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Morphometric comparisons of plant-mimetic juvenile fish associated with plant debris observed on Kuchierabu-jima Island, southern Japan

Alexya C. Queiroz¹, Yoichi Sakai², Marcelo Vallinoto¹,³, and Breno Barros¹,²,⁴

¹ Universidade Federal do Pará - campus de Bragança, Laboratório de Evolução, Instituto de Estudos Costeiros, Alameda Leandro Ribeiro, s/n, Aldeia, CEP 68600-000 Bragança, Pará, Brazil

² Graduate School of Biosphere Science, Hiroshima University, 1-4-4, Kagamiyama, 739-8528, Higashi-Hiroshima, Japan

³ CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, Universidade do Porto, 4485-661, Vairão, Portugal

⁴ Ichthyology Division, Coordination of Zoology. Museu Paraense Emílio Goeldi Av. Perimetral s/n, Montese, CEP 66077-830, Belém, Pará, Brazil

Running title: Comparative fish and plant morphometrics

Corresponding author: breno_eduardo@terra.com.br

Tel: +55 91 3075 6125
Abstract

The general morphological shape of plant-resembling fish and plant parts were compared using a geometric morphometrics approach. *Lobotes surinamensis* (Lobotidae), *Platax orbicularis* (Ephippidae) and *Canthidermis maculata* (Balistidae), three plant-mimetic fish species, were compared during their early developmental stages with accompanying plant parts (i.e. leaves of several taxa) in the coastal subtropical waters of Kuchierabu-jima Island, closely facing the Kuroshio Current. The degree of similarity shared between the plant parts and co-occurring fish species was quantified, however fish remained morphologically distinct from their plant models. Such similarities were corroborated by a linear model, in which relative body areas of fish and plant models were strongly interdependent. Our results strengthen the paradigm that morphological clues can lead to ecological evidence to allow predictions of behavioural and habitat choice by mimetic fish, according to the degree of similarity shared with their respective models. The resemblance to plant parts detected in the three fish species may provide fitness advantages via convergent evolutionary effects.

Key-words: Protective camouflage, Masquerade, Coastal Environments, Morphometrics, Shape analysis, Convergent evolution
1. Introduction

Mimesis is defined as a phenotype evolved in response to selective pressures favouring individuals that can disguise their identity by masquerading as another organism (Wickler, 1968; Pasteur, 1982; Moland, Eagle & Jones, 2005; Skelhorn, Rowland & Ruxton, 2010a; Skelhorn et al., 2010b). Therefore, many organisms have never been evaluated as mimetic examples despite their high resemblance to plant parts because of insufficient data suggesting an adaptive function. Mimesis in fish is a relatively well-studied subject (Wickler, 1968; Moland, Eagle & Jones, 2005; Robertson, 2013), particularly regarding deceptive resemblance to plant parts via protective camouflage, which is a known feature in several freshwater and marine fish species, as extreme crypsis examples of protective resemblance (Breder, 1942, 1946, 1949, 1955; Randall, 1965, 2005a; Vane-Wright, 1980; Sazima et al., 2006; Barros & Higuchi, 2007). Although these reports have addressed the patterns and general similarities in morphology or colouration of model plant parts and mimetic fish, few studies have examined similarities among them based on morphological and/or ethological details (Barros et al., 2008, 2011, 2012).

Studies focusing on morphology and geometric morphometrics frequently used fish species as models, and several authors have suggested that morphological clues can be used as ecological predictors from basic behavioural constraints, such as swimming mode (Walker, 2004; Comabella, Hurtado & García-Galano, 2010; Xiong & Lauder, 2014), feeding behaviour (Galis, 1990; Franssen, Goodchild & Shepard, 2015) and habitat choice (Loy et al., 1998; Gibran, 2010; Soares, Ruffeil & Montag, 2013), especially in juvenile fish, suggesting that such changes are important for improving fitness and increasing the chance for survival during subsequent ontogenetic stages (Barros et al., 2011; Comabella et al., 2013). Nevertheless, such a
tool has not been used to establish comparisons among distant taxa belonging to completely
different groups (i.e. fish and plants). In the present study, previously well-known plant-mimetic
juvenile fish, the tripletail, *Lobotes surinamensis* (Bloch, 1790), the orbicular batfish, *Platax
orbicularis* (Forsskål, 1775) and the ocean triggerfish, *Canthidermis maculata* (Bloch, 1786)
were compared with their respective plant models co-occurring in the field to objectively
evaluate their resemblance in shape to their respective models.

