Exwave HAD digital camera mounted on an Olympus CX41 light microscope, at $\times 400$ magnification. Images were loaded into IMAGEJ (Abramoff *et al.*, 2004) to perform measurements. Measurements were taken using the landmarks method by taking distances between two points

Table 1. Host–parasite associations investigated

	<i>Diplodus sargus</i> (7)	<i>Diplodus vulgaris</i> (6)	<i>Diplodus puntazzo</i> (1)	<i>Diplodus annularis</i> (3)	<i>Oblada melanura</i> (1)	<i>Lithognathus mormyrus</i> (1)	<i>Salpa salpa</i> (1)
<i>Lamellodiscus ignoratus s.l.</i>	23	11	2	1		10	4
<i>Lamellodiscus elegans</i>	33	10		2	5		
<i>Lamellodiscus ergensi</i>	12	5	6	*			
<i>Lamellodiscus kechemirae</i>	6	16					

*Association reported in the literature (Desdevises *et al.*, 2002a; Amine *et al.*, 2007b), but not found in our sample. Composition of the morphotypes: *L. ignoratus s.l.* *L. ignoratus s.s.*, *L. confusus*, *L. neifari*, *L. falcus*; *L. ergensi*: *L. ergensi*, *L. tomentosus*; *L. kechemirae* and *L. elegans* are individual species. The number of sampled host individuals is reported in the first line. Numbers in the body of the table indicate the occurrence of each association (number of parasites in a given host species) in our sample.

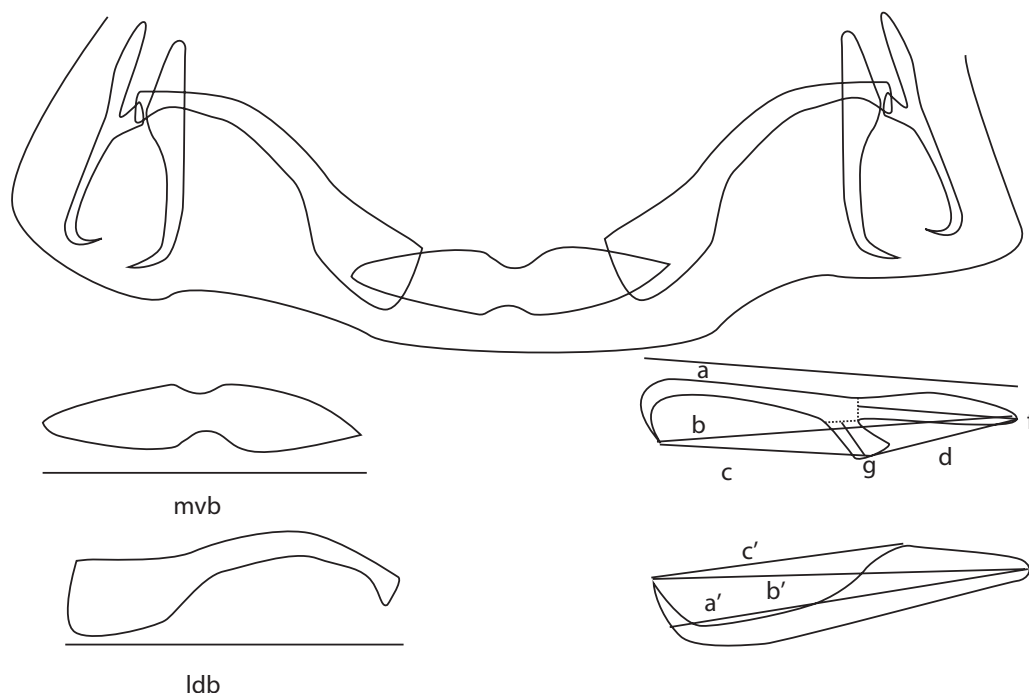


Figure 1. Overview (upper part) and measurements (lower part) of the haptor of *Lamellodiscus* (*ignoratus* type, lamellodiscs and marginal hooks are not shown). mvb, median ventral bar; ldb, lateral dorsal bar; a, a', length of hamuli; b, b', point length; c, c', hook opening; d, guard opening; f, root length; g, guard length.

(Fig. 1). The mean ± SD for each measurement (for each morphotype, on each host) are provided in the Supporting Information (Table S1).

Species were grouped into four morphotypes on the basis of a high level of morphological similarity. Similarity was defined by reviewing the shapes of the haptoral parts (mostly median ventral and lateral dorsal bars) and male copulatory organs, and species

differing only in minor details were grouped. The composition of the morphotypes was (1) *Lamellodiscus ignoratus s.l.* *L. ignoratus s.s.*, *L. confusus*, *L. neifari*, *L. falcus*; (2) *L. ergensi*: *L. ergensi*, *L. tomentosus*. Two other morphotypes are formed by a single species; (3) *L. kechemirae* and (4) *L. elegans*, whose morphology is clearly differentiated from the others *Lamellodiscus* species.

STATISTICAL ANALYSIS

$P < 0.05$ was considered statistically significant. Analyses were performed using the R 2.9.1 (R Development Core Team, 2008). Homogeneity of variances was first tested using Bartlett's test by permutation (999 permutations) to determine which test was to be subsequently used. Permutational tests were used when variances were homogeneous, and nonparametric tests were used otherwise (Sheskin, 2004).

Preliminary investigation

Preliminary studies using a subset of our data, via measurements on nonfixed and unflattened parasites, allowed us to rule out the hypothesis that variability in haptor part lengths is driven by parasite size because there is little correlation between body size and most of the other measurements (see Supporting Information, Table S2). Moreover, preparation for observation under a light microscope tends to flatten the parasite's body, making measurements of total body length potentially unreliable. We therefore chose not to include this variable in our analyses.

