Phenotypic plasticity in haptoral structures of *Ligophorus cephali* (Monogenea: Dactylogyridae) on the flathead mullet (*Mugil cephalus*): a geometric morphometric approach

Abril Rodríguez-González[*], Raúl Míguez-Lozano, Cristina Llopis-Belenguer, Juan Antonio Balbuena

Post-print version (ie final draft post-refereeing)

Published in International Journal for Parasitology (2015), vol. 45, issue 5, pp. 295-303.

Science Direct link: http://www.sciencedirect.com/science/article/pii/S0020751915000399

doi:10.1016/j.ijpara.2015.01.005

Phenotypic plasticity in haptoral structures of *Ligophorus cephali* (Monogenea: Dactylogyridae) on the flathead mullet (*Mugil cephalus*): a geometric morphometric approach✭

Abril Rodríguez-González*, Raúl Míguez-Lozano, Cristina Llopis-Belenguer, Juan Antonio Balbuena

Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, P.O. Box 22085, 46071 Valencia, Spain

*Corresponding author. Tel.: +34 963543685; fax: +34 963543733.

E-mail address: arogon5@uv.es

✭Note: The nucleotide sequence data reported in this paper are available in the GenBank database under accession numbers **KP294376 - KP294383**.

Note: Supplementary data associated with this article.

Abstract

Evaluating phenotypic plasticity in attachment organs of parasites can provide information on the capacity to colonize new hosts and illuminate evolutionary processes driving host specificity. We analysed the variability in shape and size of the dorsal and ventral anchors of *Ligophorus cephali* from *Mugil cephalus* by means of geometric morphometrics and multivariate statistics. We also assessed the morphological integration between anchors and between the roots and points in order to gain insight into their functional morphology. Dorsal and ventral anchors showed a similar gradient of overall shape variation, but the amount of localized changes was much higher in the former. Statistical models describing variations in shape and size revealed clear differences between anchors. The dorsal anchor/bar complex seems more mobile than the ventral one in *Ligophorus*, and these differences may reflect different functional roles in attachment to the gills. The lower residual variation associated with the ventral anchor models suggests a tighter control of their shape and size, perhaps because these anchors seem to be responsible for firmer attachment and their size and shape would allow more effective responses to characteristics of the microenvironment within the individual host. Despite these putative functional differences, the high level of morphological integration indicates a concerted action between anchors. In addition, we found a slight, although significant, morphological integration between roots and points in both anchors, which suggests that a large fraction of the observed phenotypic variation does not compromise the functional role of anchors as levers. Given the low level of genetic variation in our sample, it is likely that much of the morphological variation reflects host-driven plastic responses. This supports the hypothesis of monogenean specificity through host-switching and rapid speciation. The present study demonstrates the potential of geometric morphometrics to provide new and previously unexplored insights into the functional morphology of attachment and evolutionary processes of host-parasite coevolution.

Keywords: Geometric morphometrics, Phenotypic plasticity, Haptor, Monogenean, Western Mediterranean, Mugilidae

1. Introduction

Establishing the determinants of host specificity in parasites has both theoretical and applied implications. The former pertain to the study of evolutionary patterns between hosts and parasites and revolve around a central problem in evolutionary ecology (Gemmill et al., 2000): when does natural selection favour the evolution of specialists over generalists? On the applied side, delineating the host range of a given parasite is fundamental for both the design and implementation of control strategies (Murphy, 1998), and the evaluation and forecast of the impact of parasites associated with host introductions (Woolhouse et al., 2005).

Classically, the specificity of a host-parasite system is commonly believed to be the result of an adaptive process (Brooks and McLennan, 1991) and it has been suggested that high degrees of host specificity might be explained by the tight coevolutionary interaction between hosts and parasites (Poulin, 1992). Thus parasites would tend to optimize exploitation by adapting locally to the environment provided by their hosts and developing specific morphological, physiological and behavioural traits (Bush, 2009). However, other evolutionary processes might also lead to tight host specificity. Desdevises (2007) proposed that host switching could be a major driver of host specificity in some parasites such as monogeneans and particularly in marine systems. Under such a scenario, phenotypic variability could increase the spectrum of hosts available; this provides switching opportunities which, coupled with rapid speciation by parasites, could account for high host specificity, as frequently observed in marine monogeneans (Desdevises, 2007).

Many monogeneans are characterized as being highly specific, restricted to certain gill arches and certain parts of gill filaments, and having developed different strategies in adapting to this microhabitat (Whittington and Kearn, 1991; Vignon et al., 2011). This adaptive process suggests that the high morphological variability of attachment organs in monogeneans is possibly linked to host specificity (Morand et al., 2002). Thus the evaluation of phenotypic plasticity of the organs responsible for attachment to the gills can inform us on the capacity to colonize new hosts and would eventually cast light on evolutionary forces driving host specificity in monogeneans and other parasites in general (Poisot and Desdevises, 2010).

Despite this, few studies have focused on this topic (i.e., Olstad et al., 2009; Mladineo et al., 2013). Caltran et al. (1995a, b) observed that populations of *Ligophorus imitans* Euzet & Suriano, 1977 from *Liza ramada* Risso, 1827 display high morphological and anatomical variability of haptoral structures and genitalia, and revealed that variations in these organs are independent of each other. This variability was higher than that originally described by Euzet and Suriano (1977) for the other *Ligophorus* spp., but similar to that observed in *Dactylogyrus* (Dactylogyridae) and *Diplectanum* (Diplectanidae) (Belova, 1988; Silan and Maillard, 1989). In addition, the evaluation of environmental and demographic variables in morphological plasticity was reflected in the correlation between the size of haptoral anchors and host size, which the authors related to an increase in gill heterogeneity in larger fish.

These studies, similar to most others to date (except Olstad et al., 2009), have been based on linear measurements. The problem with this approach is that the pure shape information is frequently not obtained, making it impossible to partition size and shape for separate analyses (Corti et al., 2001). Geometric morphometrics can address this issue effectively and in additional provide visualization tools to better appreciate morphological variability (Bastir and Rosas, 2005; Vignon and Sasal, 2010; Zelditch et al., 2012). This technique has been successfully utilized in monogeneans to study ecological and evolutionary questions (Vignon and Sasal, 2010; Vignon et al., 2011), including phenotypic plasticity in *Gyrodactylus* spp. (Olstad et al., 2009).

We adopted this approach herein to examine the intraspecific variability and phenotypic plasticity of the ventral and dorsal anchors of *Ligophorus cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006 on the gills of *Mugil cephalus* L., 1758. Our focus was on the dorsal and ventral anchors as structures primarily responsible for attachment to the host gills. Specifically, we (i) describe, quantify and test patterns of shape and size variation in relation to site attachment on the host individual, and (ii) evaluate the morphological integration between ventral and dorsal anchors, and between the roots and points of anchors, in order to gain insight into their functional morphology.

2. Materials and methods

2.1. Study site, host and parasite collection

Flathead grey mullets (*Mugil cephalus*) were collected in L'Albufera, Spain (39º 20' N, - 0º 21' W), in April-May 2011. L'Albufera is a 23.2 km^2, shallow, eutrophied, Mediterranean lagoon surrounded by marshlands mainly devoted to rice crops, orchards, scattered country houses and coastline resorts (Soria et al., 2000; Soria, 2006). Fishes (n = 31) were purchased from local fishermen and were immediately transported to the laboratory for examination. Their total length ($\bar{x}$ ± S.D.: 32.5 ± 3.5 cm) and weight (404.2 ± 130.5 g) were recorded.

The gills were surveyed for monogeneans under a stereomicroscope on the day of capture. Infected gills were then fixed in a plastic container with 4% formalin for 3 - 4 h to keep the monogeneans attached at their sites before being stored in 70% alcohol (Rubio-Godoy, 2008).

For the morphometric analyses, an enzymatic digestion technique was used to obtain the sclerotized structures. A mixture of 300 µl of TE9 buffer (500 mM Tris-HCl, 200 mM EDTA, 10 mM NaCl, pH 9) and 100 - 200 µl of proteinase K (10 mg/ml) was used (Mo and Appleby, 1990; Paladini et al., 2011). Slides were then mounted in Kaiser's glycerol-gelatin and examined under a microscope at 100× magnification. The specimens were identified as *L. cephali* on the basis of morphological traits (haptoral and copulatory structures) based on Rubtsova et al. (2006), Dmitrieva et al. (2009) and Sarabeev et al. (2013).

