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Phylogeny of the Atlantic and Pacific species of *Ligophorus* (Monogenea: Dactylogyridae): Morphology vs. molecules

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ABSTRACT

Within ectoparasitic fish monogeneans, the genus *Ligophorus* contains a high number of species from which several were recently described. The precise determination of their taxonomic status requires robust diagnostic morphologic features that rely predominantly on a restricted set of sclerotized structures. In the present study, these morphological characters were used for the reconstruction of a phylogenetic tree, which was compared with a tree built from molecular data (28S and ITS1 DNA sequences). Thirty-eight morphological characters were used in 29 species of *Ligophorus* from the Atlantic and Pacific regions and 5 species within close genera of Dactylogyridae. The morphological and molecular phylogenetic trees are congruent and suggest that the genus *Ligophorus* is monophyletic, and that species parasitizing *Liza* spp. and *Chelon labrosus* occupy basal positions. The present study suggests that host switching is a common event in this host–parasite association, because about half of the species infecting the same host species are not close relatives. Following host switching, dispersal with vicariance is probably an important force shaping the present distribution and diversity of *Ligophorus*. The pattern of occurrence of *Ligophorus* spp. on *Mugil cephalus* supports that reproductive isolation and therefore parallel speciation are taking place among these parasitic organisms.

1. Introduction

In dactylogyrid monogenean ectoparasites, there are at least three genera completely restricted to the grey mullets (Teleostei: Mugilidae). Among them *Ligophorus* is the most diverse and widely distributed. The last revision of this genus recognized 33 species from ten mullet hosts in the Atlantic and Pacific regions [1]. Furthermore, 16 species were recently described from four mullets sampled in the Indian Ocean [2,3]. The other two genera, *Ergenstrema* Paperna, 1964 and *Kriboetrema* Sarabeev, Rubtsova, Tingbao and Balbuena, 2013, less diverse and represented by two species in each, seem to have a geographic distribution restricted to the NE Atlantic, including the Mediterranean, and the NW Pacific region, respectively [1,4–6]. However, our knowledge of the diversity of dactylogyrids from mullets remains imperfect because most studies have concentrated on a limited range of hosts and geographic areas. Moreover, considering that dactylogyrid monogeneans on mullets exhibit a high host-specificity and are common and abundant, it is likely that many additional species remain to be discovered [1,7].

The increasing number of species described within *Ligophorus* points out the problem of differential species diagnosis that relies on a restricted set of morphological traits. These traits are predominantly represented by morphology and size of sclerotized elements of the haptor and genital structures [e.g. 1–3,8–10]. The ranges of variation of metric characters between *Ligophorus* species overlap widely, making their identification difficult and rising the question of species validity within the genus. It prompts to use of multivariate analyses such as stepwise linear discriminant or principal component analyses to help species discrimination and then a dimensional reduction of the original dataset [2,3,10–15]. Some attempts were made to group morphologically similar species of *Ligophorus* to investigate their taxonomic status: Mariniello et al. [9] relied on distance matrices obtained from morphometric data to describe the phenetic relationships between species; Soo and Lim [3] used numerical taxonomy based on morphological and morphometric characters to group 42 species of *Ligophorus* to distinguish them from 8 newly described species.

In dactylogyrid monogeneans from mullets, ribosomal DNA sequences are currently available only for a limited number of species. It includes *Ligophorus vanbenedenii* (Parona and Perugia, 1890) and *Ligophorus leporinus* (Zhang and Ji, 1981) both from flathead mullet *Mugil cephalus* L. in Guangdong, China [16,17], *Ergenstrema mugilis* Paperna, 1964, and 14 out of 16 nominal *Ligophorus* species from five mullet hosts in the Mediterranean, Black and Azov Seas [7]. Phylogenetic reconstructions for all these species were carried out by Blasco-Costa et al. [7]. The analysis of these molecular data helped to clarify the position of *Ligophorus* and *Ergenstrema* within the Dactylogyridae,

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supported the validity of 15 species, and provided the first clues to understand the diversification of *Ligophorus* spp. in relation to their hosts.

