Morphometric comparisons of plant-mimetic juvenile fish associated with plant debris observed in the coastal subtropical waters around Kuchierabu-jima Island, southern Japan

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The general morphological shape of plant-resembling fish and plant parts were compared using a geometric morphometrics approach. Three plant-mimetic fish species, *Lobotes surinamensis* (Lobotidae), *Platax orbicularis* (Ephippidae) and *Canthidermis maculata* (Balistidae), were compared during their early developmental stages with accompanying plant debris (i.e. leaves of several taxa) in the coastal subtropical waters around 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 analysis of covariance and linear discriminant analysis, in which relative body areas of fish were strongly related to plant models. 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.

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22   Abstract

23   The general morphological shape of plant-resembling fish and plant parts were compared using a

24   geometric morphometrics approach. Three plant-mimetic fish species, *Lobotes surinamensis*

25   (Lobotidae), *Platax orbicularis* (Ephippidae) and *Canthidermis maculata* (Balistidae), were

26   compared during their early developmental stages with accompanying plant debris (i.e. leaves of

27   several taxa) in the coastal subtropical waters around Kuchierabu-jima Island, closely facing the

28   Kuroshio Current. The degree of similarity shared between the plant parts and co-occurring fish

29   species was quantified, where fish and plants presented highly similar morphometric associations,

30   yet remaining morphologically distinct. Such similarities were corroborated by analysis of

31   covariance and linear discriminant analysis, in which relative body areas of fish were strongly

32   related to plant models. Our results strengthen the paradigm that morphological clues can lead to

33   ecological evidence to allow predictions of behavioural and habitat choice by mimetic fish,

34   according to the degree of similarity shared with their respective models. The resemblance to

35   plant parts detected in the three fish species may provide fitness advantages via convergent

36   evolutionary effects.

37

38   Key-words: Protective camouflage, Masquerade, Coastal Environments, Shape analysis,

39   Convergent evolution

40

1. Introduction

42
43
44   Mimesis is defined as a phenotype evolved in response to selective pressures favouring

45   individuals that can disguise their identity by masquerading as another organism (Pasteur, 1982;

46   Skelhorn, Rowland & Ruxton, 2010a; Skelhorn *et al.*, 2010b). Mimesis in fish is a relatively

47   well-studied subject (Wickler, 1968; Moland*,* Eagle & Jones, 2005; Robertson, 2013),

48   particularly regarding deceptive resemblance to plant parts via protective camouflage, which is a

49   known feature in several freshwater and marine fish species, as extreme crypsis examples of

50   protective resemblance (Breder, 1946; Randall, 1965, 2005a; Vane-Wright, 1980; Sazima *et al.*,

51   2006). Although these reports have addressed the patterns and general similarities in morphology

52   or colouration of model plant parts and mimetic fish, few studies have examined similarities

53   among them based on morphological and/or ethological details (Barros *et al*. 2008, 2011, 2012).

54   Studies focusing on morphology and geometric morphometrics frequently used fish species

55   as models, and several authors have suggested that morphological clues can be used as

56   ecological predictors from basic behavioural constraints, such as swimming mode (Walker,

57   2004; Comabella, Hurtado & García-Galano, 2010; Xiong & Lauder, 2014), feeding behaviour

58   (Galis, 1990; Franssen, Goodchild & Shepard, 2015) and habitat choice (Loy *et al.*, 1998;

59   Gibran, 2010; Soares, Ruffeil & Montag, 2013), especially in juvenile fish, suggesting that such

60   changes are important for improving fitness and increasing the chance for survival during

61   subsequent ontogenetic stages (Barros *et al.*, 2011; Comabella *et al.*, 2013). Nevertheless, such a

62   tool has not been used to establish comparisons among distant taxa belonging to completely

63   different groups (i.e. fish and plants). In the present study, previously well-known plant-mimetic

64   juvenile fish, the tripletail, *Lobotes surinamensis* (Bloch, 1790), the orbicular batfish, *Platax*

65 *orbicularis* (Forsskål, 1775) and the ocean triggerfish, *Canthidermis maculata* (Bloch, 1786)

66 were compared with their respective plant models co-occurring in the field to objectively

67 evaluate their resemblance in shape to their respective models. All fish and plant models were

68 observed and sampled from Kuchierabu-jima Island and its surrounding waters, which are

69 subjected to a strong influence of Kuroshio Current.