*Lobotes surinamensis* is generally found in shallow brackish water habitats but may occur
far offshore with drifting algae or flotsam, and juveniles may lie on their side matching the
colour of the plant material with which they are drifting, from near black to yellow (Randall,
2005b). Juveniles are usually dark-coloured, presenting drifting swimming patterns among dry
leaves, exhibiting similar movements to their associated plant model (Uchida, 1951; Randall,
2005b). Uchida (1951) also described that young *C. maculata* resemble pieces of pine bark and
were observed drifting among pieces of bark in a horizontal swimming posture, suggesting
mimetic effects. Juveniles of *P. orbicularis* look similar to yellow waterlogged jack tree leaves
(genus *Rhizophora*) and greatly resemble floating dead leaves (Wiley, 1904; Breder, 1946).
Randall (1960) reported that larger individuals (87 mm standard length [SL]) resemble large sea
hibiscus leaves (*Hibiscus tiliaceus*) with a yellowish-brown colouration, with dorsal and anal fins
appearing to lengthen with growth. Such drastic changes in morphological shape occur in
juvenile *P. orbicularis* while they maintain a resemblance to drifting leaves (Barros et al., 2008,
2011).

The novel comparative methods presented herein may provide useful associations between
behavioural ecology and morphological studies. We tested the null hypothesis of a lack of shape
similarity among the studied fish and plant parts, considering both classic and geometric
morphometrics comparative approaches. We briefly discuss the functional contributions of
camouflage characteristics to fish fitness using mimetic shape attributes as a disguise based on
morphological resemblance data among fish and model plants, adopting the concepts of cryptic
mimesis as synonym of protective camouflage or masquerading, following the definitions as
proposed by Pasteur (1982), where all fish samples are defined as "mimetic fish" and all plant
part samples as "models", instead of adopting the terminology as proposed by Skelhorn,
Rowland & Ruxton (2010a). This is due to the highly dynamic environments such fish usually
occur, where mimetic behaviour is achieved not only by appearance, but also through actively
behaving alike the drifting models (Barros et al., 2008).

2. Material and Methods

2.1. Sampling

Sampling was conducted in the port of Honmura, Kuchierabu-Jima Island (Ohsumi Group, 30°
28′ N, 130° 10′ E), southern Japan, during diurnal observations July 3–14, 2011 (S1 Fig.). The
island closely faces the Kuroshio Current and maintains a rich subtropical fish fauna (Gushima &
Murakami, 1976). Fish samples and plant debris were collected using hand nets, and the sampled
fish were euthanized using 5 ml 95% eugenol in 1 L ethanol as a stock solution. All plant
material were sampled along with their associated fish. Of this, 20 ml was added to each 1 L of
water containing the fish to be euthanized to minimise suffering, following international ethical
standards (Jenkins et al., 2014). As there is no national Japanese licensing framework, samples
were collected following the “Guidelines for Proper Conduct of Animal Experiments” set out by
the Hiroshima University Animal Research Committee, which are based on international ethical
standards, and only after obtaining local community permission.

The fish were identified to as low a taxonomic category as possible, according to available literature (Nakabo, 2002; Nelson, 2006; Okiyama, 2014). Fifteen mimetic fish specimens of three species (Fig. 1A–C) were analysed: *Lobotes surinamensis* (Lobotidae; n = 6, TL = 2.71 ± 0.46 cm), *Canthidermis maculata* (Balistidae; n = 2, TL = 2.02 ± 0.98 cm) and *Platax orbicularis* (Ephippidae; n = 7, TL = 1.80 ± 0.42 cm).

Floating plant debris (hereafter, models, n = 52) were collected using hand nets and sorted, then visually subdivided using two subjective criteria (round shapes, as for the Podocarpaceae *Nageia nagi* and the Sapindaceae *Acer morifolium*; or elongated shapes, as for the Laureaceae *Neolitsea sericea* and for the Fagaceae *Castanopsis sieboldii*; Fig. 1D–E), regardless of taxonomy and dried in paper envelopes until they were photographed for further analysis.

