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1 **Gender identification in farmed giant gourami (*Osphronemus goramy*):**
2 **a methodology for better broodstock management**

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17 **Abstract:**

18 The giant gourami *Osphronemus goramy* Lacepède (1801) is one of the main freshwater
19 commodities of economic importance in Indonesia. This species has been produced in small-
20 scale farms for decades. Although giant gourami aquaculture has grown exponentially during
21 the last 15 years, there are still limitations in the availability of fry, in part due to difficulties
22 in sexing broodfish, which leads to non-optimal sex-ratios for breeding. In this study,
23 morphological and behavioral criteria for sex identification based on the Indonesian National
24 Standard and a field survey were assessed on more than 400 giant gourami broodfish using a
25 random forest algorithm. The actual sex of the fish was confirmed using a urogenital
26 cannulation technique. This analysis demonstrated that, for the so-called “black” phenotype
27 fish, observations of the hump on the forehead, thickening of the lower jaw, and the
28 pigmentation on the pectoral fin are highly reliable for sexing (about 95% success). However,
29 pectoral fin pigmentation was not useful for sexing the so-called “white” phenotype fish. This
30 study also revealed that the criteria often used by fish farmers do not improve sexing success.
31 Based on these findings, recommendations are made for optimizing the sexing of mature giant
32 gourami fish and for selecting preadults as future broodfish.

33

34 Keywords: Fish reproduction, Indonesia, Random forest algorithm, Sexing.

35

36 **1. Introduction**

37 Worldwide, there is increasing willingness to develop the production of local species in
38 aquaculture (FAO, 2016; Ross et al., 2008; Saint-Paul, 2017), an inclination that is backed by
39 governmental policies in Southeast Asian countries (Pengseng and Claude, 2011). The giant
40 gourami *Osphronemus goramy* Lacepède (1801), native to Southeast Asia, is one of the main
41 freshwater commodities of economic importance in Indonesia owing to its high price and its
42 high local demand (Budi and Suprayudi, 2015; Pengseng and Claude, 2011; Rimmer et al.,
43 2013). Its annual production in Indonesia has grown exponentially over the past 15 years,
44 reaching over 119,000 t in 2014. However, for the first time in 2015, Indonesian production of
45 this species decreased slightly (113,400 t; FAO, 2017). Although the giant gourami has been
46 reared for decades and national production is fulfilled by approximately 100,000 fish farmers
47 mainly located on Java Island (79%; BPS, 2013), there are gaps in knowledge on several
48 aspects of its biology. One of the main impediments in giant gourami aquaculture is ensuring
49 a regular supply of fry, the availability of which should be improved through more reliable
50 production methods (Amornsakun et al., 2014a, b).

51 Giant gourami fry production relies on the natural spawning of captive broodfish in ponds.
52 The overall recruitment (i.e. number of fry produced per broodfish and per spawn in the
53 breeding ponds) remains generally low partly due to variable spawning frequency or success,
54 and variable egg number and quality (Azrita, 2015). One of the main causes of this low fry
55 production is the lack of an objective and reliable sex identification method in giant gourami
56 broodfish. The male-to-female ratio for gourami egg production recommended by the
57 Indonesian National Standard is 1:3-4 (SNI, 2000a; SNI, 2000b). However, an on-farm
58 inquiry carried out on 40 farms of the West Java province revealed that the actual sex-ratio
59 used for gourami egg and fry production is highly variable, ranging from 1:1 to 1:9

60 (unpublished data). Occasionally the sex-ratio is biased in favor of males, revealing sexing
61 errors in broodfish sold to farmers.