In the present study, we decided to build a morphological-based phylogenetic tree of these monogenean species for several reasons. First, there are taxa that are still very difficult to include in molecular studies [18]. For example, some species of *Ligophorus* and *Kriboetrema* are known from a very limited number of species (i.e. *Ligophorus zhangi* Dmitrieva, Gerasev, Gibson, Pronkina and Gally, 2012; *Ligophorus miroshnichenki* Sarabeev, Rubtsova, Yang, Balbuena, 2013; *Kriboetrema ellochelon* (Zhang, 2001); *Kriboetrema rectangulus* Sarabeev, Rubtsova, Yang, Balbuena, 2013), and are stored in a way that makes impossible DNA extraction (i.e. fixation on slides in Canada balsam, glycerin jelly or in other mediums). Second, it is important to have accurate, morphological-based phylogenies to compare them to molecular-based trees [19]. Unfortunately, we are very far from describing all living species on Earth, and much less to obtain molecular data for all of them [18].

There is a current discussion regarding the validity of some taxonomic characters diagnosed in *Ligophorus* or applicable for species identification within this genus [1,3,14,15]. This discussion concerns the structure and shape of the base of the copulatory organ, the accessory piece of the male copulatory complex and the ventral bar. A description of the main morphological types of key sclerotized structures useful for species discrimination was recently provided by Sarabeev et al. [1]. In the present study, we examine the validity of morphological features considered in the latter paper: i) to carry out a cladistic analysis within Dactylogyridae from the Atlantic and Pacific mullets based on morphological characters and ii) to test the link between morphological and molecular interspecific similarities of *Ligophorus* spp.

2. Material and methods

2.1. Taxa chosen

We have been studying *Ligophorus* spp. for several years [1,11–13,20] and had access to all the essential material. The taxonomic revision of the Atlantic and Pacific representatives of the genus by Sarabeev et al. [1] recognized 33 valid species and erected a new genus, *Kriboetrema*, for two Dactylogyridae species. 29 revised species of *Ligophorus* and two species of *Kriboetrema* were selected for the present phylogenetic analysis, all treated below as ingroup taxa (Table 1). The remaining four species, *Ligophorus chenzhenensis* Hu and Li, 1992, *Ligophorus hamulosus* Pan and Zhang, 1999, *Ligophorus guanduensis* Abdallah, Azevedo and Luque, 2009 and *Ligophorus lizae* Abdallah, Azevedo and Luque, 2009, were not available for loan [1], then an unambiguous definition of many morphological characters for these species was problematic to achieve. Whenever possible, at least three specimens were examined for each taxon (in most cases 15 or more), and only mature worms were considered (Table 1).

The outgroup taxa were selected based on a recent molecular phylogenetic analysis of *Ligophorus* provided by Blasco-Costa et al. [7] and include i) *E. mugilis* Paperna, 1964 as the sister taxon of *Ligophorus* and ii) several species (see below) of *Cichlidogyrus* Paperna, 1960 and *Euryhaliotrema* Kritsky and Boeger, 2002 as the putative closest basal genera of *Ligophorus.* Character argumentation for outgroup species was based on published information on *Euryhaliotrema chaoi* Kritsky and Boeger, 2002 and the examination of: i) representative specimens of *Cichlidogyrus agnesi* Pariselle and Euzet, 1995 mounted in ammonium picrate–glycerine, which were kindly provided by Antoine Pariselle (Chargé de recherche IRD, CNRS, Université Montpellier II), and ii) our own specimens of *E. mugilis* samples mounted unstained directly in glycerin jelly [21] or stained in iron acetocarmine [22], dehydrated through different ethanol concentrations (from 70 to 100%), cleared in dimethyl phthalate and mounted as whole mounts in Canada balsam to ascertain details of their soft internal anatomy.

Table 1
Zoogeographical and host information on the specimens of Dactylogyridae used in the present study.