Exploratory analysis

Two exploratory methods were used to assess morphometrical differences between or within host species. First, a principal component analysis (PCA; Legendre & Legendre, 1998) was conducted using all variables measured on the haptor. Second, the Euclidian standardized morphometric distance between individuals of *L. elegans* found on *D. sargus* was computed using all measurements made on the haptor, and a clustering analysis was carried out, using the unweighted pair group method with arithmetic mean, implemented in APE (Paradis *et al.* 2004) on all morphometric distances after standardization. The reliability of each node of the dendrogram, which is assumed to be an indication of the difference between the groups, was estimated using a nonparametric bootstrap analysis (1000 replicates).

Quantification of differences between groups

To test whether or not the global morphometry of parasite individuals from the same species were significantly different between hosts, we extracted the first component of the PCA, which was considered as an integrative variable, summing up the morphometry of each individual (hereafter referred to as the 'morphometric value'). A comparison was made between hosts using the Kruskal–Wallis test (a Mann–Whitney test was used when there were only two groups to compare; Sokal & Rohlf, 1995).

When exploratory analyses revealed the existence of two populations of a parasite species within a single host species, their morphometric values were

compared using a Student's *t*-test, with 999 permutations. The Hartigan–Hartigan dip test of unimodality (Hartigan & Hartigan, 1985) was applied to ensure that the distribution of morphometric values was truly bimodal.

Variance comparison

To assess whether the variability of populations of *L. elegans* on *D. sargus* was comparable with the values found for specialist *Lamellodiscus* species, variances for each morphometric character were computed for each sub-population of *L. elegans* on *D. sargus*, and compared with the variance of the whole population of *L. elegans* on *D. sargus*. Values were also compared with weighted average variances found by Kaci-Chaouch *et al.* (2008). Variances were compared with *F*-tests, and the *F*-statistic was doubled to control for a type 1 error (Bruning & Kintz, 1987).

RESULTS

One hundred and forty-seven *Lamellodiscus* parasites belonging to nine putative *Lamellodiscus* species were regrouped into four morphotypes based on high morphological similarity (for distribution of morphotypes on host species, see Table 1).

We consider that the value for the first component is summing up the morphometry of each individual because of the high percentage of variance explained [eigenvalue of the first axis for each morphotype (%): *L. elegans* = 74.5; *L. kechemirae* = 54.5; *L. ignoratus s.l.* = 71.1; *L. ergensi* = 66.7]. No correlation was found between host size and morphometric value (for all parasites together as well as for each morphotype). Out of the four morphotypes, only three presented a significant difference in morphometry between the different hosts (Fig. 2): *L. ergensi* ($P = 0.003$), *L. elegans* ($P = 0.007$), and *L. kechemirae* ($P = 0.02$). No significant difference was found for *L. ignoratus* ($P = 0.19$). However, the comparison of morphometry of *L. ignoratus* from *D. puntazzo* and *L. mormyrus* showed a significant difference ($P = 0.03$).

Two populations of *L. elegans* on *D. sargus* were observed (Fig. 3). However, these two *L. elegans* populations were not found on the same *D. sargus* host individual. The difference between means in morphometric value was tested using a Student's *t*-test with 999 permutations, and was found to be significant ($P = 0.0001$). This fragmentation in two groups is visible on the dendrogram built using morphometric distances (Fig. 4). Bootstrap values delineate two groups of *L. elegans* individuals within the same host species (with an internal branch with 100% bootstrap support). Within each group, the branches are poorly supported, which indicates that the global population of *L. elegans* on *D. sargus* is formed by two morpho-