Only the anchors (i.e., ventral and dorsal, from each specimen) on both sides were considered for geometric morphometric techniques because they are not subject to large variation due to contraction or flattening on fixation (Lim and Gibson, 2009). The bars were not studied because they are more difficult to observe flat and more prone to distortion during fixation and mounting (Vignon and Sasal, 2010). Specifically, one anchor from each pair (left or right) from each different specimen was chosen for analysis. Thus, the differences between the right and left side of each pair of ventral and dorsal anchors were not assessed.

The anchors were drawn using a drawing tube at 100× (under immersion oil) under a Nikon Optiphot-2 microscope equipped with interference contrast.

2.2. Molecular data

Evaluating phenotypic plasticity requires assessment of the degree of genetic variation in the sample. To this end, we sequenced and compared the internal transcribed spacer 1 region (ITS1) of rDNA. Ten specimens were unmounted and transferred into 200 μL of TE9 buffer (500 mM Tris-HCl, 200 mM EDTA, 10 mM NaCl, pH 9) (Wu et al., 2007) to clean the glycerol-gelatin from the specimens. The DNA was extracted using an Qiagen DNeasy® Blood & Tissue Kit following the manufacturer's instructions (Qiagen, Germany). ITS1 sequences were amplified using primers Lig18endF (5'-GTC TTG CGG TTC ACG CTG CT-3') and Lig5.8R (5'-GAT ACT CGA GCC GAG TGA TCC-3') (Blasco-Costa et al., 2012). PCR amplifications were performed in 20 μL reactions containing 2 μL of extracted DNA, the ready-to-use 2x MyFi Mix (Bioline Ltd., United Kingdom) and 5 pmol/μL of each primer. The following thermocycling profile was applied: denaturation of DNA at 95ºC for 3 min, 35 cycles of amplification with 40 s of denaturation at 94ºC, 30 s primer annealing at 56ºC and 45 s at 72ºC for primer extension, and a final extension step of 4 min at 72ºC. PCR amplicons were purified using a Macherey-Nagel NucleoSpin® Gel and PCR Clean-Up kit (Macherey-Nagel, Germany), and PCR primers were used for sequencing. Sequencing was performed by the commercial sequence provider Macrogen (Netherlands) using ABI BigDye™ Terminator v3.1 chemistry and run on an ABI 3730XL automated sequencer. Contiguous sequences were assembled and edited using VectorNTI advance 10 (Lu and Moriyama, 2004), and the resultant sequence identities were checked using the Basic Local Alignment Search Tool (BLAST) available from GenBank (Benson et al., 2005). The eight most complete new sequences generated in this work (GenBank accession numbers **KP294376 - KP294383**) and a previously published sequence of *L. cephali* from Blasco-Costa et al. (2012) (GenBank accession number **JN996865**) were aligned for comparison using MUSCLE (Edgar, 2004) implemented in MEGA v5.1 (Tamura et al., 2011).

2.3. Geometric morphometrics

Anchor shape was analysed using landmark-based geometric morphometrics (Rohlf and Marcus, 1993; Zelditch et al., 2004; Klingenberg, 2011), which facilitates subsequent multivariate analyses (Adams et al., 2004). The anchor shape variables were obtained using eight homologous landmarks (Fig. 1) from a sample of 213 anchors (114 ventral and 99 dorsal from 16 and 14 hosts, respectively) of 136 *L. cephali* considered as adults. The eight landmarks were chosen to represent the same biological locations and their location could be readily established in each individual (Rosenberg et al., 2002; Mitteroecker and Gunz, 2009). Landmark *x* and *y* coordinates of each anchor were obtained from digitized images with tpsDig (Rohlf, F.J., 2013, tpsDig digitize landmarks and outlines. Version 2.17. Department of Ecology and Evolution, State University of New York at Stony Brook, New York, USA) and tpsUtil (Rohlf, F.J., 2012, tpsUtility. Version 1.52. Department of Ecology and Evolution. State University of New York at Stony Brook, New York, USA) from the thin-plate spline (TPS) packages.

In order to remove all of the information unrelated to shape, the configurations were superimposed using Generalized Full Procrustes Analysis (Cox and Cox, 2001; Zelditch et al., 2012; Klingenberg, 2013), using the Least Squares criterion that minimizes bending energy with respect to a mean reference form (Sarris et al., 2012). This analysis was performed with MorphoJ 1.05f (Klingenberg, 2011).

A Relative Warp Analysis (Rohlf, 1993) was performed with the Procrustes coordinates using tpsRelw (Rohlf, F.J., 2010, tpsRelw. Version 1.49. Department of Ecology and Evolution. State University of New York at Stony Brook, New York, USA) to examine shape variations in anchors among monogeneans, thereby generating a data set of shape variables. In order to give all landmarks equal weighting, the scaling option was set $\alpha = 0$. The shape changes modelled onto a TPS can be separated into two parts, the uniform and non-uniform components (Rohlf and Slice, 1990). The former (*U1* and *U2*) express global variations in shape, whereas the latter describe local shape changes at different geometric scales (Vignon et al., 2011). To visualize localized anchor shape differences, TPS deformation grids and grey-scaled coded Jacobian expansion factors, which measure the degree of local expansion or contraction of the grid (black for factors >1, indicating expansion; grey for factors between 0 and 1, indicating contraction) were used (Bookstein, 1993; Viscosi and Cardini, 2011).

All analyses were performed separately for the two-dimensional projections of the ventral and dorsal anchor shapes. However, since we observed shape differences between the dorsal and ventral anchors, covariation in shape between them was tested in 80 specimens with matching dorsal and ventral anchors according to a two-block Partial Least Square (PLS) analysis (Rohlf, F.J., 2006, tpsPLS. Version 2.17. State University of New York at Stony Brook, New York, USA; Rohlf and Corti, 2000; Klingenberg et al., 2001). In addition, since anchors work as levers where the effort to open/close them is applied at the roots, whereas the force against the gill is applied at the point root, we also used PLS to test the covariation in shape between the root and point of dorsal and ventral anchors. For the analysis, we established two functional blocks: the "root block" (corresponding to landmarks 1 - 4) and the "point block" (landmarks 5 - 8) (Fig. 1). The PLS analyses yielded a RV Escoufier's coefficient, which quantifies morphological integration between the blocks on a scale between 0 and 1 (the latter meaning total integration), and can be interpreted as a multivariate analogue of the coefficient of correlation (Klingenberg, 2009; Püschel, 2014).

In addition, the geometric size of each anchor was estimated as its centroid size (CS), defined as the square root of the sum of squared distances of each landmark from the centroid of the configuration (Bookstein, 1991; Zelditch et al., 2012). CS was calculated with tpsRelw 1.49 (Rohlf, 2010). Correlations analysis was used to evaluate the relationship between CS in dorsal and ventral anchors. To explore how shape variables (all relative warps) vary with CS, a multivariate regression was used for the assessment of allometric localized shape variation in ventral and dorsal anchors. In addition, the uniform component was regressed on CS to evaluate the uniform shape variation. These analyses were carried out with tpsRegr (Rohlf, F.J., 2009, tpsRegr, Shape regression. Version 1.37. Department of Ecology and Evolution. State University of New York at Stony Brook, New York, USA).

2.4. Data analysis with shape and size

We used Permutational Multivariate Analysis of Variance (PERMANOVA)+ for PRIMER (Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. *PERMANOVA+* for PRIMER: Guide to Software and Statistical Methods. Versión 6. PRIMER-E: Plymouth, UK) to evaluate to what extent specific gill site variables and individual hosts accounted for shape variability in the dorsal and ventral anchors. For this purpose the gill apparatus was divided into four gill arches. Each arch was divided into four equidistant sections and three gill areas (internal, medial and external) (for details see Fig. 3 in Šimková et al. (2002)).