70   *Lobotes surinamensis* is generally found in shallow brackish water habitats but may occur

71 far offshore with drifting algae or flotsam, and juveniles may lie on their side matching the

72 colour of the plant debris, from near black to yellow (Randall, 2005b). Juveniles are usually

73 dark-coloured, presenting drifting swimming patterns among dry leaves, exhibiting similar

74 movements to their associated plant model (Uchida, 1951; Randall, 2005b). Uchida (1951) also

75 described that young *C. maculata* resemble pieces of pine bark and were observed drifting

76 among pieces of bark in a horizontal swimming posture, suggesting mimetic effects. Juveniles of

77 *P. orbicularis* look similar to yellow waterlogged jack tree leaves (genus *Rhizophora*) and

78 greatly resemble floating dead leaves (Wiley, 1904; Breder, 1946). Randall (1960) reported that

79 larger individuals (87 mm standard length [SL]) resemble large sea hibiscus leaves (*Hibiscus*

80 *tiliaceus*) with a yellowish-brown colouration, with dorsal and anal fins appearing to lengthen

81 with growth. Such drastic changes in morphological shape occur in juvenile *P. orbicularis* while

82 they maintain a resemblance to drifting leaves (Barros *et al.*, 2015).

83   The novel comparative methods presented herein may provide useful associations between

84 behavioural ecology and morphological studies. We tested the null hypothesis of a lack of shape

85 similarity among the studied fish and plant parts, considering both classic and geometric

86 morphometrics comparative approaches. We briefly discuss the functional contributions of

87 camouflage characteristics to fish fitness using mimetic shape attributes as a disguise based on

88 morphological resemblance data among fish and model plants, adopting the concepts of cryptic

89 mimesis as synonym of protective camouflage or masquerading, following the definitions as

90 proposed by Pasteur (1982), where all fish samples are defined as "mimetic fish" and all plant

91 part samples as "models", instead of adopting the terminology as proposed by Skelhorn,

92 Rowland & Ruxton (2010a). This is due to the highly dynamic environments such fish usually

93 occur, where mimetic behaviour is achieved not only by appearance, but also through actively

94 behaving alike the drifting models (Barros *et al.*, 2008).

95

96 **2. Material and Methods**

97

98 2.1. Sampling

99 Sampling was mainly conducted in the port of Honmura, Kuchierabu-jima Island (Ohsumi

100 Group, 30° 28′ N, 130° 10′ E), southern Japan, during diurnal observations July 3–14, 2011 (S1

101 Fig.). The island closely faces the Kuroshio Current and maintains a rich subtropical fish fauna

102 (Gushima & Murakami, 1976). Fish samples and plant debris were collected using hand nets,

103 and the sampled fish were euthanized using 5 ml 95 % eugenol in 1 L ethanol as a stock solution

104 All fish samples were preserved in order to maintain integrity of peripheral structures and

105 general shape, and were photographed as soon as possible, in order to avoid any arching or

106 deformation effect from the fixation protocols established (Valentin *et al*., 2008). All plant

107 materials were sampled at the island along with their associated fish. Of this, 20 ml was added to

108 each 1 L of water containing the fish to be euthanized to minimise suffering, following

109 international ethical standards (Jenkins *et al.,* 2014). As there is no national Japanese licensing

110 framework, samples were collected following the "Guidelines for Proper Conduct of Animal

111   Experiments" set out by the Hiroshima University Animal Research Committee, which are based

112   on international ethical standards, and only after obtaining local community permission.

113   Fish samples from Kuchireabu-jima Island were identified to as low a taxonomic category as

114   possible, according to available literature (Nakabo, 2002; Nelson, 2006; Okiyama, 2014). Fifteen

115   mimetic fish specimens of three species (Fig. 1A–C) were observed to drift around plant debris:

116   *Lobotes surinamensis* (Lobotidae; $n = 6$, TL = 3.89 ± 0.46 cm; AVE ± SDEV values), *Platax*

117   *orbicularis* (Ephippidae; $n = $ 7 TL = 2.05 ± 0.42 cm) and *Canthidermis maculata* (Balistidae; n

118   $= 2$, TL = 3.15 ± 0.98 cm). An additional 24 fish specimens (n = 14 for *L. surinamensis*, n = 10

119   for *C. maculata*) sampled in subtropical waters of Kagoshima Prefecture were also obtained from