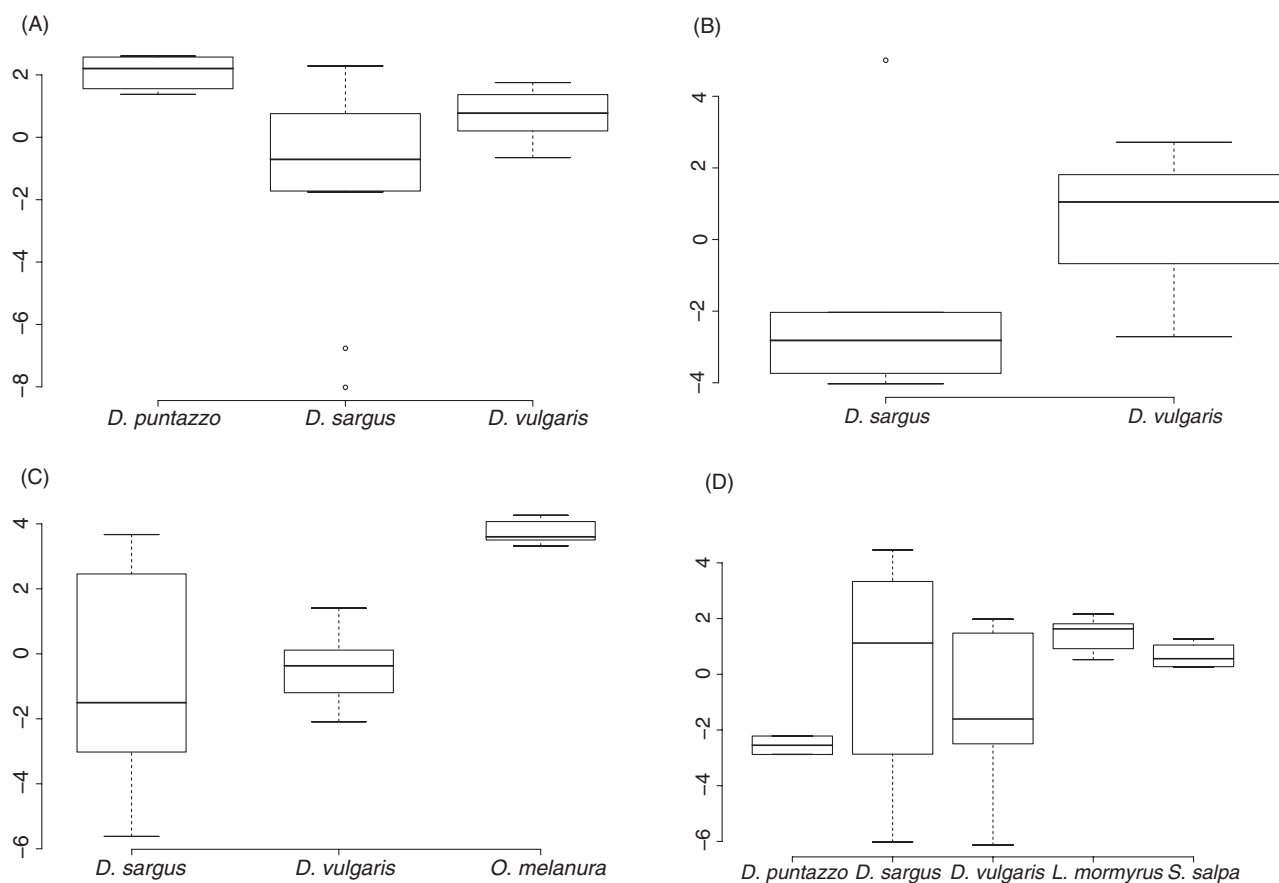


Figure 2. Differences in morphometrics in the four *Lamellodiscus* morphotypes on different hosts (*Diplodus puntazzo*, *D. sargus*, *D. vulgaris*, *Oblada melanura*, *Lithognathus mormyrus*, and *Sarpa salpa*). After Kruskal-Wallis test, morphometrics are different between hosts for *Lamellodiscus ergensi* (A), *L. elegans* (B) and *L. kechemirae* (C), but not for *L. ignoratus* (D).

metrically distinct sub-populations, each one being homogeneous. Variances for *L. elegans* on *D. sargus* (Table 2) are similar to those found for specialist species [*L. virgula*, *L. baeri*, *L. drummondi*, *L. erythrini*, except for the root length (f) and the dorsal hamuli length (a') in sub-population 1, and root (f) and guard (g) length for sub-population 2; Fig. 1], whereas the variance of the whole population was similar to the one found for generalist species (except for the length of the median ventral bar). Moreover, the distribution of morphometric values of *L. elegans* on *D. sargus* is significantly different from unimodality (Dip > 0.1).

DISCUSSION

Morphometric analysis has already been used in several monogean genera. Shinn *et al.* (2001) used morphometric tools to discriminate between the pathogenic *Gyrodactylus salaris* and other congeneric species on salmonids. Similarly, Mariniello *et al.*

(2004) differentiated several species of *Ligophorus*, and demonstrated considerable morphological variation between allopatric populations of *Ligophorus angustus*. Huyse & Volckaert (2002) used morphometry to reveal the existence of a species complex within *Gyrodactylus* spp. However, these studies were mostly descriptive, and did not use morphometric analysis to assess diversification below the species level.

Kaci-Chaouch *et al.* (2008) showed that, within *Lamellodiscus*, morphometric variability was higher in generalist than in specialist species. Beyond this result, the present study emphasizes that, except for *L. ignoratus*, generalist *Lamellodiscus* species are not similar (in their morphometry) on their different hosts, but tend to form clusters (Fig. 1). The observation that *L. ignoratus* is the morphotype with the greatest host range (Euzet *et al.* 1993) could account for the lack of group formation if the rate of specialization is not equal in all host species, or if specialization is not occurring in all host species. This