In order to estimate the components of variation in anchor shape, the relative warp datasets of dorsal and ventral anchors were used to construct respective Euclidean distance matrices. Then, we performed a PERMANOVA on the distance matrices using a crossed design with three fixed factors: gill arch (four levels), gill section (four levels) and gill area (three levels). Pseudoreplication was accounted for by considering host individual as a random factor. Due to the small sample size with respect to the number of variables and levels, our initial model included all terms up to two-way interactions. Log-transformed CS and worm size (WS), the latter measured as the area of body contours computed from digitized images, were included as covariates to control for size effects on shapes on anchors, but were tested in alternative models, and not simultaneously, to avoid the effect of collinearity (Zuur et al., 2010). The significance of each term was established based on 9,999 permutations. To identify a parsimonious model of shape variation, we followed the procedure set up by Anderson et al. (2008). First, terms having negative and /or associated P values > 0.25 were pooled (one at a time and beginning with the term having the smallest mean square residual) with the term (or terms) having equivalent expected mean squares after the component of variation of the term to be pooled was set to zero. Then, the pooling of terms was repeated until all the estimates of component variation associate to each term remaining in the model were positive (Anderson et al., 2008) . We used a Type-I sum of squares, where each term is fitted after taking into account all previous terms in the model. Therefore results may vary depending on the order of the terms listed in the design file (Anderson et al., 2008). However, we tried different input orders to ensure that this factor did not substantially change the resulting model (see Supplementary data S1).

Variation in CS was analysed as a function of the same gill-site factors considered above and host individual as random factor (for ventral anchors) with Generalized Linear Mixed Models (Bates, D., Maechler, M., Bolker, B., Walker, S., 2010, lme4: Linear mixed-effects models using S4 classes .Version 0.999999-0, http://CRAN.R-project.org/package=lme4). For dorsal anchors, preliminary analyses indicated that the variance component associated with host individual was negligible and thus a Generalized Lineal Model (GLM) with the fixed factors was used instead. In addition, log-transformed WS was considered as a covariate in the models. To evaluate the influence of these explanatory variables, we first developed a series of alternative models that included different combinations of variables using a stepwise process. Model selection was based on values of the Akaike Information Criterion (AIC). Models with a difference in AIC < 2, compared with the best model, were retained (Burnham and Anderson, 2002). A model weight was computed for each of the retained models based on the value this difference following Burnham and Anderson (2002), and a measurement of importance of each explanatory variable was obtained by summing the weights of all the models that included the given variable (Burnham and Anderson, 2002).

These analyses were performed using the lnme (Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2011. R Development Core Team nlme: linear and nonlinear mixed effects models. R Package Version 3, 1 – 102) and GLM packages in R 3.0.1 (R Development Core Team, 2011). In a preliminary analysis, the uniform components and relative warps for ventral and dorsal anchors were not significantly related to CS ($r = 0.001$; $P = 0.990$ and $r = 0.004$; $P = 0.593$, uniform components) and ($r = 0.057$, $P = 0.545$ and $r = 0.047$; $P = 0.643$, relative warps). This indicates no allometric shape variation in our dataset and allows consideration of shape and size as independent factors.

3. Results

3.1. Molecular identification

The aligned dataset of nine ITS1 sequences (eight sequences from this study and one from Blasco-Costa et al. (2012) representing *Ligophorus cephali*) was composed of 630 nucleotide (nt) positions, after trimming the end parts to match the shortest sequence. This aligned sequence set showed exactly the same pattern of nts.

3.2. Shape variation

A relative warp analysis was run on the total shape matrix. The first two relative warps (RW1 and RW2) accounted for 47.17% of the total variance (25.54% and 21.63%, respectively) for ventral anchors, and 45.72% (26.56% and 19.16%, respectively) for dorsal anchors. A scatter plot of RW1 and RW2, TPS and Jacobian expansion grid factors for both anchors (ventral and dorsal) are shown in Fig. 2.

In the ventral anchors, RW1 conveyed variation in positions of the outer shaft base and inner shaft base of anchors (landmarks 4 and 8 respectively, Fig. 2A), defining a gradient of shaft width along this axis. In the most extreme positive values, the TPS and coded Jacobian plots indicate narrow and elongated shapes. RW2 corresponded to variation in the outer point base and inner point base (landmarks 5 and 7, respectively) of anchors, leading to different curvatures at the tips of anchors, and displayed short and wide anchors in the extreme negative values.

Regarding the dorsal anchors, the variation along RW1 mainly concerned the positions of the maximum point of the inner root, and the outer and inner point bases (landmarks 1, 5 and 7, respectively, Fig. 2B). Shape variation was much higher than in the ventral anchors as denoted by the TPS and Jacobian expansion grids (Fig. 2B). The plot indicated shortening anchor tips at the extreme positive values, similar to the ventral anchors. RW2 reflected marked variation in the maximum point of the inner root, inflection between the outer root and the inner root, the outer shaft base, the anchor point and the inner shaft base (landmarks 1, 2, 4, 6 and 8, respectively, Fig. 2B). The extreme negative values showed a similar shape variation to that of ventral anchors.

3.3. Shape and size models

Table 1 summarizes the results of the multivariate analyses of anchor shape measurements (relative warps). Gill arch accounted for a significant part of the variation in shape of the dorsal anchors. Additionally, the variable ‘gill section’ explained differences in the shape of the ventral anchors, but not in a consistent manner across hosts (Table 1). The variation explained by model terms in the dorsal anchors was much larger (two orders of magnitude). with respect to those of the ventral anchors. Similarly residual variation was approximately three orders of magnitude larger in the dorsal anchors (Table 1).

Of the 11 candidate models considered for CS, seven were retained for the ventral anchors (Table 2) and three for the dorsal anchors (Table 3). CS of ventral anchors appeared to be mainly driven by WS and host individual, as evidenced by the inclusion of only these two variables in the most parsimonious model (AIC: 507.1) and the estimates of relative importance (1 and 0.84 for host individual and WS, respectively), which were clearly larger than the corresponding estimates of gill area (0.35), arch (0.25) and section (0.23). The variation associated with host individual as a random factor in the best model was 3.94, which was similar to the residual variation (3.73).

Following the same criteria, gill arch was the main determinant of CS in the dorsal anchors, with a relative importance of 1 versus 0.28 and 0.21 for gill area and WS, respectively (Table 3).

Whereas host individual accounted for a marginal part of the variation, residual variation was clearly larger than in the ventral-anchor model (15.34).

Although these results indicate high variation in the CS of ventral anchors among hosts, there was no statistical evidence that CS of either ventral or dorsal anchors was related to host size ($r = 0.13$; $P = 0.14$ and $r = 0.079$; $P = 0.44$), which is further corroborated by scatterplots showing no clear increase in CS with host weight (Fig. 3).

3.4. Morphological integration

There was a slight, although significant, morphological integration between the root and point block in the same anchor (ventral anchor: *RV* coefficient = 0.40; $P \leq 0.001$; dorsal anchor: $RV = 0.34$; $P \leq 0.001$). In addition, the degree of shape integration between both the ventral anchor blocks and the dorsal anchor blocks was high ($RV = 0.70$; $P \leq 0.0001$), denoting a relatively high level of morphological integration between anchors of *L. cephali*. However, CS of ventral and dorsal anchors were not correlated ($r = -0.13$; $P = 0.18$).

4. Discussion

The study of sclerotized haptoral structures of monogenean morphology and phenotypic plasticity with geometric morphometrics is a poorly explored field. In the present study we believe that we use this approach for the first time to document the total morphological integration between ventral and dorsal anchors, and between the roots and points of anchors of *Ligophorus cephali*, to provide detailed information on shape variations among these anchors and to model the morphological shape and size as a function of host variables (gill arch, gill area, gill section and host individual).

The warps determine and decompose the shape variation into uniform components (global variation) and non-uniform components (local variation) (Zelditch et al., 2012). Globally, the pattern of shape variation observed herein was similar in ventral and dorsal anchors, defining a gradient ranging from narrow and elongated anchors to wide and short anchors. Similar global changes have been observed in *Gyrodactylus salaris* Malmberg, 1957 (Olstad et al., 2009). Localized shape variation in the anchors has also been reported in monogeneans of the Dactylogyridae and the Diplectanidae (Vignon and Sasal, 2010), but information is still scarce. In *Ligophorus llewellyni* Dmitrieva, Gerasev and Pron'kina, 2007, Dmitrieva et al. (2007) showed localized changes in the anchor roots and point anchors, but their study was based on linear measurements and therefore the results are not directly comparable with those of the present study.