62 In cultures of fish species without clearly apparent sexual dimorphism, sexing can be
63 performed using methods such as hormone dosage (Feist et al., 2004; Webb et al., 2002),
64 vitellogenin detection (Chu-Koo et al., 2009) or ultrasonography (Holm, 1994; Martin et al.,
65 1983; Martin-Robichaud and Rommens, 2001). These methods require specific tools, skilled
66 technicians and have a cost incompatible with current practices in gourami culture. Thus,
67 external observation remains the method the most adapted to the local context for giant
68 gourami sexing and often the only one that can be used by fish farmers. To provide guidance
69 for farmers, the Indonesian National Standard (SNI, 2000b) lists several morphological
70 criteria for sexing giant gourami. At a body weight of about 300 g, gourami males show
71 morphometric changes such as a hump on the forehead and thickening of the lips (Sularto et
72 al., 2017). Nevertheless, these criteria are not well characterized and there are still errors in
73 sexing. To overcome these difficulties, gourami egg producers employ additional
74 morphological and behavioral criteria, identified from a previous on-farm survey. For
75 example, males appear to exhibit specific behavior, bending the body and contracting their
76 muscles to produce tremors when held horizontally out of the water. This behavior has been
77 suggested as a way to distinguish males and females in giant gourami (Sularto et al., 2017).
78 Nevertheless, to our knowledge, no in-depth scientific investigation has been conducted thus
79 far to validate these sexing criteria.

80 Therefore, the objectives of the present study were: (1) to test the applicability in actual
81 production conditions of the gender identification criteria based on either the Indonesian
82 National Standard or gourami farmers; (2) to better characterize putative sex-related
83 morphological criteria using a morphometric approach; (3) to compare the usefulness of the
84 various sexing criteria recognized in the Indonesian National Standard or additionally used by

85 fish farmers; and finally (4) to propose a reliable sexing methodology for giant gourami
86 broodfish. We assessed these criteria on more than 400 sexually mature giant gourami that
87 were individually sexed using a validated urogenital cannulation technique.

88 **2. Materials and Methods**

89 2.1. Fish origin and maintenance

90 The experiment was carried out at the Tasikmalaya “BPPSIGN” Center (West Java Center for
91 the Development of Giant Gourami Culture, 7°19’37.992’’N, 108°6’101.155’’E) over a
92 period of 21 months from May 2016 to February 2018. In giant gourami culture, there are two
93 different phenotypes: the so-called “black” phenotype with scale pigmentation identical to
94 wild individuals and the so-called “white” phenotype characterized by scale depigmentation
95 (Arifin et al., 2018). In this study, 293 black individuals and 120 white individuals were used.
96 The females (n=311) were 3-5 years old and weighed 3.063 ± 0.424 kg (mean \pm SD), whereas
97 males (n=102) were 5-7 years old and weighed 4.042 ± 0.572 kg.

98 Giant gourami breed throughout the year and sexually mature broodfish (at a sex-ratio of 1
99 male to 3 females) were placed in four spawning ponds (earthen bottom and concrete banks)
100 of 525 to 570 m² at a stocking density of one fish for 5 m². Fish were kept in the spawning
101 structures for 3 to 6 months before a 1-month resting period (i.e. 2-3 egg production periods
102 year⁻¹). During the egg production periods, the fish were fed with leaves of elephant ear plants
103 (*Alocasia macrorrhizos*) and commercial floating pellets (32% proteins, 5% lipids) distributed
104 at a daily feeding rate of 2% and 1% of fish biomass, respectively.

105 2.2. Experimental procedures

106 2.2.1. Fish observations and sexing by urogenital cannulation

107 At the beginning and at the end of each reproduction period in the ponds (approx. every 3
108 months), all fish were caught, individually weighed with a digital scale (to the nearest 10 g)

109 and examined to score the sexing criteria recommended by the Indonesian National Standard
110 (SNI, 2000b) and additional criteria corresponding to those used by gourami farmers
111 themselves (see section 2.2.2). The color of each fish (black and white phenotypes, see
112 section 2.1) was recorded and pictures were taken of the fish. To confirm the attribution of the
113 values for each criterion, two observers were present during sampling and scoring.

114 To reliably determine the sex of each gourami individual, a urogenital cannulation was made
115 using a “pipelle de Cornier” (a thin, flexible polypropylene pipette with a soft rounded end,
116 containing an inner piston and a lateral opening for tissue biopsy; Laboratoire CCD, Paris,
117 France) inserted through the urogenital papilla of each fish (e.g. Lewis, 1962; Ross, 1984;
118 Cacot et al., 2002; Slembrouck et al., 2004; Legendre et al., 2012; Smith et al., 2014). The
119 collection of oocytes from cannulation allowed unequivocal female identification. Regarding
120 males, preliminary trials have shown that the genital papilla of the males is too narrow to be
121 able to gently introduce the “pipelle de Cornier”, even when the individual body weight
122 exceeded 3 kg. For such individuals, we gently stripped the gonad to obtain sperm and thus
123 identify the individual as male with certainty. Nevertheless, gourami males do not produce
124 sperm all year round and, in most cases, the amount of sperm obtained by stripping is very
125 low. Therefore, the certain identification of males is less straightforward than that of females.
126 For this reason, sexing was performed several times on the same individuals at intervals of a
127 few months and results were cross-checked by recording the occurrence of fertilized spawns
128 in compartmentalized broodfish ponds (sex-ratio of 1:3 in each compartment).