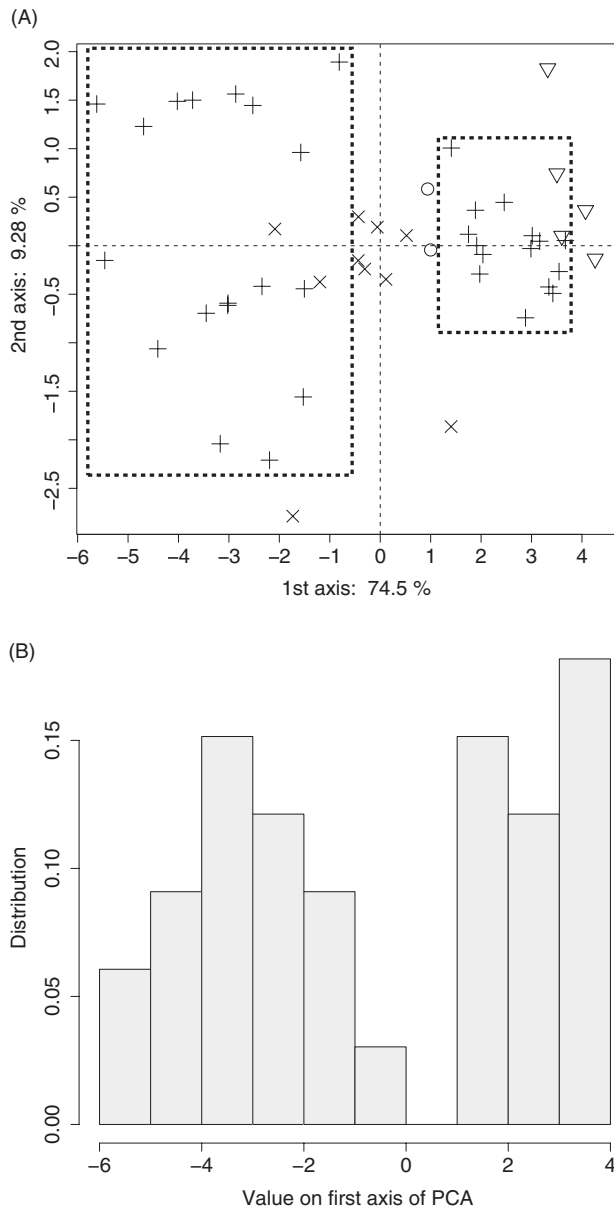


Figure 3. Principal component analysis (PCA) and repartition of the values on the first axis of PCA conducted on morphometric measures (cf. Fig. 1) of *Lamellogadus elegans* parasites from *Diplodus sargus*. A, principal component analysis of all morphometric variables measured on *L. elegans* parasites of *D. sargus*. Dashed boxes indicate the two different groups observed. B, repartition of the values on the first axis of the principal component analysis, showing the existence of two distinct sub-populations of *L. elegans* on *D. sargus*. This distribution shows a significant deviance from unimodality (Dip > 0.1). Each symbol in (A) corresponds to a host species: *Diplodus annularis*, + *D. sargus*, × *D. vulgaris*, *Oblada melanura*.

Table 2. Variances in each morphometric variable from *Lamellogadus elegans* from *Diplodus sargus*, and of specialist and generalist *Lamellogadus* species

	<i>N</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>f</i>	<i>g</i>	<i>a'</i>	<i>b'</i>	<i>c'</i>	<i>mvb</i>	<i>ldb</i>
<i>Lamellogadus elegans</i>	V	85.77	98.72	62.06	16.62	23.24	6.92	70.75	84.26	29.64	120.08	314.35
	Pop 1	17.58	18.29	16.04	5.19	18.21	6.16	16.35	21.56	10.05	75.93	96.90
	Pop 2	8.10	9.35	6.53	6.12	2.33	1.46	9.79	10.43	3.03	22.41	23.11
Specialists	WAV	11.68	—	13.60	9.15	7.34	3.68	6.21	—	6.50	48.19	46.61
Generalists	WAV	87.96	—	68.17	13.36	24.69	12.66	62.98	—	22.16	270.97	232.64

The first line reports the variance (V) of the two populations of *L. elegans* on *D. sargus* (see Fig. 3). Population 1 (line 2) is formed by the parasites collected on hosts 1, 8, and 9. Population 2 (line 3) is formed by the parasites collected on hosts 2, 3, 6, and 7. Weighted average variance (WAV) for specialist (line 4) and generalist species was taken from Kaci-Chaouch *et al.* (2008). Bold values on line 1 indicate a significant difference between variances of all *L. elegans* on *D. sargus* and weighted average variances of generalists (line 5). Bold values on lines 2 and 3 indicate a significant difference between variances in each population of *L. elegans* on *D. sargus* and weighted average variance of specialists. No differences in variances for the two populations of *L. elegans* on *D. sargus* were found, except for the root (f), guard (g), and lateral dorsal bars (ldb). *a*, *a'*, length of hamuli; *b*, *b'*, point length; *c*, *c'*, hook length; *d*, guard opening; *f*, root length; *g*, guard length.

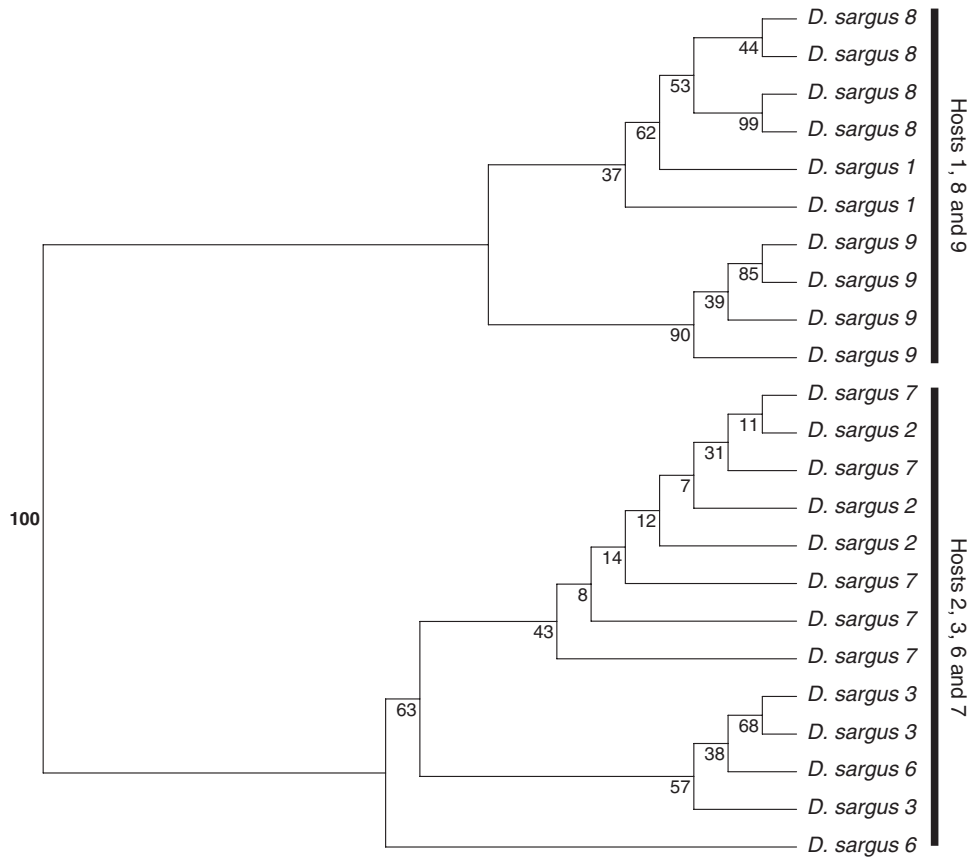


Figure 4. Dendrogram reconstructed using the unweighted pair group method with arithmetic mean on the standardized Euclidian distances based on morphometric variables between individuals of the morphotype *Lamellogdiscus elegans* on *Diploodus sargus*. Each host is identified by a number. The two sub-populations are formed by parasites from different hosts. Node labels indicate bootstrap values (1000 replicates, expressed in %). Hosts identification numbers are given for information purposes.