120   the collections of the Kagoshima University Museum (KAUM) to enhance and equalize sample

121   size of our data set for the statistical analyses (see below). The KAUM samples were all

122   juveniles, with relatively similar standard length as those observed (*L. surinamensis* TL = 4.92 ±

123   2.02 cm, and *C. maculata* TL = 3.95 ± 0.98 cm) and collected near to the present study area, i.e.,

124   Satsuma Peninsula of mainland Kagoshima, Tanega-shima Island, and Yaku-shima Island

125   (31º28'-31º33'N, 130º11'-130º51'E) (for details refer to S2 Dataset). Of these, the most images

126   were provided by the KAUM (N = 5 for *C. maculata* and N = 11 for *L. surinamensis*), taken

127   from fresh specimens. All other samples were photographed in the Laboratory of Biology of

128   Aquatic Resources, at the Hiroshima University, and only those with all peripheric structures

129   intact were considered in the analysis. No arched or deformed specimens were used during the

130   analyses, in order to prevent from any misinterpretation of data, as inconclusive attempts to

131   explain such posture variations by any possible biological factors, as allometric growth or even

132   sexual dimorphism (Valentin *et al.*, 2008).

133 Also, additional twelve samples of *P. orbicularis* (TL = 2.05 ± 0.91 cm) collected during

134 previous surveys on Kuchierabu-jima Island (Barros *et al.* 2008, 2011) were eventually

135 employed, in order to equalize N size. These were also fixed using the same protocol as

136 standardized herein, being photographed soon after sampling. A total 52 individual mimetic

137 fishes were analysed.

138 Floating plant debris (hereafter, models, n = 43) were collected using hand nets and sorted,

139 then visually subdivided using two subjective criteria (round shapes, as for the Podocarpaceae

140 *Nageia nagi* and the Sapindaceae *Acer morifolium*; or elongated shapes, as for the Laureaceae

141 *Neolitsea sericea* and for the Fagaceae *Castanopsis sieboldii*; Fig. 1D–E), regardless of

142 taxonomy and dried in paper envelopes until they were photographed for further analyses.

143 High resolution digital pictures of the left lateral view of the mimetic fish and model

144 samples were taken over a black background using a Nikon D700 equipped with AF-S 60-mm

145 immersive lens and a stand table with a reference scale of 1 cm for the fish and models. The left

146 lateral view of the models was defined as the "dorsal view of leaves with the petiole oriented to

147 the right". Artificial light was used to avoid shading morphological structures.

148

149 2.2. Data Analyses

150 Sixteen landmarks (LM) were established for the mimetic fish and models using ImageJ v. 1.47

151 software for geometric morphometrics purposes (Abramoff, Magelhaes & Ram, 2004).

152 Homologous LM for the mimetic fish were marked obeying the morphological structures

153 constrained or related to mimetic behaviour to cover the fish general outline profile, including

154 peripheral structures (Fig. 1A, Table I). We established equidistant 16 semilandmarks (SLM) for

155 each model using the ImageJ grid tool to cover all lateral profiles of the model, obeying the same

156   marking distribution as for the mimetic fishes (Fig. 1D). Raw coordinates LM and SLM data

157   were implemented in MorphoJ v. 1.02n software (Klingenberg, 2011), where preliminary

158   adjustments, such as the Procrustes fit, and creation of the data matrix, were done. The

159   morphometric comparisons among the fish and models were not intended to analyse homologous

160   patterns, as we were interested in shape similarities randomly shared among the mimetic fish and

161   their respective models distributed in the same environment, from a geometric morphometrics

162   perspective. Therefore, the necessity of marking peripheral anatomic structures in the mimetic

163   fish, instead of fins insertions only, in order to check for general appearance of mimetic fish with

164   the plant models.

165   Data analyses were performed with Geomorph v. 2.0 software (Adams & Otarola-Castillo,

166   2013). A post-hoc general Procrustes analysis (GPA) and principal components analysis (PCA)

167   were run followed by analysis of variance (ANOVA) to compare the mimetic fish and models

168   plotted together in the analyses. Also, a linear discriminant function was run, in order to visualize

169   how close were these group associations, using the package MASS v. 7.3-42 (Venables &

170   Ripley, 2002).

171   In addition, individual TL and relative body area (BA, cm^2/TL) of the fish and models

172   were calculated using ImageJ to establish interdependent comparisons among the fish species

173   and plant debris via analysis of covariance (ANCOVA), followed by a linear discriminant

174   analysis (LDA), to accurately predict whether the mimetic fish can be misclassified as a model.