High resolution digital pictures of the left lateral view of the mimetic fish and model samples were taken over a black background using a Nikon D700 equipped with AF-S 60-mm immersive lens and a stand table with a reference scale of 1 cm for the fish and models. The left lateral view of the models was defined as the “dorsal view of leaves with the petiole oriented to the right”. Artificial light was used to avoid shading morphological structures.

2.2. Data Analyses

Sixteen landmarks (LM) were established for the mimetic fish and models using ImageJ v. 1.47 software for geometric morphometrics purposes (Abramoff, Magelhaes & Ram, 2004). Homologous LM for the mimetic fish were marked obeying the morphological structures constraining or related to mimetic behaviour to cover the fish general outline profile, including peripheral structures (Fig. 1A, Table I). The data set used in the present analysis is made...
available in Supplementary Information 1. We established equidistant 16 semilandmarks (SLM)
for each model using the ImageJ grid tool to cover all lateral profiles of the model (Fig. 1D).
Raw coordinate LM and SLM data were implemented in MorphoJ v. 1.02n software
(Klingenberg, 2011), where preliminary adjustments, such as the Procrustes fit, and creation of
the data matrix, were done. The morphometric comparisons among the fish and models were not
intended for use to analyse homologous patterns, as we were interested in shape similarities
randomly shared among the mimetic fish and their respective models distributed in the same
environment, from a geometric morphometrics perspective. Therefore, the necessity of marking
peripheral anatomic structures in the mimetic fish, instead of fins insertions only, in order to
check for general appearance of mimetic fish with the plant models.

Data analyses were performed with Geomorph v. 2.0 software (Adams & Otarola-Castillo,
2013). A post-hoc general Procrustes analysis (GPA) and principal components analysis (PCA)
were run followed by analysis of variance (ANOVA) to compare the mimetic fish and models
plotted together in the analyses. Also, a linear discriminant function was run, in order to visualize
how close were these group associations, using the package MASS v. 7.3-42 (Venables &
Ripley, 2002).

In addition, individual TL and relative body area (BA, cm²/SL) of the fish and models were
calculated using ImageJ to establish interdependent comparisons among the fish species and
plant debris via analysis of covariance (ANCOVA). BA was chosen because of its importance
for discriminating teleost aggregations (Gómez-Laplaza & Gerlai, 2013). Fish were measured
from the tip of the snout to the edge of the caudal fin (TL), and models were measured from edge
to edge and considered TL. All statistical analyses were conducted in ‘R’ v. 3.1.3 (R
Development Core Team, 2015), and all relevant data for the current analysis are available within this paper (S2 dataset).

3. Results

Mimetic fish were observed mimicking plant debris near the water surface in all extensions of the port of Honmura. The mimetic assemblages resembled the models in shape, colour and drifting movements, having shared the same environment during the entire sampling period. All fish drifted among fallen plant debris near the water surface.

The visual GPA analysis indicated significant variance in the shape configurations among the different models (Fig. 2A) and mimetic fish (Fig. 2B). All-pooled data showed a relative tendency of the mimetic fish to resemble plant debris with ~50% of the variation explained in PC1 and ~40% of the variation explained in PC2 (ANOVA $F_{1,49} = 53.34, P < 0.001$, Fig. 2C).

Discriminant function analysis has revealed that while mimetic fish and models present morphometric similarities, they do also maintain their “morphological identity”.

While analogous LM in fish are associated with the same structures, shape differs amongst the three species of mimetic fishes and the shape of fishes overall is substantially different from the shape of plant models, where function 1 explains 94.14%, and function 2 responsible for 4.76% of the groupings, respectively (Discriminant Function Analysis, $F_{93,96} = 10.29, P<0.001$; Fig. 3).

BA of the mimetic fish and models regressed against TL revealed a highly significant interdependency (ANCOVA, $F_{2,67} = 112.1, P < 0.001$; Fig. 4), where juvenile *L. surinamensis*
and \textit{P. orbicularis} have shown a size gradient, sharing similar BA with round and elongated leaves of different sizes, accordingly to different growth stages of each mimetic fish species.