statement is confirmed by the fact that *L. ignoratus* on *Diploodus puntazzo* and *Lithognathus mormyrus* are morphometrically different. Desdevises *et al.* (2002a) suggested a host switch followed by speciation as the main mechanism of evolutionary radiation in *Lamellogdiscus*. Therefore, depending on the age of the association (time at which the host was acquired), specialization may not have started yet. This result also indicates that inter-host speciation, although readily found in our association, is in no way a general trend, and that specialization is not the unavoidable result of the exploitation of several hosts.

The morphometric analysis revealed the existence of distinct groups of a parasite species within a host species: *L. elegans* on *D. sargus*. This result can be interpreted in several ways (Brooks & McLennan, 1993; Poulin, 1999; McCoy, 2003). It could be an indication that two populations of hosts are coexisting in the study area, each harbouring a distinct parasite population, stressing the need to consider hosts when studying parasite populations (i.e. the host population

might not be homogeneous; Secord & Kareiva, 1996). This claim is supported by the fact that none of the hosts investigated during the present study carried parasites from both sub-populations (Fig. 4). However, without more work on the host population, trying to determine which host features account for this heterogeneity would remain speculative. Intra-host speciation has been studied in *Dactylogyrus* spp., and it was suggested that specialization to exploit different parts of host gills (reflected by the modification of morphometric characters in the parasites) was a factor initiating speciation (Šimková *et al.*, 2002, 2006). In the case of *L. elegans* on *D. sargus*, the observed separation in two populations could be linked to an adaptation to different parts of the gills (Šimková *et al.*, 2004).

An alternative hypothesis is that host populations might have been geographically isolated at some time, and then undergone a secondary contact, triggering resource partitioning among the parasites. In this situation, we expect to observe the same pattern

as in true sympatric speciation. A more thorough study of this association is required to investigate this idea. The fact that the two populations of *L. elegans* on *D. sargus* are not evenly distributed on all hosts but, instead, correspond to two groups of hosts, suggests that this speciation is not ongoing at the intra-host level: if the factor initiating speciation is specialization in the exploitation of different parts of the gills, one would expect to find individuals from each population within the same hosts. The finding in the present study could also be explained by the fact that *L. elegans* parasites from *D. sargus* are undergoing an intra-host speciation event, resulting in the emergence of two groups that are distinguishable by their morphometry. The low bootstrap values within sub-groups suggest that the individuals within each group are homogeneous on the basis of their global morphometry.

As previously noted, recent descriptions of *Lamellodiscus* species have been based on very small and sometimes inconsistent morphological differences (Amine *et al.*, 2007a, b). The results obtained in the present study did not reveal morphometric groups to support these species distinctions, raising doubt as to their validity. However, we did not use the samples of Amine *et al.* (2007a, b), nor did we sample in the same geographic area. Because it has been suggested that monogeneans are good models to study speciation and adaptation to the host (McCoy, 2003), the results obtained in the present study emphasize that it is important to be especially careful when describing new monogenean species. This issue needs to be more thoroughly investigated using molecular data.

Morphometric variability was shown to be a good correlate of host-specificity in *Lamellodiscus* (Kaci-Chaouch *et al.*, 2008). We found that *L. elegans* on *D. sargus* (1) forms two sub-populations and that (2) within each sub-population, the morphometric variance is similar to the weighted average variance found for specialist *Lamellodiscus* species. This finding supports an ongoing intra-host speciation event, reflected by an adaptation, either to different parts of the gills or to different host populations. However, an exhaustive analysis of how *Lamellodiscus* individuals distributes on the gills of their hosts, which would allow one of these hypotheses to be ruled out, remains to be conducted. We found that variance within each sub-population of *L. elegans* on *D. sargus* was similar to the weighted average variance of specialist *Lamellodiscus* species, whereas the variance for the whole population was similar to the variance observed for generalist *Lamellodiscus* species. Kaci-Chaouch *et al.* (2008) suggested that morphometric variability within generalist species was higher than within specialist species. The results obtained in the present study suggest that the greater variance

observed in generalist species is a result of their trend to form groups, even within a host species. The difference of variance between generalist and specialist species could be at least partially a result of this group formation in generalists, which is not observed in specialists (Kaci-Chaouch *et al.*, 2008), thus suggesting that different factors act in phenotypic evolution in generalist and specialist species. Moreover, whether the host-parasite assembly is formed by colonization or descent might impact group formation, and remains to be investigated.

A similar phenomenon could act for generalist species between their different hosts. The formation of groups, according to morphometry, could indicate an adaptation to each host species, leading to specialization. This result is consistent with the study by Desdevises *et al.* (2002a) claiming that evolutionary radiation in *Lamellodiscus* is the result of a host switch followed by speciation. Desdevises *et al.* (2002b) suggested that specialization in *Lamellodiscus* is not an evolutionary 'dead-end', but rather an ancestral state, and that *Lamellodiscus* species tend to acquire new hosts. The observation that individuals belonging to generalist species are forming groups on at least some of their different hosts may reflect ongoing specialization events, along with adaptation to the new hosts.