Interestingly, localized shape variation was much higher in the dorsal anchors (compare Jacobian grids in Fig. 2), which are in line with the higher residual variation associated with dorsal anchors in the shape models (Table 1). Note also that in the size models the residual variations of the dorsal anchors were much higher than those of the ventral anchors. In addition, we showed that random effects (gill section × host individual) were an important determinant of shape in ventral, but not in dorsal, anchors and size models of dorsal and ventral anchors were clearly different (Tables 2, 3).

All of this evidence points to differences between dorsal and ventral anchors in the factors determining both shape and size, which perhaps reflects different functional roles in attachment to the gills. To our knowledge, detailed functional studies of the hard haptoral structures in *Ligophorus* are lacking and it is therefore difficult to interpret our results in the light of current evidence. However, in *Ligophorus* the pairs of ventral anchors and dorsal anchors are connected, respectively, by ventral and dorsal transverse bars. In *L. cephali*, as in other species of the genus, these bars are dissimilar in shape and size (Siquier and Ostrowski de Núñez, 2009; Sarabeev et al., 2013) and the ventral bar appears to be more rigid than the dorsal one (Dmitrieva et al., 2012). In fact, the curvature of the dorsal bar can vary sharply (Mariniello et al., 2004; Dmitrieva et al., 2007; Sarabeev et al., 2013). Thus morphology suggests that the dorsal anchor/bar complex is more mobile than the ventral one, at least in this genus.

Vrat and Shanker (2013) observed the movement and change in position/orientation of various haptoral elements with respect to the dorsal anchors in *Mizelleus indicus* (Jain, 1957) (Dactylogyridae) from *Wallago attu* (Bl. and Schn.). Although the morphology of the bars and anchors in this species is quite different from that of *Ligophorus* spp., some of their findings appear useful in understanding some aspects on the functional dynamics of anchors and bars of *L. cephali*. In *M. indicus* the process of achieving attachment to the host tissue involves movements of the ventral bar together with the ventral anchors. This movement is achieved with or without the aid of the supporting dorsal bar, which moves upwards and downwards, resulting in the spreading the points of the dorsal anchors. Thus the dorsal bar appears to be primarily involved in the movement of the dorsal anchors.

In light of this evidence, the differences in forces generated for attachment by the respective bars might account for the differences between the dorsal and ventral anchors observed in the present study. The lower residual variation associated with the ventral anchors suggests a tighter control of their shape and size, perhaps because these anchors are the most important for attachment and their size and shape would more closely fit the characteristics of the individual host microenvironment (Šimková et al., 2001; Mancheva et al., 2009; Sarabeev et al., 2013). This is also in line with the significant fraction of variation accounted for by host-associated random effects in the ventral anchors in the models of anchor shape and size.

Despite these putative functional differences, we observed high integration in shape between the ventral and dorsal anchors, indicating a concerted action between dorsal and ventral structures. Vignon et al. (2011) also suggested strong coordination and integration among the different parts of the haptoral structure in *Cichlidogyrus* spp., (Monogenea, Dactylogyridae) considering three main morphological configurations in the parts of attachment organs as modules: marginal hooks, anchors and bars. Thus, their results revealed that the shapes of haptoral parts are not independent characters and furthermore suggest morphological integration, which is in line with our findings.

This coordination among parts of the haptor could be due to host specificity and the attachment mechanism (Vignon et al., 2011). Klingenberg (2008) suggested that this kind of morphological covariationcan be a substantial player determining the evolutionary potential of traits within populations. Although the haptoral structures have long been studied in various environmental and evolutionary contexts, our study highlights the importance of morphological integration analyses for better understanding of the variability among haptoral anchors.

A considerable part of the variation in the shape and size models was attributable to either random factors or remained unaccounted for by the variables considered. Thus there is a large unpredictable component in the models imputable to a combination of measurement error, genetic variation, ontogenetic changes and plastic responses to environmental factors. The molecular analyses showed that the ITS1 sequences of our specimens were identical to those of *L. cephali* previously reported in a nearby locality (Cullera) by Blasco-Costa et al. (2012). ITS1 sequences have previously shown some level of intraspecific divergence within species of monogeneans, including members of *Gyrodactylus* (0.09 - 3.5% intraspecific divergence, Bueno-Silva et al., 2011), *Lamellodiscus* (0.27%, Desdevises et al., 2000) and *Furnestinia* (0.05 - 1.38%, Mladineo et al., 2013). This evidence does not completely rule out some level of genetic variation in our sample and it is therefore possible that not all of the phenotypic variation revealed in the present investigation is environmentally induced. However, we ensured that all of the specimens of *L. cephali* used in the present study came from fish captured within 1 day in a single locality in L'Albufera (El Palmar), thereby reducing the possibility of important genetic differences. Note also that anchor shape was independent of WS and therefore ontogenetic changes do not seem to contribute substantially to anchor shape. This lack of relationship with WS was also observed by Dmitrieva and Dimitrov (2002) in haptoral structures in gyrodactylids. They observed that the size of the anchors is the most variable, whereas the size of the marginal hooks is the most stable. This is associated with the order of appearance of these structures in ontogeny. Marginal hooks, which appear first, can reach their final size long before the birth, whereas the size of the anchors, which appear later, is essentially dependent on the duration of embryogenesis. It is therefore likely that much of the random variation reported herein reflects environmentally driven plastic responses.

In addition, we found that host gill arch was an important determinant of anchor shape and size in the dorsal anchors. Shape variability related to the host gill arch has also been observed in *L. imitans* and others monogeneans (Caltran et al., 1995b; Roberts and Janovy, 1996). This is perhaps not surprising given that hydrodynamic processes are associated with the spatial position of each gill and this can determine the leverage applied for attachment (Soler-Jiménez and Fajer-Ávila, 2012). In fact, maintaining high phenotypic plasticity can be advantageous in monogeneans given the diversity of microhabitats provided by fish gills (Šimková et al., 2002; 2004; Verneau et al., 2009) and thus selective forces can promote the maintenance of this feature.

Phenotypic plasticity allows organisms to respond rapidly to changing environmental conditions without the time lag required for responses to natural selection (Zhou et al., 2012). The evidence presented herein points to phenotypic plasticity in anchor morphology, which could confer on *Ligophorus* spp. the ability to instantly colonize a new host when the occasion arises. In fact, straggling seems common in this genus due to the usual co-occurrence of several sympatric host populations that overlap in habitat and behaviour, and which, due to their phylogenetic relatedness, can provide a similar physiological environment for the parasites (Sarabeev et al., 2013; Sarabeev and Desdevises, 2014). Eventually straggling would make host switching and subsequent speciation in the newly colonized host possible, as postulated by Desdevises (2007).

We found no evidence of correlation between dorsal or ventral anchors size and host size. This relationship has been much studied in monogeneans, and most evidence points to a significant positive correlation between these traits (Perera, 1992; Šimková et al., 2006; Mendlová and Šimková, 2014), including species of *Ligophorus* (Caltran et al., 1995b; Rubtsova et al., 2005). This pattern has often been explained in terms of water currents and the secondary lamella lengths that tend to increase with host gill size and the performance of the parasite's attachment to the host gill that is associated with parasite anchor size (Kearn, 1970; Caltran et al., 1995b; Turgut et al., 2006;

Soler-Jiménez and Fajer-Ávila, 2012). However, other studies do not support this relationship (Fuentes and Nasir, 1990; Matejusová et al., 2002). In *Metamicrocotyla macracantha* Alexander, 1954 from *M. cephalus,* the unique perpendicular attachment of the parasite haptor to the host gill filament seems to limit the ability of the haptor to grow past the maximum width of the host gill filament, even while the body of the worm continues to grow relative to the haptor (Baker et al., 2005).

However, it seems unlikely that this type of constraint affects *L. cephali*. Note, however, that the range of host sizes in the present study is quite narrow, which could determine the lack of relationship with anchor size. In addition, previous studies based on linear measurement did not explicitly separate size and shape. Therefore this question deserves further exploration within a geometric morphometric framework.