4. Discussion

The present results show significant shape heterogeneity among mimetic fish and the models, with a significant level of similarity shared in their general external shape profile, yet maintaining each group identity as fishes and plant structures, as observed through the grouping by linear discriminant function. Such results are highly expected, as mimetic behaviour is more likely to be driven by a combination of factors (i.e.: shape, colour and movements) than solely by morphological attributes (Wickler, 1968; Pasteur, 1982). Although the importance of floating plant debris for passive transportation, providing shelter and feeding grounds for fish in coastal environments has been evaluated (Castro, Santiago & Santana-Ortega, 2001; Vandendriessche \textit{et al.}, 2007), the closeness of these interactions has not been investigated, particularly regarding plant resemblance by fish. According to observed shape similarities shared among the mimetic fish and models, it was clear that the present fish assemblage accompanied their respective models, being probably dependent on drifting plant material for survival, also suggested by the linear model of covariance shared amongst drifting fish and plants. While not the main goal of the present study, such association might suggest an allometric dependence for the plant mimetic species, at least until a given ontogenetic stage when such fish species suffer significant changes in both morphology and behaviour, cessing with the mimetic association with plants (Barros \textit{et al.}, 2015).
The concepts regarding mimetic behaviour are still a matter of discussion, as it is difficult
to define a case of mimetic association using only a shape resemblance to another
animal/inanimate object (Skelhorn, Rowland & Ruxton, 2010a; Skelhorn et al., 2010b),
especially in marine systems (Roberston, 2013; 2015). The observed species herein not only
presented good shape similarity with the models, but also behaved alike, via drifting movements
along with their respective models, far away of being “inanimate” (BBarros, personal
observation; data not shown). Close resemblance of fish to their models in shape and drifting
behaviour at the water surface environment could confuse visually oriented predators through the
camouflage effect. Thus, “mimetic behaviour” was a valid classification in the present case.

All species tested in the present study, such as *L. surinamensis* (Lobotidae), *C. maculata*
(Balistidae) and *P. orbicularis* (Ephippidae) have been described previously as resembling dried
leaves in shallow water (Uchida, 1951; Breder, 1942, 1946, 1949; Randall & Randall, 1955;
Barros et al., 2008, 2011, 2012). Although coastal fish resembling a plant via cryptic colouration
has been an intriguing subject since the early reports, the present study is the first attempt to
establish analytical comparisons between mimetic fish and models at the morphometrics level.
Kelley & Merilaita (2015) suggested that successful crypsis in fish is more likely achieved
through colouration, via a background matching effect. Although we did not test the predation
rate of mimetic fish, our results add relevant information, in which background matching is
achieved not only by cryptic colouration (Breder, 1946; Randall & Randall, 1960; Randall,
2005b), but also through shape and behavioural resemblance of mimetic fish to their respective
models. The present level of protective camouflage shared by the fish assemblage analysed
herein might be important against potential aerial and bottom predators, as background colour
matches surrounding environments (Donnelly & Whoriskey Jr., 1991; Cortesi et al., 2015; Kelley
& Merilaita, 2015). However, no predatory attempt by a bird species has been observed. Further experiments and field observations of all observed species are necessary to test this assumption.

The co-occurring mimetic assemblages observed herein are a typical example of convergent evolution in a coastal environment (Endler, 1981; Hamner, 1995; Johnsen, 2014).

Some taxa analysed undergo numerous morphological and ethological changes. For example, *P. orbicularis* adults inhabit deeper environments, changing in both shape and behaviour within the settlement (Kuiter & Debelius, 2001; Barros *et al*., 2011). As major morphological changes are usually expected through ontogeny of several fish groups (Galis, 1990; Loy *et al*., 1998; Comabella, Hurtado & García-Galano, 2010; Leis *et al*., 2013; Nikolioudakis, Koumoundouros & Somarakis, 2014; Barros *et al*., 2015), resemblance to leaves by the fish species observed here may be crucial for first settlement, as it could improve survival chances (Johnsen, 2014).

Nevertheless, our results are based solely on morphometrics data, our observations support fundamental information on the distributions of these fish species during early stages, their life history and evolutionary paths if combined with mimetic fish and model ethological and ecological data that are available for some taxa (Barros *et al*., 2008, 2011, 2012). Strictly morphological studies are ineffective for providing all of the clues necessary to interpret the natural history of most living organisms (Scholtz, 2010). Our results provide a novel approach using morphological data to interpret complex ecological interactions under a convergent evolution perspective to understand the shape similarities shared by mimetic fish and models. Although refinements to the methodologies are necessary, this new comparative approach may stimulate discussion of morphology as a predictor of ecology (Douglas & Matthews, 1992; Gibran, 2010; Oliveira *et al*., 2010). More experimental studies are expected to understand how important plant models are for plant-mimetic fish species in coastal waters.
Acknowledgements

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We dedicate this study to the memory of Dr. Kenji Gushima (Hiroshima University).