175   BA was chosen because of its importance for discriminating teleost aggregations (Gómez-

176   Laplaza & Gerlai, 2013). Fish were measured from the tip of the snout to the edge of the caudal

177   fin (TL), and models were measured from edge to edge and considered TL. All statistical

178   analyses were conducted in 'R' v. 3.1.3 (R Development Core Team, 2015), and all relevant data

179   for the current analysis are available within this paper (S2 dataset).

180

181   **3. Results**

182

183   Mimetic fish were observed mimicking plant debris near the water surface in all extensions of

184   the port of Honmura. The mimetic assemblages resembled the models in shape, colour and

185   drifting movements, having shared the same environment during the entire sampling period. All

186   fish drifted among fallen plant debris near the water surface.

187   The visual GPA analysis indicated a significant variance in the shape configurations

188   among the different models (Fig. 2A) and mimetic fish (Fig. 2B). All-pooled data showed a

189   relative tendency of the mimetic fish to resemble plant debris with ~24% of the variation

190   explained in PC1 and ~10% of the variation explained in PC2 (ANOVA $F_{2,52} = 40.97$, $P < 0.001$,

191   Fig. 2C), yet remaining morphologically distinct, as observed in the GPA analyses.

192   BA of the mimetic fish and models regressed against TL revealed a significant

193   interdependency (ANCOVA, $F_{2,96} = 92.06$, $P < 0.001$; Fig. 3), where juvenile *L. surinamensis, P.*

194   *orbicularis* and *C. maculata* have shown a size gradient, sharing similar BA with round and

195   elongated leaves of different sizes, accordingly to different growth stages of each mimetic fish

196   species, with some deviation observed for the round leaf models. These results were

197   corroborated by LDA, which has shown high similarities in shape of mimetic fish and models,

198   with a 52.52% probability of misclassification among the observed individuals. Details on both

199   ANCOVA and LDA can be found in S2 dataset.

200

201   **4. Discussion**

202

203   The present results show shape heterogeneity among mimetic fish and plant models, with a

204   significant level of similarity shared in their general external shape profile. Such results are

205   highly expected, as mimetic behaviour is more likely to be driven by a combination of factors

206   (i.e.: shape, colour and movements) than solely by morphological attributes (Wickler, 1968;

207   Pasteur, 1982). Although the importance of floating plant debris for passive transportation,

208   providing shelter and feeding grounds for fish in coastal environments has been evaluated

209   (Castro, Santiago & Santana-Ortega, 2001; Vandendriessche *et al.*, 2007), the closeness of these

210   interactions has not been investigated, particularly regarding plant resemblance by fish.

211   Arching effects due to fixation protocols are known to strongly influence geometric

212   morphometric analyses (Valentin *et al.*, 2008). Although we have combined data from museum

213   specimens with our own samples, we have selected only intact individuals for the present

214   analyses. According to observed shape similarities shared among the mimetic fish and models, it

215   was clear that the present fish assemblage accompanied their respective models, being probably

216   dependent on drifting plant material for survival, also suggested by the linear model of

217   covariance shared amongst drifting fish and plants. While not the primary goal of the present

218   study, such association might suggest an allometric dependence for the plant mimetic species, at

219   least until a given ontogenetic stage when such fish species suffer significant changes in

220   morphology and behaviour, cessing with the mimetic association with plants (Barros *et al.*,

221   2015).

222   The concepts regarding mimetic behaviour are still a matter of discussion, as it is difficult

223   to define a case of mimetic association using only a shape resemblance to another

224 animal/inanimate object (Skelhorn, Rowland & Ruxton, 2010a; Skelhorn *et al.*, 2010b),

225 especially in marine systems (Robertson, 2013; 2015). The observed species herein not only

226 presented good shape similarity with the models, but also behaved alike, via drifting movements

227 along with their respective models, far away from being "inanimate" (BBarros, personal

228 observation; S3 video). Close resemblance of fish to their models in shape and drifting behaviour

229 at the water surface environment could confuse visually oriented predators through the

230 camouflage effect. Thus, "mimetic behaviour" was a valid classification in the present case.