We observed shape integration within parasite anchors (point and root blocks), which is not surprising given the functional relationship between points and roots. However, in line with previous findings in *L. imitans*, Caltran et al. (1995a) reported that, in the same anchor, not all of the metric variables are systematically positively correlated ,which the authors interpreted as resulting from asynchronous growth of the different anchor parts. In any case, the low integration observed herein indicates that a large fraction of the phenotypic variation observed does not compromise the functional role of anchors as levers.

The present study demonstrates that geometric morphometrics can be an extremely useful technique in analysing intraspecific shape and size variations in haptoral structures in monogeneans and illustrates the potential to provide new insight into the functional morphology of parasite attachment to the host and evolutionary processes of host-parasite coevolution. Additionally, future studies should assess the patterns of shape evolution in the genus, assessing the quantitative genetics of shape variation.

Acknowledgements

A.R.G. benefited from a PhD student grant from the Consejo Nacional de Ciencia y Tecnología (CONACyT-CONCYTEY) of the Mexican Government and Yucatan State, Mexico (Scholarship No. 204397). This study was funded by the National Plan for Scientific Research, Development and Technological Innovation of Spain (CGL2008-02701) and the Generalitat Valenciana, Spain (Prometeo Project 2011-040).

References

Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric Morphometrics: Ten Years of Progress Following the ‘Revolution’. Ital. J. Zool. 71, 5 - 16.

Baker, T.G., Viricel, A., Meraziz, L., De Buron, I., 2005. Size variation of adult polyopisthocotylid *Metamicrocotyla macracantha* (Monogenea) in relation to host size. Comp. Parasitol. 72, 179 - 182.

Bastir, M., Rosas, A., 2005. Hierarchical nature of morphological integration and modularity in the human posterior face. Am. J. Phys. Anthropol. 128, 26 - 34.

Belova, S.V., 1988. Variability of chitinoid structures of *Dactylogyrus hypophtalmichthys* (Monogenoidea) in relation to host size and season. In: Skarlato, O.A. (Ed.), Investigations of monogeneans in the USSR. Balkema, Rotterdam, pp. 38 - 43.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Wheeler, D.L., 2005. GenBank. Nucleic Acids Res. 33, D34 - D38.

Blasco-Costa, I., Míguez-Lozano, R., Sarabeev, V., Balbuena, J.A., 2012. Molecular phylogeny of species of *Ligophorus* (Monogenea:Dactylogyridae) and their affinities within the Dactylogyridae. Parasitol. Int. 61, 619 - 627.

Bookstein, F.L., 1991. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, Cambridge.

Bookstein, F.L., 1993. A brief history of the morphometric synthesis. In Marcus, L.F., Bello, E., García-Valdecasas, A. (Ed.), Contributions to morphometrics. Monografías del Museo Nacional de Ciencias Naturales 8, CSIC, Madrid, pp. 15 - 40.

Brooks, D.R., McLennan, D.A., 1991. Phylogeny, Ecology and Behaviour: A Research Program in Comparative Biology. The University of Chicago Press, Chicago.

Bueno-Silva, M., Boeger, W.A., Pie, M.R., 2011. Choice matters: incipient speciation in *Gyrodactylus corydori* (Monogenoidea: Gyrodactylidae). Int. J. Parasitol. 41, 657 - 667.

Burnham, K., Anderson, D., 2002. Models selection and multi-model inference. Springer, Berlin.

Bush, S.E., 2009. Does behavioural flexibility facilitate host switching by parasites? Funct. Ecol. 23, 578 - 586.

Caltran, H., Silan, P., Roux, M., 1995a. *Ligophorus imitans* (Monogenea) Ectoparasite de *Liza ramada* (Teleostei) I. Populations Naturelles Et Variabilité Morphologique. Ecologie 26, 95 - 104.

Caltran, H., Silan, P., Roux, M., 1995b. *Ligophorus imitans* (Monogenea) Ectoparasite de *Liza ramada* (Teleostei) II. Variabilité Morphologique Et Contraintes Environnementales Ecologie 26, 105 - 113.

Corti, M., Aguilera, M., Capanna, E., 2001. Size and size changes in the skull accompanying speciation of South American spiny rats (Rodentia: *Proechimys* spp.). J. Zool. 253, 537 - 547.

Cox, T.F., Cox, M.A.A., 2001. Multidimensional Scaling. Chapman and Hall, Florida.

Desdevises, Y., 2007. Cophylogeny: insights from fish-parasite systems. Parassitologia 49, 41 - 42.

Desdevises, Y., Jovelin, R., Jousson, O., Morand, S., 2000. Comparison of ribosomal DNA sequences of *Lamellodiscus* spp. (Monogenea, Diplectanidae) parasitising *Pagellus* (Sparidae, Teleostei) in the North Mediterranean Sea: species divergence and coevolutionary interactions. Int. J. Parasitol. 30, 741 - 746.

Dmitrieva, E., Dimitrov, G., 2002. Variability in the taxonomic characters of Black Sea gyrodactylids (Monogenea). Syst. Parasitol. 51, 199 - 206.

Dmitrieva, E.V., Gerasev, P.I., Gibson, D.I., Pron'kina, N.V., Galli, P., 2012. Descriptions of eight new species of *Ligophorus* Euzet & Suriano, 1977 (Monogenea: Ancyrocephalidae) from Red Sea mullets. Syst. Parasitol. 81, 203 - 237.

Dmitrieva, E.V., Gerasev, P.I., Merella, P., Pugachev, O.N., 2009. Redescriptions of *Ligophorus cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa, Euzet, 2006, *L. chabaudi* Euzet &

Suriano, 1977 (Monogenea: Ancyrocephalidae), with notes on the functional morphology of the copulatory organ. Syst. Parasitol. 73, 175 - 191.

Dmitrieva, E.V., Gerasev, P.I., Pron'kina, N.V., 2007. *Ligophorus llewellyni* n. sp. (Monogenea: Ancyrocephalidae) from the redlip mullet *Liza haematocheilus* (Temminck & Schlegel) introduced into the Black Sea from the Far East. Syst. Parasitol. 67, 51 - 64.

Edgar, R.C., 2004. MUSCLE: A multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5.

Euzet, L., Suriano, M.D., 1977. *Ligophorus* n. g. (Monogenea, Ancyrocephalidae) parasite de Mugilidae (Teleosteens) in Mediterranee. Bull. Mus. Natl. Hist. Nat. 3e Sér. Zool. 472, 799 - 821.

Fuentes, J.L., Nasir, P., 1990. Descripción y ecología de *Ligophorus mugilinus* (Hargis, 1955) Euzet y Suriano, 1977 (Monogenea: Ancyrocephalinae) en *Mugil curema* (Val. 1936) de la Isla de Margarita, Venezuela. Sci. Mar. 54, 187 - 193.

Gemmill, A.W., Viney, M.E., Read, A.F., 2000. The evolutionary ecology of host-specificity: experimental studies with *Strongyloides ratti*. Parasitology 120, 429 - 437.

Jain, S.L., 1957. *Mizellus indicus* n. gn. (subfamiliy Tetraonchinae) from the gill filaments of *Wallagonia attu* (Bloch.). Ann. Zool. 2, 56 - 62.

Kearn, G.C., 1970. The attachment of the ancyrocephalid monogenean *Haliotrema balisticus* to the gills of trigger fish, *Balistes capriscus* (= carolinensis). Parasitology 63, 157 - 162.

Klingenberg, C.P., 2008. Morphological integration and developmental modularity. Annu. Rev. Ecol. Syst. 39, 115 - 132.

Klingenberg, C.P., 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a priori hypotheses. Evol. Dev. 11, 405 - 421.

Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Mol. Ecol. Resour. 11, 353 - 357.

Klingenberg, C.P., 2013.Visualizations in geometric morphometrics: how to read and how to make graphs showing shape changes. Hystrix 24, 1 - 10.

Klingenberg, C.P., Badyaev, A.V., Sowry, S.M., Beckwith, N.J., 2001. Inferring developmental modularity from morphological integration: analysis of individual variation and asymmetry in bumblebee wings. Am. Nat. 157, 11 - 23.