Species	N	Host[a]	Locality[b]
Ingroup			
Ligophorus abditus Dmitrieva, Gerasev, Gibson, 2013	14	MC	KB
L. acuminatus Euzet and Suriano, 1977	19	LS	ED
L. angustus Euzet and Suriano, 1977	28	CL	JE
L. brasiliensis Abdallah, Azevedo and Luque, 2009	3	ML	GR
L. cephali Rubtsova, Balbuena, Sarabeev, Blasco-Costa and Euzet, 2006	57	MC	KC
L. chabaudi Euzet and Suriano, 1977	21	MC	JE
L. cheleus Rubtsova, Balbuena and Sarabeev, 2007	28	MC	KB, RD
L. confusus Euzet and Suriano, 1977	14	LR	ED
L. domnichi Rubtsova, Balbuena and Sarabeev, 2007	32	MC	KB
L. heteronchus Euzet and Suriano, 1977	42	LS	ED, SB
L. huitrempe Fernández, 1987	4	MC	BRC
L. imitans Euzet and Suriano, 1977	25	LR	SPG
L. kaohsianghsieni (Gussev, 1962)	18	LH	KC
L. llewellyni Dmitrieva, Gerasev and Pron'kina, 2007	14	LH	AD, RD
L. macrocolpos Euzet and Suriano, 1977	17	LS	ED
L. mediterraneus Sarabeev, Balbuena and Euzet, 2005	37	MC	KC
L. minimus Euzet and Suriano, 1977	39	LS	ED
L. miroshnichenki Sarabeev, Rubtsova, Yang, Balbuena, 2013	7	LH	PB
L. mugilinus (Hargis, 1955)	5	MC	CH
L. pacificus Rubtsova, Balbuena and Sarabeev, 2007	31	MC	KB, RD
L. parvicirrus Euzet and Sanfilippo, 1983	13	LR	ED, SPG
L. pilengas Sarabeev and Balbuena, 2004	15	LH	PB
L. rectus Sarabeev, Rubtsova, Yang, Balbuena, 2013	4	LT	ZH
L. saladensis Marcotegui and Martorelli, 2009	1	MP	SBB
L. szidati Euzet and Suriano, 1977	19	LA	JE, SPG
L. tainhae Abdallah, Azevedo and Luque, 2009	3	ML	GR
L. triangularis Sarabeev, Rubtsova, Yang, Balbuena, 2013	19	LH	AD, PB, RD
L. uruguayense Failla Siquier and Ostrowski de Núñez, 2009	2	MP	LF
L. vanbenedenii (Parona and Perugia, 1890)	91	LA	ED
Kriboetrema ellochelon (Zhang, 2001)	4	LV	ZH
K. rectangulus Sarabeev, Rubtsova, Yang, Balbuena, 2013	3	VC	ZH
Outgroup			
Cichlidogyrus agnesi Pariselle and Euzet, 1995	4	TG	BR
Ergenstrema mugilis Paperna, 1964	5	LR	ED

[a] Host abbreviations: CL, *Chelon labrosus* (Risso); LA, *Liza aurata* (Risso); LH, *Liza haematocheilus* (Temminck and Schlegel); LR, *Liza ramada* (Risso); LS, *Liza saliens* (Risso); LT, *Lz. tade* (Forsskål); LV, *Liza vaigiensis* (Quoy and Gaimard); MC, *Mugil cephalus* L.; ML, *Mugil liza* Valenciennes; MP, *Mugil platanus* Günther; TG, *Tilapia guineensis* (Bleeker, 1862); VC, *Valamugil cunnesius* (Valenciennes).

[b] Locality abbreviations: AD, Artemovka Delta, Sea of Japan, Russia; BR, Tiassalé, Bandama River, Ivory Coast; BRC, Biobio River, Concepción, SE Pacific, Chile; CH, Charleston, South Carolina, NW Atlantic, USA; ED, Ebro Delta, Mediterranean Sea, Spain; GR, Guandu River, State of Rio de Janeiro, SW Atlantic, Brazil; JE, Júcar Estuary, Mediterranean Sea, Spain; KB, Kiyevka Bay, Sea of Japan, Russia; KC, Kerch Channel, Black Sea, Ukraine; LF, Las Flores, Department of Maldonado, SW Atlantic, Uruguay; PB, Posiet Bay, Sea of Japan, Russia; RD, Razdol'naya Delta, Sea of Japan, Russia; SB, Sozopol Bay, Black Sea, Bulgaria; SBB, Samborombón Bay, SW Atlantic, Argentina SPG, ZH, Zhanjiang, Guangdong Province, South China Sea, China.

2.2. Character chosen and phylogenetic analysis

The phylogenetic analysis was performed with PAUP 4.0 Beta 6 for PC [23] based on a parsimony criterion. Homologous series and character states were defined trough the study of voucher and type specimens or according to previous phylogenetic studies on monogeneans [24,25], in particular Dactylogyrids [26–28]. An initial phylogenetic reconstruction was based on 57 transformation series. The original set of characters was evaluated using consistency index (CI) and character changes at each node to reveal characters with high homoplasy. After phylogenetic analyses with PAUP, a list of apomorphies was obtained, which includes CI for each character. The program was rerun excluding one character at a time, beginning with the lowest CI, and observing the effect, if any, that the removal of that character had on the results. Nineteen characters with lowest CI (position of vaginal aperture; presence