231   All species tested in the present study, such as *L. surinamensis* (Lobotidae)*, C. maculata*

232 (Balistidae) and *P. orbicularis* (Ephippidae) have been described previously as resembling dried

233 leaves in shallow water (Uchida, 1951; Breder, 1942, 1946, 1949; Randall & Randall, 1955;

234 Barros *et al.*, 2008, 2011, 2012), and are commonly found in the surveyed area (Motomura *et al.*,

235 2010).

236   Although coastal fish resembling a plant via cryptic colouration has been an intriguing

237 subject since the early reports, the present study is the first attempt to establish analytical

238 comparisons between mimetic fish and models at the morphometrics level. Kelley & Merilaita

239 (2015) suggested that successful crypsis in fish is more likely achieved through colouration, via a

240 background matching effect. Although we did not test the predation rate of mimetic fish nor for

241 any colour influence, our results add relevant information, in which background matching is

242 achieved not only by cryptic colouration (Breder, 1946; Randall & Randall, 1960; Randall,

243 2005b), but also through shape and behavioural resemblance of mimetic fish to their respective

244 models. The present level of protective camouflage shared by the fish assemblage analysed

245 herein might be important against potential aerial and bottom predators, as background colour

246 matches surrounding environments (Donnely & Whoriskey Jr., 1991; Cortesi *et al.*, 2015; Kelley

247 & Merilaita, 2015). However, no predatory attempt by a bird species has been observed. Further

248 experiments and field observations of all observed species are necessary to test this assumption.

249   The co-occurring mimetic assemblages observed herein are a typical example of

250 convergent evolution in a coastal environment (Endler, 1981; Hamner, 1995; Johnsen, 2014).

251 Some taxa analysed undergo numerous morphological and ethological changes. For example, *P.*

252 *orbicularis* adults inhabit deeper environments, changing in both shape and behaviour within the

253 settlement (Kuiter & Debelius, 2001; Barros *et al.*, 2011). As major morphological changes are

254 usually expected through ontogeny of several fish groups (Galis, 1990; Loy *et al.*, 1998;

255 Comabella, Hurtado & García-Galano, 2010; Leis *et al.* 2013; Nikolioudakis, Koumoundouros &

256 Somarakis, 2014; Barros *et al.*, 2015), resemblance to leaves by the fish species observed here

257 may be crucial for first settlement, as it could improve survival chances (Johnsen, 2014).

258   The Kuroshio Current is regarded as a key factor for passive transportation of masses of

259 plant and algae material and juvenile fishes closely associated with, as such ichthyofauna use the

260 plant debris as both shelter and food source (Kimura *et al.*, 1998). Strictly morphological studies

261 are ineffective for providing all of the clues necessary to interpret the natural history of most

262 living organisms (Scholtz, 2010). The present observations support fundamental information on

263 the distributions of these fish species during early stages, their life history and evolutionary paths

264 if combined with mimetic fish and model ethological and ecological data that are available for

265 some taxa (Barros *et al.*, 2008, 2011, 2012). Although refinements to the methodologies are

266 necessary, this new comparative approach may stimulate discussion of morphology as a

267 predictor of ecology (Douglas & Matthews, 1992; Gibran, 2010; Oliveira *et al.*, 2010).

268

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276

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422

423   **Table legends**

424   Table I – List of homologous landmarks and criteria adopted for selecting each landmark used

425   for the mimetic fish.

426

427   **Supporting Information**

428   S1 – Schematic map showing the geographic position of Kuchiearbu jima Island in southern

429   Japan.

430   S2 - Dataset used for the GM, ANCOVA and LDA analyses, with the respective scripts for data

431   replication using R. Specific information on vouchers references from the Kagoshima

432   University Museum are also included.

433 S3 – Video file containing examples of plant-mimetic interactions by several fish species

434   observed at Kuchierabu jima Island, including *Platax orbicularis*.