Lim, L.H.S., Gibson, D.I., 2009. A new monogenean genus from an ephippid fish off Peninsular Malaysia. Syst. Parasitol. 73, 13 - 25.

Lu, G., Moriyama, E.N., 2004. Vector NTI, a balanced all-in-one sequence analysis suite. Brief. Bioinformatics 5, 378 - 388.

Mancheva, K., Karaivanova, E., Atanasov, G., Stojanovski, S., Nedeva, I., 2009. Analysis of the influence of the host body size on morphometrical characteristics of *Ancylodiscoides siluri* and *Ancylodiscoides vistulensis*. Biotechnol. Biotechnol. Equip. 23, 735 - 741.

Mariniello, L., Ortis, M., D'Amelio, S., Petrarca, V., 2004. Morphometric variability between and within species of *Ligophorus* Euzet and Suriano, 1977 (Monogenea: Ancyrocephalidae) in the Mediterranean Sea. Syst. Parasitol. 57, 183 - 190.

Matejusová, I., Koubková, B., Gelnar, M., Cunningham, C., 2002. *Paradiplozoon homoion* Bychowsky and Nagibina, 1959 versus *P. gracile* Reichenbach-Klinke, 1961 (Monogenea): two species or phenotypic plasticity? Syst. Parasitol. 53, 39 - 47.

Mendlová, M., Šimková, A., 2014. Evolution of host specificity in monogeneans parasitizing African cichlid fish. Parasit. Vectors 7, 1 - 14.

Mladineo, I., Šegvić-Bubić, T., Stanić, R., Desdevises, Y., 2013. Morphological Plasticity and Phylogeny in a Monogenean Parasite Transferring between Wild and Reared Fish Populations. PLoS ONE 8, e62011.

Mitteroecker, P., Gunz, P., 2009. Advances in Geometric Morphometrics. Evol. Biol. 36, 235 - 247.

Mo, T.A., Appleby, C., 1990. A special technique for studying haptoral sclerites of monogeneans. Syst. Parasitol. 17, 103 - 108.

Morand, S., Simková, A., Matejusová, I., Plaisance, L., Verneau, O., Desdevises, Y., 2002. Investigating patterns may reveal processes: evolutionary ecology of ectoparasitic monogeneans. Int. J. Parasitol. 32, 111 - 119.

Murphy, F.A., 1998. Emerging zoonoses. Emerg. Infect. Dis. 4, 429 - 435.

Osltad, K., Bachmann, L., Bakke, T.A., 2009. Phenotypic plasticity of taxonomic and diagnostic structures in gyrodactylosis-causing flatworms (Monogenea, Platyhelminthes). Parasitology 136, 1305 - 1315.

Paladini, G., Huyse, T., Shinn, A.P., 2011. *Gyrodactylus salinae* n. sp. (Platyhelminthes: Monogenea) infecting the south European toothcarp *Aphanius fasciatus* (Valenciennes) (Teleostei, Cyprinodontidae) from a hypersaline environment in Italy. Parasit. Vectors 4, 1 - 12.

Perera, L., 1992. The effect of host size on large hamuli length of *Kuhnia scombri* (Monogenea: Polyopisthocotylea) from Eden, New South Wales, Australia. Int. J. Parasitol. 22, 123 - 124.

Poisot, T., Desdevises, Y., 2010. Putative speciation in *Lamellodiscus* (Monogenea: Diplectanidae) assessed by a morphometric approach. Biol. J. Linn. Soc. 99, 559 - 569.

Poulin, R., 1992. Determinants of host-specificity in parasites of freshwater fishes. Int. J. Parasitol. 22, 753 - 758.

Püschel, T., 2014. Modularidad e Integración Morfológica en Cráneos Humanos: un enfoque Morfométrico Geométrico. Int. J. Morphol. 32, 299 - 304.

Roberts, L.S., Janovy, J.Jr., 1996. Foundations of Parasitology. Wm C. Brown Co, Dubuque. Iowa.

Rohlf, F.J., 1993. Relative warps analysis and an example of its application to mosquito wings. In: Marcus, L.F., Bello, E., Garcia-Valdecasas, A. (Ed.), Contributions to morphometrics, Museu Nacional de Ciencias Naturales Madrid, pp. 131 - 159.

Rohlf, F.J., Corti, M., 2000. Use of Two-Block Partial Least-Squares to study covariation in shape. Syst. Biol. 49, 740 - 753.

Rohlf, F.J., Marcus, L.F., 1993. A revolution in morphometrics. Trends. Ecol. Evol. 8, 129 - 132.

Rohlf, F.J., Slice, D.E., 1990. Extensions of the Procrustes Method for the Optimal Superimposition of Landmarks. Syst. Biol. 39, 40 - 59.

Rosenberg, N.A., Pritchard, J.K., Weber, J.L., Cann, H.M., Kidd, K.K., Zhivotovsky, L.A., Feldman, M.W., 2002. Genetic structure of human populations. Science 298, 2381 - 2385.

Rubio-Godoy, M., 2008. Microhabitat selection of *Discocotyle sagittata* (Monogenea: Polyopisthocotylea) in farmed rainbow trout. Folia Parasitol. 55, 270 - 276.

Rubtsova, N.Y., Balbuena, J.A., Sarabeev, V.L., Blasco-Costa, I., Euzet, L., 2006. Description and morphometrical variability of *Ligophorus cephali* n. sp. and *Ligophorus chabaudi* Euzet and Suriano, 1977 (Monogenea:Dactylogyridae) on *Mugil cephalus* (Teleostei) from the Mediterranean Basin. J. Parasitol. 92, 486 - 495.

Rubtsova, N.Y., Sarabeev, V.L., Shvidka, S.V., Balbuena, J.A., Borisenkov, B.S., 2005. Morphometric variability of *Ligophorus vanbenedenii* (Ancyrocephalidae: Monogenea) and its relationship with host characteristics and infrapopulation size. Bull. Zaporizhzhia Natl. Univ. Biol. Sci. 1, 151 - 158 [In Ukrainian].

Sarabeev, V., Desdevises, Y., 2014. Phylogeny of the Atlantic and Pacific species of *Ligophorus* (Monogenea: Dactylogyridae): Morphology vs. molecules. Parasitol. Int. 63, 9 - 20.

Sarabeev, V., Rubtsova, N., Yang, T., Balbuena, J.A., 2013. Taxonomic revision of the Atlantic and Pacific species of *Ligophorus* (Monogenea, Dactylogyridae) from mullets (Teleostei, Mugilidae) with the proposal of a new genus and description of four new species. J. Schmalhausen Inst. Zool. 28, 1 - 112.

Sarris, I., Marugán-Lobón, J., Chamero, B., Buscalioni, A.D., 2012. Shape Variation and Allometry in the Precloacal Vertebral Series of the Snake *Daboia russelli* (Viperidae). Int. J. Morphol. 30, 1363 - 1368.

Silan, P., Maillard, C., 1989. Biologie comparée du développement et discrimination des Diplectanidae ectoparasites du Bar (Teleostei). Ann. Sci. Nat. Zool. Paris, 13ème série 10, 31 - 45.

Šimková, A., Desdevises, Y., Gelnar, M., Morand, S., 2001. Morphometric correlates of host specificity in *Dactylogyrus* species (Monogenena) parasites of European Cyprinid fish. Parasitology 123, 169 - 177.

Šimková, A., Kadlec, D., Gelnar, M., Morand, S., 2002. Abundance - prevalence relationship of gill congeneric ectoparasites: testing the core satellite hypothesis and ecological specialisation. Parasitol. Res. 88, 682 - 686.

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., Ondračková, M., Gelnar, M., Morand S., 2002. Morphology and coexistence of congeneric ectoparasite species: reinforcement of reproductive isolation? Biol. J. Linn. Soc. 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.

Siquier, G.F., Ostrowski de Núñez, M., 2009. *Ligophorus uruguayense* sp. nov. (Monogenea: Ancyrocephalidae), a gill parasite from *Mugil platanus* (Mugiliformes, Mugilidae) in Uruguay. Acta Parasitol. 54, 95 - 102.