129 2.2.2. Description of morphological and behavioral criteria

130 All the criteria used in this study were based on the Indonesian National Standard (SNI,
131 2000b) and from a field survey of West Java gourami fish farms (n=40). A total of seven
132 potential sexing criteria, described hereafter (character names given in *italics*), were evaluated
133 (Fig. 1; Table 1).

134 According to the SNI (2000b), sexually mature males are characterized by a marked hump on
135 the upper part of the head (*Forehead*) and a thickening of the lower jaw (*Lower.jaw*). The
136 same arbitrary scale with 5 developmental stages was used to score the importance of the
137 hump on the forehead and the thickening of the lower jaw (Table 1).

138 This scale is easy to use on fish farms but requires some experience because the stages are
139 more or less relative to one another. To provide a more objective characterization of the
140 different levels, morphometric measurements were carried out on photographs of the anterior
141 part of the body of 43 live fish (preadults, males and females) randomly sampled. The photos
142 were taken with a camera fixed on a tripod and set horizontally with a bubble level to take
143 photos perpendicular to the plane of the fish and avoid any parallax effect. Measurements
144 were made using Image-J software (Abramoff and Magalhaes, 2004; see Fig. 1). The
145 development of the hump was measured by the distance between the top of the hump and the
146 upper end of the opercula opening (F-OP; for details see Fig. 1). The thickening of the lower
147 jaw was measured by the distance between the upper end of the opercula opening and the
148 furthest point of the lower jaw and passing through the center of the eye (Lj-OP). These two
149 measurements were expressed as the percentage of the distance between the upper end of the
150 opercula opening and the top of the upper lip (Upl-OP), the allometry of which is not
151 influenced by the development of secondary sexual characters (no significant relationship
152 between the ratio Upl-OP/fish total length and the developmental stages used to score either
153 the importance of the hump on the forehead or the thickening of the lower jaw; $P > 0.1$ in
154 both cases). Alternatively, the development of the hump was also measured by the angle
155 between Upl-OP and the tangent to the hump from the top of the upper lip (Tg 1; Fig. 1). The
156 corresponding measurement value ranges for each stage of the arbitrary scale are given in
157 Table 1.

158 According to the SNI (2000b), females can be identified by black pigmentation at the base of
159 their pectoral fins. In this study, from broodfish observations, three distinguishable
160 pigmentation patterns were identified: (1) no pigmentation (white); (2) presence of dotted
161 black melanophores (black spots); and (3) very intense black patch (full black) (Fig. 2). In
162 addition, these observations showed that, for a given individual, differential pigmentation can
163 occur between the outer and the inner part of the pectoral fin base. These differences were not
164 considered by the SNI but previous surveys have shown that fish farmers always observe the
165 pigmentation on the inner part of the pectoral fin base for sexing. For these reasons, in this
166 study we considered both pigmentation on the outer part (*Col.out.pec*; Fig. 2) and on the inner
167 part of the pectoral fin peduncle (*Col.in.pec*; Fig. 2).

168 In addition to the criteria defined by the SNI (2000b), our survey revealed that aquaculture
169 extension services and fish farmers have identified several other morphological or behavioral
170 criteria used to try to improve sexing success (Sendjaja and Rizki, 2002). Among these, three
171 objectively observable criteria were included in this study: body shape, caudal fin edging and
172 particular aspects of fish behavior. Thus, for fish farmers, the general body shape of the fish
173 (*Shape*) varies, with a flattened and slender abdomen for males and a rounded abdomen for
174 females. Observation of the caudal fin (*Caudal.fin*) is also a criterion used; farmers generally
175 consider that the posterior edge of the caudal fin has a straight-shape in males whereas it has
176 an arched-shape in females. In addition to these morphological observations, fish behavior is
177 also examined by fish farmers. When males are held horizontally out of water, the caudal fin
178 curves upwards and the muscles of the whole fish body contract and cause tremors (*Tremor*),
179 whereas this particular behavior is not observed in females (Sularto et al., 2017).