The clustering of individuals according to different hosts may be seen as phenotypic plasticity. Current knowledge, however, does not support this hypothesis. Previous studies focusing on phenotypic plasticity of haptor parts (Mo, 1991, 1993; Matejusová *et al.*, 2002) indicate that this phenomenon, when detected, does not induce variations of the same amplitude to that observed in the present study (i.e. several factors are also known to modify the size of haptor parts; Ergens & Gelnar, 1985, Dmitrieva & Dimitrov, 2002), although additional work is needed to investigate the role of phenotypic plasticity in morphometric variation because this phenomena alone could explain the pattern observed in the present study. In the case of phenotypic plasticity, no genetic differentiation between parasite populations would be seen, as would exist in the case of true differentiation following assortative mating. Because we ensured that environmental conditions known to potentially impact the development of sclerotized parts of monogeneans were constant during sampling, the results obtained support the hypothesis that the observed differences are a result of the host. However, the possibility remains that the hosts studied belong to populations that have experienced different environmental conditions in the past. Even in morphotypes in which morphometry suggested the existence of different groups, no single discriminant morphological feature could be used to identify these groups. However, the

objective of the present study was to assess the existence of groups within, and not between, species.

Moreover, even in studies indicating that phenotypic plasticity might be acting in some monogenean genera, parasite populations were not different (according to morphometry) between their hosts (e.g. *Paradiplozoon homoion*; Matejusová *et al.*, 2002). The fact that speciation would be reflected in modification of the size of haptor parts can be seen as a consequence of a strong evolutionary pressure acting on the shape of the haptor, resulting in a better adaptation to host. One might also expect that competition between different *Lamellodiscus* populations within a single host would affect their development, and could introduce nonhost-induced variation in the length of haptor parts. However, many studies (Euzet & Combes, 1998; Morand *et al.*, 1999; Šimková *et al.*, 2000, 2001b; Lo & Morand, 2001; Rohde, 2002) have suggested that monogenean niches are not saturated, indicating that competition is low or absent amongst monogeneans. However, these studies mostly relate to interspecific competition, and intraspecific competition is far less known in monogeneans. We cannot rule out its possible effects on parasite development.

Futuyma & Moreno (1988) stressed that causes and consequences of specialization should be considered distinctly. However, this distinction is not always straightforward (especially in the case of morphometry): adaptation to a host may be the result of specialization driven by other factors (thus being a consequence) but could also be a constraint for further specialization (thus acting as a cause). It is known that specialist monogeneans are found on larger hosts compared to generalists (Sasal *et al.*, 1999; Šimková *et al.*, 2001a; Morand *et al.*, 2002; Desdevises *et al.*, 2002b). Because size is a life-trait correlated with longevity and proximity to the top of the food web in fish (Winfield & Nelson, 1991; Winemiller & Rose, 1992), this finding supports the hypothesis of specialization on predictable resources (Ward, 1992). However, the fact that generalist species tend to form clusters on their different hosts was not taken into account in these studies and should receive proper attention in any future investigations.

Finally, morphometrics alone cannot fully answer the question of the occurrence of speciation events, and some other factors may play an important role. Chemical stimuli from the host are responsible for recognition by oncomiracidium (Buchmann & Lindstrøm, 2002), and the host triggers an immune response against the parasite (Jones, 2001), especially during parasite growth (Faliex *et al.*, 2008). Before morphometry could act in host–parasite compatibility, these factors may modify the encounter or compatibility filters defined by Combes (2001), thus regulating specificity, the potential for the the acquisition of new

hosts, and the chances for the parasite to properly develop on its host. Moreover, anterior adhesive areas play a role during infection (Whittington *et al.*, 2000). Changes in factors regulating the infection of host by infective stages are certainly important in the determinism of specificity, whereas morphometry could at least in part be a consequence of such changes. In addition, it is possible that parasite morphometry is influenced by the biology of the host (e.g. via host populations fragmentation leading to parasites' morphometric divergence, followed by a mixing of these host populations in a sympatric distribution). However, investigating this point requires an extensive amount of data on host populations.

In summary, a morphometric analysis of 147 generalists *Lamellodiscus* individuals on seven sparid host species revealed the existence of four morphometric sub-groups. Most of these groups were associated with the host species from which the parasites were recovered, suggesting that some generalist parasites could be undergoing an allopatric (inter-host) speciation event. Interestingly, we identified the existence of two subgroups of *L. elegans* on the same host species (*D. sargus*), which may represent a sympatric speciation event. By contrast, some generalist parasites (e.g. *L. ignoratus*) did not show morphometric group–host associations, suggesting that some generalist parasites do not represent intermediate forms at present.

ACKNOWLEDGEMENTS

We are grateful to Pascal Romans for invaluable help in fish sampling. We thank Louis Euzet for a discussion on the different morphotypes that we encountered during this work; Pierre Legendre and Daniel Borcard for their help with implementation of permutation tests; and Emmanuel Paradis for his assistance during application of nonparametric bootstrap to our data. We thank Jean-Lou Justine and two anonymous referees for their insightful comments on the manuscript.

