International Journal for Parasitology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

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

ARTICLE INFO

Article history:
Received 16 August 2014
Received in revised form 25 December 2014
Accepted 7 January 2015
Available online xxxx

Keywords:
Geometric morphometrics
Phenotypic plasticity
Haptor
Monogenean
Western Mediterranean
Mugilidae

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 analyzed 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.

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 favor 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 behavioral 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

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

* Corresponding author. Tel.: +34 963543685; fax: +34 963543733.
E-mail address: arogon5@uv.es (A. Rodríguez-González).

http://dx.doi.org/10.1016/j.ijpara.2015.01.005
0020-7519/

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 and 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 (*M. cephalus*) were collected in L'Albufera, Spain (39°20′ N–0°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 2× MyFi Mix (Bioline Ltd., United Kingdom) and 5 pmol/µL of each primer. The following thermocycling profile was applied: denaturation of DNA at 95 °C for 3 min, 35 cycles of amplification with 40 s of denaturation at 94 °C, 30 s primer annealing at 56 °C and 45 s at 72 °C for primer extension, and a final extension step of 4 min at 72 °C. 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)

Please cite this article in press as: Rodríguez-González, A., et al. Phenotypic plasticity in haptoral structures of *Ligophorus cephali* (Monogenea: Dactylogyridae) on the flathead mullet (*Mugil cephalus*): a geometric morphometric approach. Int. J. Parasitol. (2015), http://dx.doi.org/10.1016/j.ijpara.2015.01.005