References


in bad company”: Agonistic colour display in mimetic juveniles of two ephippid species.

*Journal of Fish Biology* **81** (3), 1032-1042.


Morphology and Morphometry of Cuban Gar (*Atractosteus tristoechus*). *Zoological Science* 27, 931-938.


comprehensive guide to Acanthuroidei. TMC Publishing: Chorleywood, UK.


Vandendriessche, S., Messiaen, M., O'Flynn, S., Vinckx, M. & Degraer, S. (2007). Hiding and feeding in floating seaweed: Floating seaweed clumps as possible refuges or feeding grounds...
for fishes. Estuarine, Coastal and Shelf Science 71, 691-703.


Table legends

Table I – List of homologous landmarks and criteria adopted for selecting each landmark used for the mimetic fish.

Supporting Information

S1 - Dataset used for geometric morphometric analysis
**Table 1** (on next page)

Table 1

List of homologous landmarks and criteria adopted for selecting each landmark used for the mimetic fish
<table>
<thead>
<tr>
<th>Landmark</th>
<th>Landmark description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tip of the snout</td>
</tr>
<tr>
<td>2</td>
<td>Nasal cavity</td>
</tr>
<tr>
<td>3</td>
<td>Posterior limit of supra-occipital</td>
</tr>
<tr>
<td>4</td>
<td>Anterior insertion of dorsal fin</td>
</tr>
<tr>
<td>5</td>
<td>Edge of last hard spine</td>
</tr>
<tr>
<td>6</td>
<td>Insertion of soft rays</td>
</tr>
<tr>
<td>7</td>
<td>Maximum height of dorsal fin</td>
</tr>
<tr>
<td>8</td>
<td>Posterior insertion of dorsal fin</td>
</tr>
<tr>
<td>9</td>
<td>Upper limit of caudal fin</td>
</tr>
<tr>
<td>10</td>
<td>Hypural joint</td>
</tr>
<tr>
<td>11</td>
<td>Lower limit of caudal fin</td>
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<tr>
<td>12</td>
<td>Posterior insertion of anal fin</td>
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<td>Maximum height of anal fin</td>
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<td>Anterior insertion of anal fin</td>
</tr>
<tr>
<td>15</td>
<td>Insertion of pelvic fin</td>
</tr>
<tr>
<td>16</td>
<td>Lower occipital edge</td>
</tr>
</tbody>
</table>
Figure 1

Examples of mimetic fish and their models (i.e. floating plant debris) occurring in the shallow water at Honmura Port, Kuchierabu-jima Island, southern Japan. a) Lobotes surinamensis, b) Canthidermis maculata and c) Platax orbicularis are the mimetic fish shown. The models were subdivided using three criteria of: d) round leaves, and e) elongated leaves. The established landmarks and semilandmarks are denoted in (a) for the mimics and in (f) for the models, respectively. White bars indicate 1 cm.

*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.
2

Figure 2

Diversity of shapes observed for the models (i.e. floating plant debris) (a) and fish mimics (b), via a general Procrustes analysis (GPA); and principal components analysis (PCA; c), of all-pooled data indicating a high tendency for shape similarities shared by the fish mimics (lower right) and models (i.e. floating plant debris) (upper left), where green plots represent leaf models (dark green representing rounded leaf models and lighter green representing elongated leaf models). Mimetic fish are represented by Lobotes surinamensis (yellow), Canthidermis maculata (white) and Platax orbicularis (red).
Distinct groups of fish species and plant models were also observed through a discriminant function analysis, according to the geometric morphometric data of each group, where mimetic fish are represented by Lobotes surinamensis (yellow), Canthidermis maculata (white) and Platax orbicularis (red), and plant models are represented by green plots (dark green representing rounded leaf models and lighter green representing elongated leaf models).
Similar relative body area values were observed among the models (i.e. floating plant debris) and mimetic fish, where mimetic fish are represented by Lobotes surinamensis (yellow), Canthidermis maculata (white) and Platax orbicularis (red), and plant models are represented by green plots (dark green representing rounded leaf models and lighter green representing elongated leaf models).