REFERENCES

- Abramoff M, Magelhaes P, Ram S. 2004.** Image processing with ImageJ. *Biophotonics International* **11**: 36–42.
- Amine F, Euzet L, Kechemir-Issad N. 2007a.** Description de *Lamellodiscus confusus* n. sp. (Monogenea: Diplectanidae), parasite de *Sarpa salpa* (Teleostei: Sparidae). *Parasite* **14**: 281.
- Amine F, Euzet L, Kechemir-Issad N. 2007b.** *Lamellodiscus theroni* sp. nov. (Monogenea, Diplectanidae), a gill parasite from *Diplodus puntazzo* (Teleostei, Sparidae) from the Mediterranean Sea. *Acta Parasitologica* **52**: 305–309.
- Bakke T, Cable J, Harris P. 2007.** The biology of Gyrodactylid monogeneans: the 'Russian-doll killers'. *Advances in Parasitology* **64**: 161–378.
- Boeger W, Kritsky D, Pie M. 2003.** Context of diversifica-

- tion of the viviparous Gyrodactylidae (Platyhelminthes, Monogenea). *Zoologica Scripta* **32**: 437–448.
- Brooks D, McLennan D. 1993.** *Parascript: parasites and the language of evolution*. Washington, DC: Smithsonian Institution Press.
- Bruning J, Kintz B. 1987.** *Computational handbook of statistics*, 3rd edn. Glenview, IL: Scott, Foresman Co.
- Buchmann K, Lindenstrøm T. 2002.** Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology* **32**: 309–319.
- Bush A, Lafferty K, Lotz J, Shostak A. 1997.** Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**: 575–583.
- Bychowsky BE. 1961.** *Monogenetic trematodes; their systematics and phylogeny*. Washington, DC: American Institute of Biological Sciences.
- Combes C. 2001.** *Parasitism: the ecology and evolution of intimate interactions*. Chicago, IL: University of Chicago Press.
- Desdevises Y, Morand S, Jousson O, Legendre P. 2002a.** Coevolution between *Lamellodiscus* (Monogenea: Diplectanidae) and Sparidae (Teleostei): the study of a complex host–parasite system. *Evolution* **56**: 2459–2471.
- Desdevises Y, Morand S, Legendre P. 2002b.** Evolution and determinants of host specificity in the genus *Lamellodiscus* (Monogenea). *Biological Journal of the Linnean Society* **77**: 431–443.
- Dmitrieva E, Dimitrov G. 2002.** Variability in the taxonomic characters of black sea gyrodactylids (monogenea). *Systematic Parasitology* **51**: 199–206.
- Ergens R, Gelnar M. 1985.** Experimental verification of the effect of temperature on the size of hard parts of opisthaptor of *Gyrodactylus katharineri* Malmberg, 1964 (Monogenea). *Folia Parasitologica* **32**: 377–380.
- Euzet L, Combes C. 1980.** Les problèmes de l'espèce chez les animaux parasites. *Bulletin de la Société Zoologique de France* **40**: 239–285.
- Euzet L, Combes C. 1998.** The selection of habitats among the monogenea. *International Journal for Parasitology* **28**: 1645–1652.
- Euzet L, Combes C, Caro A. 1993.** A checklist of Monogenea of Mediterranean fish. In: *Second international symposium on Monogenea*. Montpellier–Sète, pp. 5–8.
- Faliex E, Da Silva C, Simon G, Sasal P. 2008.** Dynamic expression of immune response genes in the sea bass, *Dicentrarchus labrax*, experimentally infected with the monogenean *Diplectanum aequans*. *Fish and Shellfish Immunology* **24**: 759–767.
- Futuyma DJ, Moreno G. 1988.** The evolution of ecological specialization. *Annual Review of Ecology and Systematics* **19**: 207–233.
- Hartigan JA, Hartigan PM. 1985.** The dip test of unimodality. *Annals of statistics* **13**: 70–84.
- Huysse T, Volckaert FAM. 2002.** Identification of a host-associated species complex using molecular and morphometric analyses, with the description of *Gyrodactylus rugiensoides* n. sp. (Gyrodactylidae, Monogenea). *International Journal for Parasitology* **32**: 907–919.
- Huysse T, Volckaert F. 2005.** Comparing host and parasite phylogenies: *Gyrodactylus* flatworms jumping from goby to goby. *Systematic Biology* **54**: 710–718.
- Johnston TH, Tiegs OW. 1922.** New gyrodactylid trematodes from Australian fishes, together with a reclassification of the superfamily Gyrodactyloidea. *Proceedings of the Linnean Society of New South Wales* **47**: 83–131.
- Jones S. 2001.** The occurrence and mechanisms of innate immunity against parasites in fish. *Developmental and Comparative Immunology* **25**: 841–852.
- Kaci-Chaouch T, Verneau O, Desdevises Y. 2008.** Host specificity is linked to intraspecific variability in the genus *Lamellodiscus* (Monogenea). *Parasitology* **135**: 607–616.
- Legendre P, Legendre L. 1998.** *Numerical ecology*. Second English edition, Developments in Environmental Modelling. 20. Amsterdam: Elsevier Science.
- Lo C, Morand S. 2001.** Gill parasites of *Cephalopholis argus* (Teleostei: Serranidae) from Moorea (French Polynesia): site selection and coexistence. *Folia Parasitologica* **48**: 30–36.
- McCoy KD. 2003.** Sympatric speciation in parasites – what is sympatry? *Trends in Parasitology* **19**: 400–404.
- Margolis L, Esch G, Holmes J, Kuris A, Schad G. 1982.** The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *The Journal of Parasitology* **68**: 131–133.
- Mariniello L, Ortis M, D'Amelio S, Petrarca V. 2004.** Morphometric variability between and within species of *Ligophorus* Euzet & Suriano, 1977 (Monogenea: Ancyrocephalidae) in the Mediterranean Sea. *Systematic Parasitology* **57**: 183–190.
- Matejusová I, Koubková B, Gelnar M, Cunningham C. 2002.** *Paradiplozoon homoion* Bychowsky & Nagibina, 1959 versus *P. gracile* Reichenbach-Klinke, 1961 (Monogenea): two species or phenotypic plasticity? *Systematic Parasitology* **53**: 39–47.
- Meinila M, Kuusela J, Zietara MS, Lumme J. 2004.** Initial steps of speciation by geographic isolation and host switch in salmonid pathogen *Gyrodactylus salaris* (Monogenea: Gyrodactylidae). *International Journal for Parasitology* **34**: 515–526.
- Mladineo I, Marsic-Lucic J. 2007.** Host switch of *Lamellodiscus elegans* (Monogenea: Monopisthocotylea) and *Sparicotyle chrysophrui* (Monogenea: Polyopisthocotylea) between cage-reared sparids. *Veterinary Research Communications* **31**: 153–160.
- Mo TA. 1991.** Variations of opisthaptor hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. *Systematic Parasitology* **20**: 11–19.
- Mo TA. 1993.** Seasonal variations of the opisthaptor hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *S. salar* L. parr in the River Sandvikselva, Norway. *Systematic Parasitology* **26**: 225–231.
- Morand S, Poulin R, Rohde K, Hayward C. 1999.** Aggregation and species coexistence of ectoparasites of marine fishes. *International Journal for Parasitology* **29**: 663–672.