435
436

List of landmarks

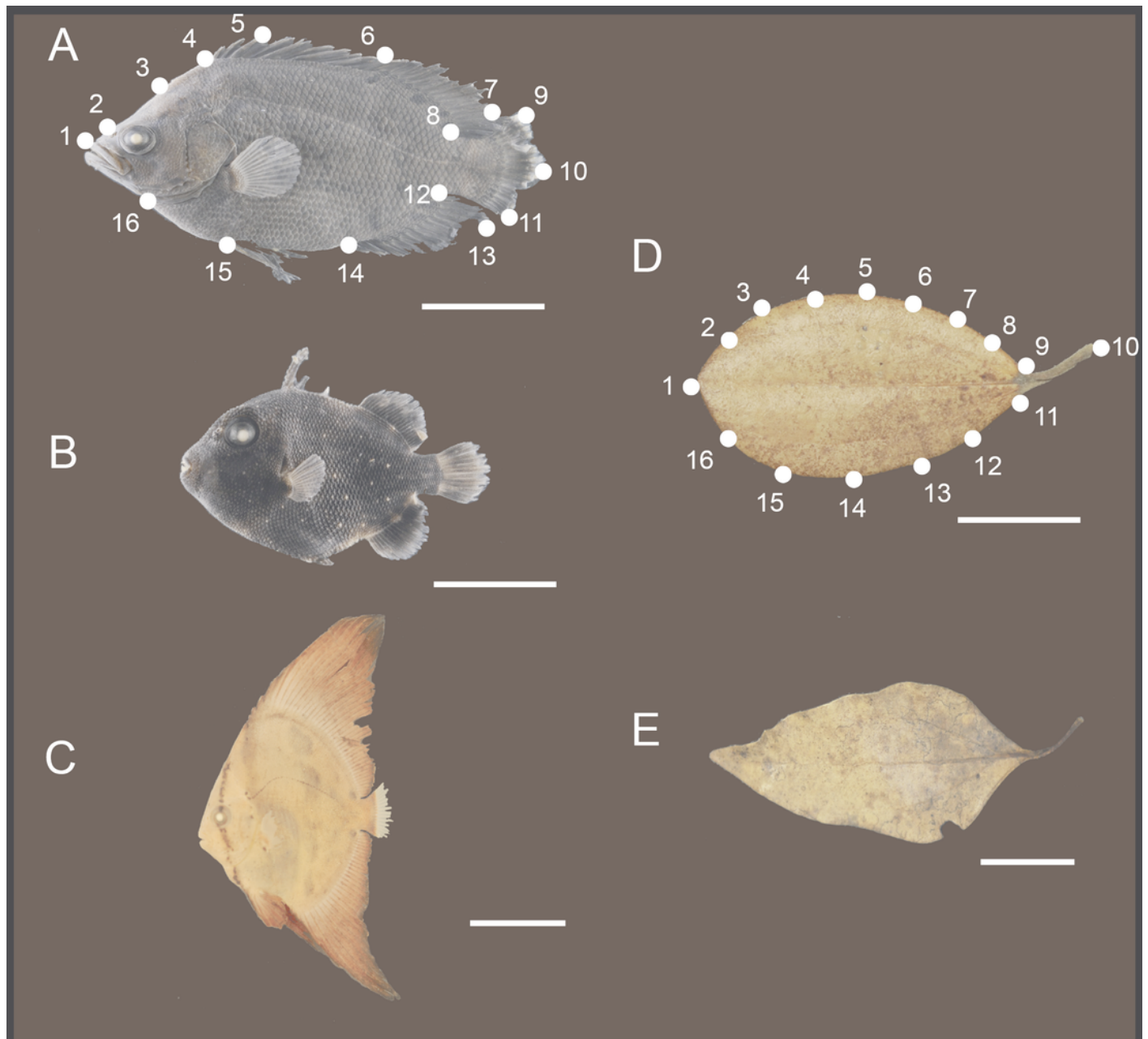
List of homologous landmarks and criteria adopted for selecting each landmark used for the mimetic fish

Landmark	Landmark description
1	Tip of the snout
2	Nasal cavity
3	Posterior limit of supra-occipital
4	Anterior insertion of dorsal fin
5	Edge of last hard spine
6	Insertion of soft rays
7	Maximum height of dorsal fin
8	Posterior insertion of dorsal fin
9	Upper limit of caudal fin
10	Hypural joint
11	Lower limit of caudal fin
12	Posterior insertion of anal fin
13	Maximum height of anal fin
14	Anterior insertion of anal fin
15	Insertion of pelvic fin
16	Lower occipital edge

Figure 1

Mimetic fish and plant models

Morphometric comparisons of plant-mimetic juvenile fish associated with plant debris observed on Kuchierabu-jima Island, southern Japan Examples of mimetic fish and their models (i.e. floating plant debris) occurring in the shallow waters of Honmura Port, Kuchierabu-jima Island, southern Japan. a) *Lobotes surinamensis*, b) *Canthidermis maculata* and c) *Platax orbicularis* are the mimetic fish observed. 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 mimetic fish 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.