180 2.3. Data analysis

181 2.3.1. Preliminary data treatment

182 Individuals with missing data were excluded ($n = 3$). The data were separated according to the
183 color (black and white) phenotypes because some criteria, such as coloration on the outer and
184 the inner part of the pectoral fin peduncle (*Col.out.pec* and *Col.in.pec*, see section 2.2.2.),
185 involved observations that can be affected by the individual phenotype. Each dataset (one per
186 phenotype) was then randomly split into two equal parts: the first half of data was analyzed to
187 identify the most useful combinations of variables for sex determination and the remaining
188 data were used to validate the results (see section 2.3.2).

189 2.3.2. Random forest algorithm

190 To determine which criteria (i.e. variables p ; see section 2.2.2) are the most useful for sexing
191 giant gourami, a random forest algorithm was used. The random forest algorithm can be
192 defined as a decision tree-type classification society (Breiman, 2001). In the algorithm, two
193 parameters must be determined: m , the number of variables to be used for each node and N ,
194 the number of trees to be created. In this study, for all the analyses, we selected $m = 2$ and $N =$
195 2000. The steps of the algorithm have been described in the literature (Breiman, 2001) and
196 can be briefly summarized as follows:

- 197 1. The dataset used to run the random forest algorithm is divided in two parts, including
198 training data (i.e. in-bag data; approx. two-thirds of the data points) used to build the decision
199 tree and remaining data (i.e. out-of-the bag data so-called OOB data; approx. one-third) used
200 to calculate the internal error rate (i.e. OOB error).
- 201 2. The m variables are selected randomly ($m < p$).
- 202 3. The best split is calculated based on the m variables. This process is repeated to create a
203 sufficiently large tree.
- 204 4. The internal error rate is calculated using the OOB data.

205 The new observation is assigned to the class with the highest vote from N trees. Random
206 forest is fast, resistant to overfit, and can be used as desired: many trees can be studied at once

207 (Breiman and Cutler, 2004). The random forest algorithm is acceptable for sexing systems
208 and has recently been used for this purpose in the literature (Akkoç et al., 2016, 2017). To
209 determine the best models, the most contributing criteria were selected based on their values
210 of the mean decrease Gini index (Breiman and Cutler, 2004). All results were validated using
211 independent data coming from broodfish not included in the previous analyses (as described
212 section 2.3.1.). Random forests were performed using R freeware version 3.3 (R Development
213 Core Team, 2016).

214 **3. Results**

215 3.1. Identification of sex-dependent criteria from broodfish observations

216 Distribution of the different levels of the criteria selected for sexing on all broodfish (n = 293
217 and n = 120 respectively for the black phenotype and the white phenotype) was examined for
218 detection of phenotype-dependent responses. Our analysis revealed that criteria *Caudal.fin*,
219 *Shape* and *Tremor* were similar in both phenotypes and no clear difference was observed
220 between males and females (Fig. 3). For the criteria related to head morphology (*Forehead*
221 and *Lower.jaw*), there were no differences between the two phenotypes. Nevertheless, these
222 secondary sexual characters were clearly less developed in females. The development of
223 humping on the forehead and the lower jaw was moderate in most females (development
224 stage ≤ 3 in more than 98% of females; see section 2.2.2), whereas about 60% of the males
225 displayed well-developed forehead and thickening of lower jaw (stage ≥ 4). However, we
226 observed up to 15% of the males at the lowest development stages (1 and 2) and 19-38% of
227 the males showed intermediate development (stage 3) for *Forehead* and *Lower.jaw*
228 irrespective of the color phenotype (Fig. 3). Criteria involving colors (*Col.out.pec* and
229 *Col.in.pec*) were the only ones that were phenotype-dependent. Among white individuals, the
230 “white” pigmentation was observed in more than 83% of females and 91% of males (Fig. 3).
231 In black individuals, the “Black spots” or “Full black” pigmentations were observed in more

232 than 76% of the females whereas the “White” pigmentation was observed in more than 78%
233 of the males (Fig. 3).