Soler-Jiménez, L., Fajer-Ávila, E.J., 2012. The microecology of dactylogyrids (Monogenea: Dactylogyridae) on the gills of wild spotted rose snapper *Lutjanus guttatus* (Lutjanidae) from Mazatlan Bay, Mexico. Folia Parasitol. 59, 53 - 58.

Soria, J.M., 2006. Past, present and future of la Albufera of Valencia Natural Park. Limnetica 25, 135 - 142.

Soria, J.M., Vicente, E., Miracle, M.R., 2000. The influence of flash floods on the limnology of the Albufera of Valencia lagoon (Spain). Verh. Int. Ver. Limnol. 27, 2232 - 2235.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731 - 2739.

Turgut, E., Shinn, A., Wootten, R., 2006. Spatial distribution of *Dactylogyrus* (Monogenean) on the gills of the host fish. Turkish J. Fish. Aquat. Sci. 6, 93 - 98.

Verneau, O., Du Preez, L., Badets, M., 2009. Lessons from parasitic flatworms about evolution and historical biogeography of their vertebrate hosts. C. R. Biol. 332, 149 - 158.

Vignon, M., Pariselle, A., Vanhove, M.P.M., 2011. Modularity in attachment organs of African *Cichlidogyrus* (Platyhelminthes: Monogenea: Ancyrocephalidae) reflects phylogeny rather than host specificity or geographic distribution. Biol. J. Linn. Soc. 102, 694 - 706.

Vignon, M., Sasal, P., 2010. The use of geometric morphometrics in understanding shape variability of sclerotized haptoral structures of monogeneans (Platyhelminthes) with insights into biogeographic variability. Parasitol. Int. 59, 183 - 191.

Viscosi, V., Cardini, A., 2011. Leaf Morphology, Taxonomy and Geometric Morphometrics : A Simplified Protocol for Beginners. PLoS One 7, 1 - 20.

Vrat, P.A., Shanker, H.S., 2013. Functional dynamics observations of haptoral armature in *Mizelleus indicus* (Jain 1957) Pandey et al. 2003. J. Parasit. Dis. 1 - 6.

Whittington, I.D., Kearn, G.C., 1991. The adhesive attitudes of some gill-parasitic capsalid monogeneans. J. Helminthol. 65, 280 - 285.

Woolhouse, M.E.J., Haydon, D.T., Antia, R., 2005. Emerging pathogens: the epidemiology and evolution of species jumps. Trends Ecol. Evol. 20, 238 - 244.

Wu, X., Zhu, X., Xie, M., Li, A., 2007. The evaluation for generic-level monophyly of Ancyrocephalinae (Monogenea, Dactylogyridae) using ribosomal DNA sequence data. Mol. Phylogenet. Evol. 44, 530 - 544.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D., 2012. Geometric Morphometrics for Biologists: A primer. Academic Press, London.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D., Fink W.L., 2004. Geometric morphometrics for Biologists: A primer. Elsevier, San Diego.

Zhou, S., Campbell, T.G., Stone, E.A., Mackay, T.F.C., Anholt, R.R.H., 2012. Phenotypic Plasticity of the Drosophila Transcriptome. PLoS Genet. 8, e1002593.

Zuur, A.F., Leno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3 - 14.

Legends to Figures

Fig. 1. Ventral (A) and dorsal (B) view of anchors of *Ligophorus cephali* (micrographs and drawings). The positions of the eight landmarks were used for morphological analyses. 1. Maximum point of inner root. 2. Inflection between outer root and inner root. 3. Mean point of outer root. 4. Outer shaft base. 5. Outer point base. 6. Anchor point. 7. Inner point base. 8. Inner shaft base. Scale bar = 20 µm.

Fig. 2. Scatterplot of relative warps 1 and 2 (RW 1 and RW 2) of the ventral (A) and dorsal (B) anchors of *Ligophorus cephali*. Points represent the positions of individual worms in the shape space. Splines associated with these first two relative warps are shown with a 2× magnification. Deformation grids indicate general shapes at the extremes of the scatterplot and grey color coded Jacobian expansion factors convey the degree of local expansion or contraction of the grid. Values >1 indicate expansions and values between 0 and 1 indicate contraction, relative to positive and negative extremes of plot.

Fig. 3. Relationship between centroid size of ventral (A) and dorsal anchors (B) of *Ligophorus cephali* with host weight. The trend lines are cubic smoothing splines.

Table 1 Factors accounting for significant variation in the shape of ventral and dorsal anchors of *Ligophorus cephali* as revealed by a Permutational Multivariate Analysis of Variance (PERMANOVA) based on pairwise Euclidean distances of relative warps coordinates.

Source of variation	Variation components	*P* (perm)
Ventral anchors		
Gill section × host	$1.95 \cdot 10^{-3}$	0.029
Residual	$7.16 \cdot 10^{-3}$	
Dorsal anchors		
Gill arch	0.39	0.001
Residual	4.98	

P (perm), *P*-value based on random permutations.

Table 2 Generalized Lineal Mixed Models that better explain the centroid size (CS) of ventral anchors of *Ligophorus cephali* according to the values of the Akaike Information Criterion (AIC). Seven (out of 11) models with differences in AIC (ΔAIC), relative to the best model, < 2 are presented (best model in bold). Weights of evidence in support of a particular model (*w*) are also listed.

Models	**AIC**	**ΔAIC**	***w***
CS ~ log(WS) + HOST	**507.1**	**0.0**	**0.25**
CS ~ 1 + HOST	508.0	0.9	0.16
CS ~ log(WS) + ARC + AREA + HOST	508.2	1.1	0.14
CS ~ log(WS) + SEC + HOST	508.0	1.3	0.13
CS ~ log(WS) + AREA + HOST	508.8	1.7	0.11
CS ~ log(WS) + ARC + HOST	508.8	1.7	0.11
CS ~ log(WS) + SEC + AREA + HOST	508.9	1.8	0.10

WS, worm size; ARC, gill arch; AREA, gill arch; SEC, gill section; HOST, host individual (random factor).

Table 3 Generalized Lineal Models that better explain the centroid size (CS) of dorsal anchors of *Ligophorus cephali* according to the values of the Akaike Information Criterion (AIC) values. Three (out of seven) models with differences in AIC (ΔAIC), relative to the best model, < 2 are presented (best model in bold). Weights of evidence in support of a particular model (*w*) are also listed.

Models	**AIC**	**ΔAIC**	***w***
CS ~ ARC	**560.2**	**0.0**	**0.50**
CS ~ ARC + AREA	561.4	1.1	0.28
CS ~ ARC + log(WS)	561.9	1.7	0.21

WS, worm size; ARC, gill arch; AREA, gill arch.