Morphometric relationships among mimetic fish and plant models

Morphometric comparisons of plant-mimetic juvenile fish associated with plant debris observed on Kuchierabu-jima Island, southern Japan 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 mimetic fish and models (i.e. floating plant debris), 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), *Platax orbicularis* (red), and *Canthidermis maculata* (white).

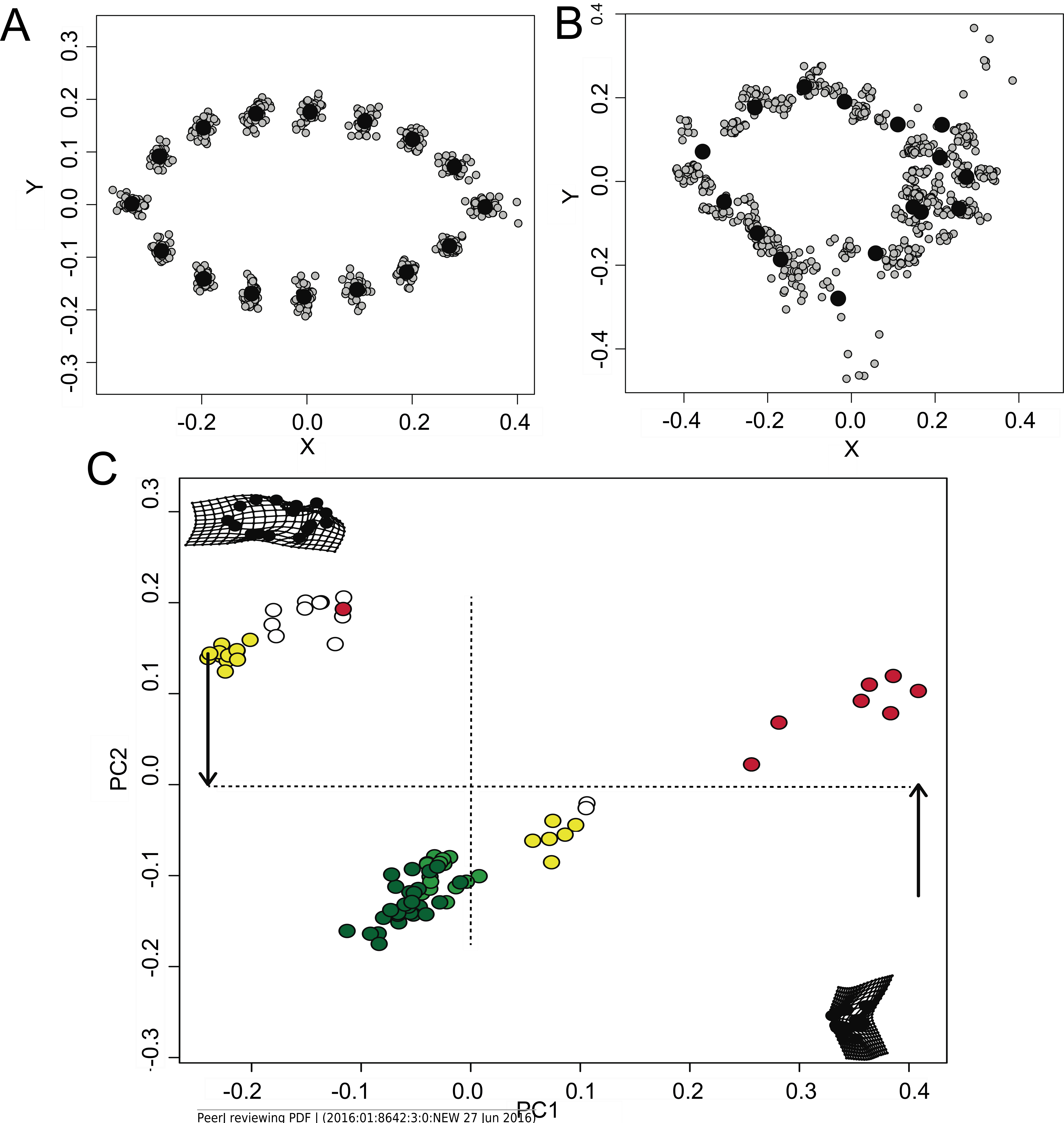



Figure 3

ANCOVA

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