234 3.2. The most useful criteria for sexing giant gourami broodfish

235 The random forest analyses were first performed on both phenotypes, including all seven
236 sexing criteria (all criteria, AC, models). For the black phenotype, the AC model yielded a
237 >99% success rate for female sexing and 96% for males. Using the AC model, sexing success
238 rates were generally lower for the white phenotype (93-100% for females and 89-96% for
239 males, Table 2). *Col.out.pec*, *Forehead* and *Lower.jaw* were the most contributing criteria
240 (Mean decrease Gini index: 9.2-13.4, Fig. 4) for black individuals and *Lower.jaw* and
241 *Forehead* (Mean decrease Gini index of 5.3 and 10.9, Fig. 4) for white individuals. These
242 criteria were then used for subsequent analyses. Thus, using models that include only the most
243 contributing criteria (MCC models), the sexing success rate reached 97-98% in females and
244 93-96% in males for black individuals. These values are similar to those obtained with the AC
245 model. For white individuals, the MCC model correctly sexed 95-100% of females and 89-
246 96% of males, with no clear-cut differences compared with the AC model (Table 2).
247 According to the classification results, the receiver operating characteristic (ROC) curves for
248 MCC models were plotted based on a class of females (Fig. 5). The areas under the ROC
249 curves (AUC) were calculated at 99.9% for black individuals and 96.7% for white
250 individuals. These results indicate that the MCC models led to an acceptably high success
251 rate. Interestingly, the most contributing variables – considered one by one – can also, in
252 some cases, lead to high percentages of correct sex discrimination. This is particularly true for
253 *Forehead* in black individuals (up to >99% for females and 100% for males) and for
254 *Lower.jaw* in white individuals (up to 100% for females and males).

255 The validation of the results from independent data indicates that when several variables are
256 included in the model (the seven criteria or the most contributing criteria), the percentages of

257 good discrimination were usually similar (max. 7% difference, Table 2). Nevertheless, in
258 comparison, the sexing appeared unreliable when only one criterion was considered (up to
259 16% difference between the two datasets, Table 2).

260 **4. Discussion**

261 The problem of broodfish sexing is common in cultures of fish species whose sexual
262 dimorphism is inconspicuous or subtle. Thus, methods of sex identification of broodfish have
263 recently been developed in species such as the beluga sturgeon, *Huso huso* (Falahatkar et al.,
264 2013, 2011; Masoudifard et al., 2011), bluefin tuna, *Thunnus* spp. (Agawa et al., 2015; Micera
265 et al., 2010), or pirarucu, *Araipama gigas* (Almeida et al., 2013; Chu-Koo et al., 2009; Torati
266 et al., 2016). Nevertheless, most of the techniques employed in these species are costly and
267 required specific skills and materials which make them practically inaccessible to traditional
268 fish farmers. For this reason, the approach developed in the present study on giant gourami
269 was to compare the reliability of gender identification using morphological and behavioral
270 criteria readily adopted by fish farmers with another convenient method based on a simple
271 technique, urogenital cannulation and intra-ovarian biopsy, already used successfully in many
272 farmed fish species (Garcia, 1989; Cacot et al., 2002; Slembrouck et al., 2004; Legendre et
273 al., 2012).

274 To date, no distinction has been made between the giant gourami broodfish of the white and
275 black phenotypes in sexing methodology (SNI, 2000b). Our results reveal that these
276 phenotypes should be considered separately because the most contributing morphological
277 criteria for sexing are different: the hump on the upper part of the head, the thickening of the
278 lower jaw and pigmentation on the pectoral fin for the black phenotype whereas the latter
279 criterion is less contributing for the white phenotype. In addition, our results demonstrate that,
280 in sexually mature fish, the combined observation of a limited number of morphological
281 criteria in broodfish improves successful sexing ($\geq 95\%$ and $\geq 89\%$ for females and males,

282 respectively). Nevertheless, we never found 100% certainty in sexing, especially in males.
283 Our analysis showed that the consideration of additional criteria such as the shape of the
284 body, the shape of the caudal fin or tremor behavior does not significantly improve sexing
285 success. To obtain high sexing success rates, observations should focus on the most
286 contributing criteria (the hump on the upper part of the head, thickening of the lower jaw and,
287 for individuals of the black phenotype only, pigmentation on the pectoral fin) in accordance
288 with the SNI (SNI, 2000b). Nevertheless, these criteria must be accurately scored, because
289 even for adult fish, secondary sexual characters can remain incompletely expressed – as
290 highlighted in the present study. The potential for misclassification is particularly pronounced
291 for males, with 19-38% having intermediate forehead plus low jaw thickening (stage 3) and
292 15% showing even lower stages of development (stages 1 and 2). The morphometric
293 measurements provided in this study contribute to a more accurate assessment of the
294 development of the hump on the upper part of the head and the thickening of the lower jaw in
295 a given group of gourami broodfish.