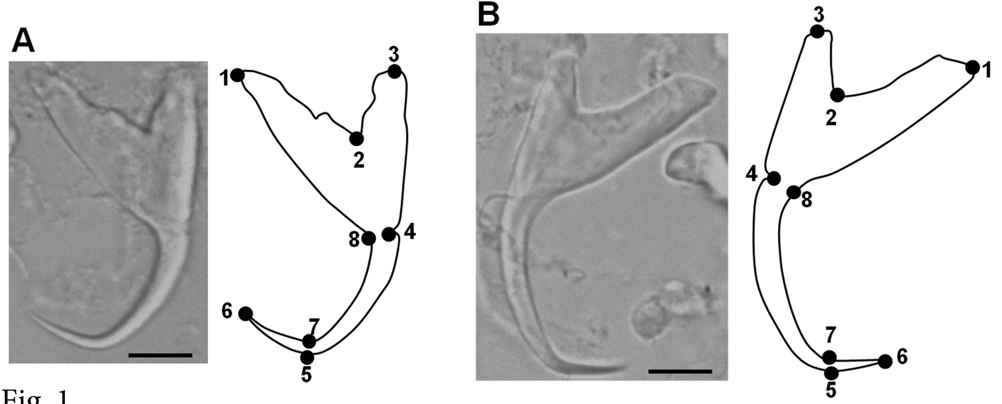

Fig. 1

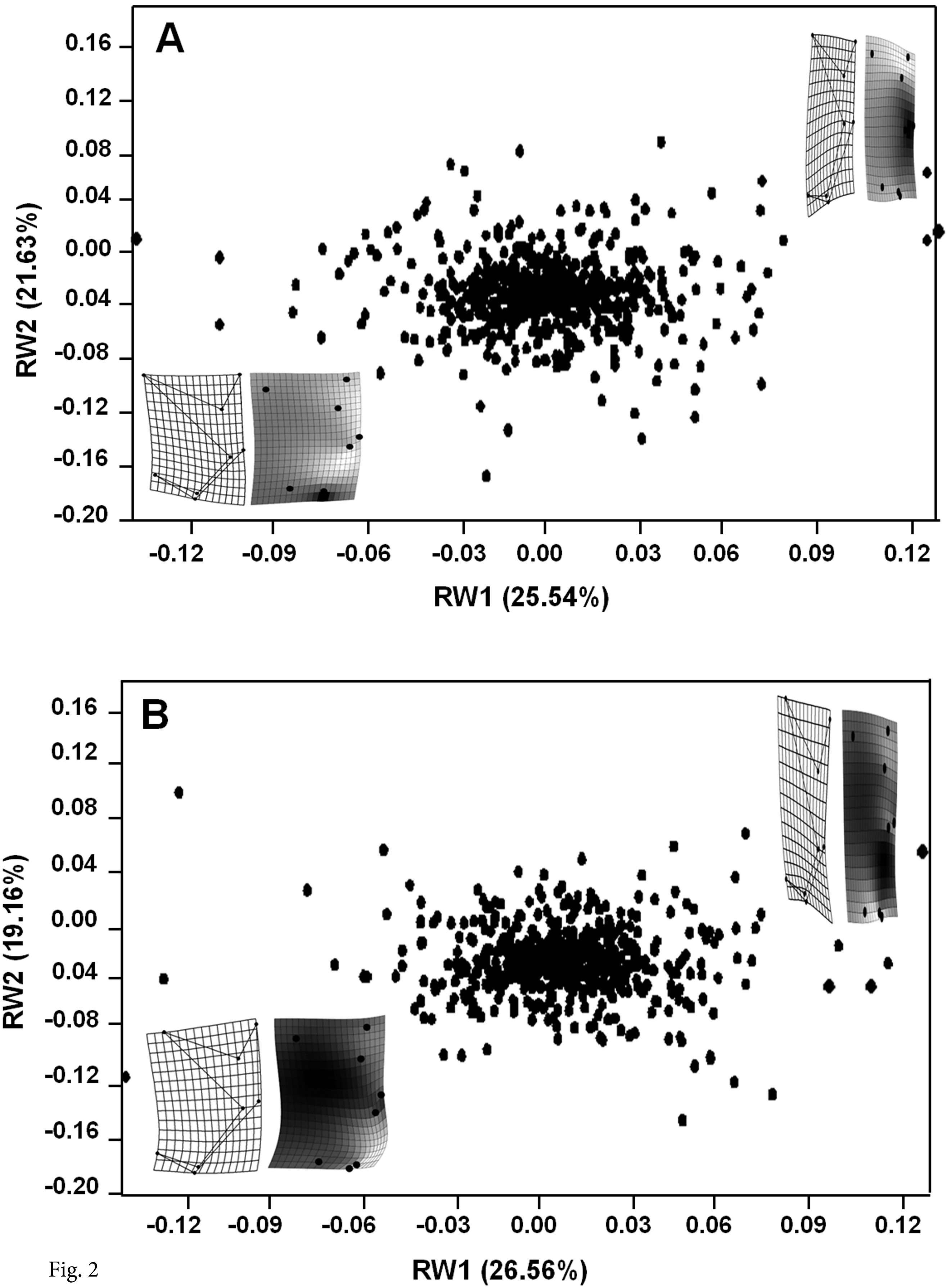
A
0.16
0.12
0.08
0.04
0.00
0.04
-0.08
-0.12
-0.16
-0.20
RW2 (21.63%)
-0.12
-0.09
-0.06
-0.03
0.00
0.03
0.06
0.09
0.12
RW1 (25.54%)
B
0.16
0.12
0.08
0.04
0.00
0.04
-0.08
-0.12
-0.16
-0.20
RW2 (19.16%)
-0.12
-0.09
-0.06
-0.03
0.00
0.03
0.06
0.09
0.12
RW1 (26.56%)

Fig. 2

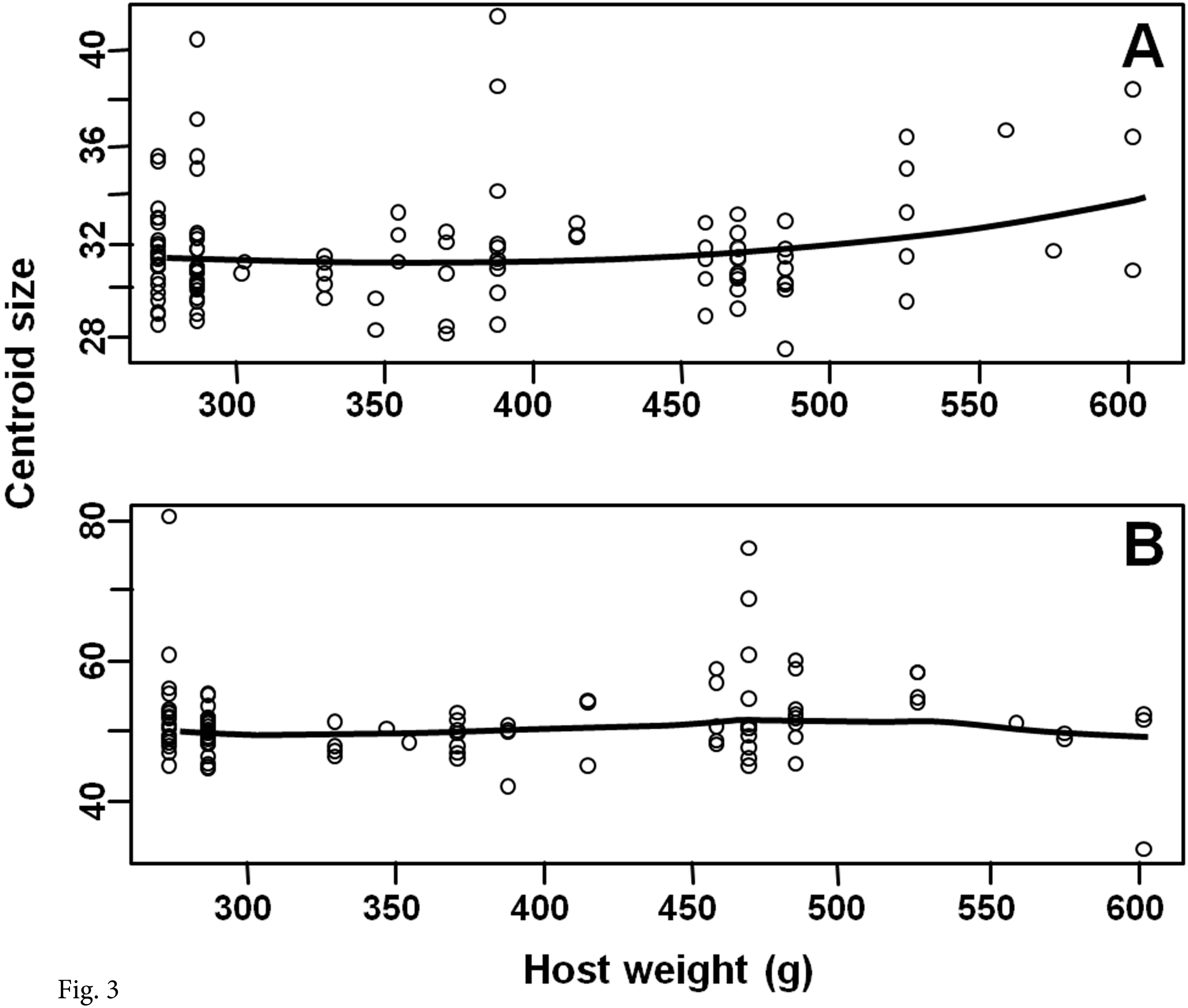
Centroid size
A
40
36
32
28
300
350
400
450
500
550
600
B
80
60
40
300
350
400
450
500
550
600
Host weight (g)

Fig. 3