- Morand S, Šimková A, Matejusova I, Plaisance L, Verneau O, Desdevises Y. 2002.** Investigating patterns may reveal processes: evolutionary ecology of ectoparasitic monogeneans. *International Journal for Parasitology* **32**: 111–119.
- Paradis E, Claude J, Strimmer K. 2004.** APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.
- Poulin R. 1999.** Speciation and diversification of parasite lineages: an analysis of congeneric parasite species in vertebrates. *Evolutionary Ecology* **13**: 455–467.
- R Development Core Team. 2008.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Rohde K. 1979.** A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. *American Naturalist* **114**: 648–671.
- Rohde K. 1994.** Niche restriction in parasites: proximate and ultimate causes. *Parasitology* **109**: S69–S84.
- Rohde K. 2002.** Ecology and biogeography of marine parasites. *Advances in Marine Biology* **43**: 1–86.
- Rohde K, Hobbs RP. 1986.** *Parasites's lives: papers on parasites, their hosts and their associations to honour JFA Sprent*. Chapter species segregation: competition or reinforcement of reproductive barriers? St Lucia: University of Queensland Press, pp. 189–199.
- Sasal P, Trouvé S, Muller-Graf C, Morand S. 1999.** Specificity and host predictability: a comparative analysis among monogenean parasites of fish. *Journal of Animal Ecology* **68**: 437–444.
- Secord D, Kareiva P. 1996.** Perils and pitfalls in the host specificity paradigm. *Bioscience* **46**: 448–453.
- Sheskin D. 2004.** *Handbook of parametric and nonparametric statistical procedures*, 4th edn. London: Chapman and Hall.
- Shinn AP, Gibson DI, Sommerville C. 2001.** Morphometric discrimination of *Gyrodactylus salaris* Malmberg (Monogenea) from species of *Gyrodactylus* parasiting British salmonids using novel parameters. *Journal of Fish Biology* **24**: 83–97.
- Šimková A, Desdevises Y, Gelnar M, Morand S. 2000.** Co-existence of nine gill ectoparasites (*Dactylogyrus*: Monogenea) parasitising the roach (*Rutilus rutilus* L.): history and present ecology. *International Journal for Parasitology* **30**: 1077–1088.
- Šimková A, Desdevises Y, Gelnar M, Morand S. 2001a.** Morphometric correlates of host specificity in *Dactylogyrus* species (Monogenea) parasites of European cyprinid fish. *Parasitology* **123**: 169–177.
- Šimková A, Gelnar M, Sasal P. 2001b.** Aggregation of congeneric parasites (Monogenea: *Dactylogyrus*) among gill microhabitats within one host species (*Rutilus rutilus* L.). *Parasitology* **123**: 599–607.
- Šimková A, Morand S, Jobet E, Gelnar M, Verneau O. 2004.** Molecular phylogeny of congeneric monogenean parasites (*Dactylogyrus*): a case of intrahost speciation. *Evolution* **58**: 1001–1018.
- Šimková A, Ondrackova M, Gelnar M, Morand S. 2002.** Morphology and coexistence of congeneric ectoparasite species: reinforcement of reproductive isolation? *Biological Journal of the Linnean Society* **76**: 125–135.
- Šimková A, Verneau O, Gelnar M, Morand S. 2006.** Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. *Evolution* **60**: 1023–1037.
- Sokal RR, Rohlf FJ. 1995.** *Biometry*. New York, NY: WH Freeman & Co.
- Ward S. 1992.** Assessing functional explanations of host-specificity. *American Naturalist* **139**: 883–891.
- Whittington I, Cribb B, Hamwood T, Halliday J. 2000.** Host-specificity of monogenean (Platyhelminth) parasites: a role for anterior adhesive areas? *International Journal for Parasitology* **30**: 305–320.
- Winemiller K, Rose K. 1992.** Patterns of life-history diversification in North American fishes: implications for population regulation. *Canadian Journal of Fisheries and Aquatic Sciences* **49**: 2196–2218.
- Winfield I, Nelson J. 1991.** *Cyprinid fishes: systematics, biology and exploitation*. Number 3 in Fish and fisheries series. London: Chapman and Hall.
- Wong WL, Tan WB, Lim LHS. 2007.** Sodium dodecyl sulphate as a rapid clearing agent for studying the hard parts of monogeneans and nematodes. *Journal of Helminthology* **80**: 87–90.
- Zietara M, Lumme J. 2002.** Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). *Evolution* **56**: 2445–2458.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean and variance of morphometric values for all parasite morphotypes.

Table S2. Correlations between length of haptor parts.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.