296 Regarding pigmentation, fish farms and broodfish production centers use the presence or
297 absence of pigmentation on the inner side of the pectoral fin base, as shown in Figure 2. In
298 this study, this pigmentation was characterized both on the outer and inner part of the pectoral
299 fin base. Interestingly, our results indicated that – conversely to traditional practice – it is
300 more useful to observe pigmentation on the outer pectoral fin.

301 The results of the present study were obtained from sexually mature individuals aged 3 to 7
302 years and weighing 2-5 kg. Nevertheless, due to the high prices of mature broodfish, most fish
303 farmers buy and select their own future broodfish from relatively small and still immature
304 individuals (< 2 kg). At this stage, some of the morphological criteria considered here for
305 sexing are not fully developed yet. Although the first changes in the hump on the upper part
306 of the head and in lower jaw thickening can be observed in fish as small as 0.3 kg (Sularto et

307 al., 2017), they are not fully developed in individuals weighing less than 2.5 kg according to
308 the West Java Gourami Center (BPPSIGN). The pigmentation on the outer part of the pectoral
309 fin begins in individuals that weigh at least 0.2 kg and is considered as completely expressed
310 in individuals of 0.5 kg (unpublished data). A more in-depth analysis of misclassified
311 individuals in our analyses reveals that more than 60% of them were in the low to
312 intermediate stages (stage 1, 2, or 3) for the variables *Forehead* and *Lower.jaw*. This
313 demonstrates the potential difficulties of sexing in immature individuals in which secondary
314 sex characters are not fully developed.

315 **5. Conclusion and Recommendations**

316 This study demonstrates the feasibility of selecting giant gourami broodfish for reproduction
317 based on observations of morphological criteria. Nevertheless, the following
318 recommendations should be taken into consideration to maximize sexing success:

- 319 • The two color-based phenotypes (white and black) should be considered separately
320 when sexing.
- 321 • For sexually mature individuals, the hump on the upper part of the head, the
322 thickening of the lower jaw and the pigmentation on the pectoral fin should be
323 observed for the so-called black phenotype, whereas the latter criterion is not relevant
324 for the so-called white phenotype. For the two criteria referring to changes in head
325 morphology, the determination of their relative importance should be carried out using
326 the arbitrary scale presented in this study.
- 327 • The observation of other morphological criteria (such as body shape, caudal fin edging
328 or fish behavior) generally used by fish farmers does not improve sexing success;
329 therefore, particular attention should be given to the examination of the criteria listed
330 above.

- 331 • Sexing should be confirmed by urogenital cannulation and intra-ovarian biopsies,
332 when the necessary skills and materials are available. Whenever possible, and under
333 good fish welfare, the cannulation method should always be preferred for sexing
334 gourami broodfish.
- 335 • Pre-selection of future broodfish from sexually immature individuals should be based
336 on the reliable morphological criteria identified in the present study, emphasizing
337 color at the base of the pectoral fin in individuals with the black phenotype. Sexing
338 should be confirmed when the sexual maturity is reached and before using these
339 individuals for reproduction.

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346

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463 **Captions to figures**

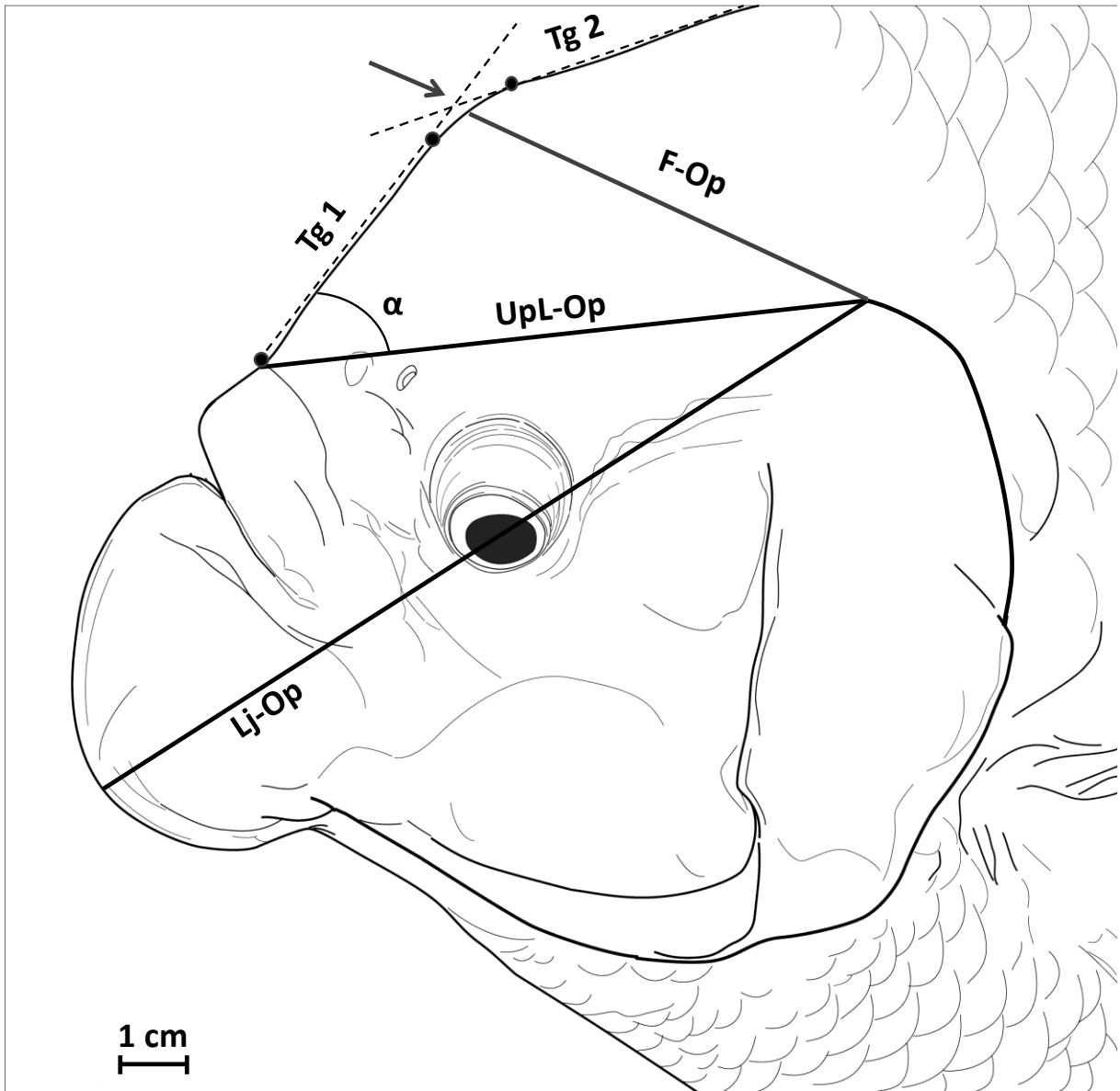
464 Figure 1. Morphometric measurements made to score the development of the humping of the
465 forehead and the thickening of the lower jaw in broodfish of the giant gourami, *Osphronemus*
466 *goramy*. F-OP: distance between the top of the hump and the upper end of the opercula
467 opening. The top of the hump (indicated by the black arrow) was determined from the
468 intersection of the tangent to the hump from the top of the upper lip (Tg 1) and the tangent to
469 the hump from the edge of the fish back (Tg 2). Lj-OP: distance between the upper end of the
470 opercula opening and the furthest point of the lower jaw (passing through the center of the
471 eye). Upl-OP: distance between the upper end of the opercula opening and the top of the
472 upper lip. α : angle between Upl-OP and Tg 1.

473 Figure 2. Pigmentation on the outer (*Col.out.pec*, on the left) and inner (*Col.in.pec*, on the
474 right) pectoral fin in mature giant gourami broodfish. (A) absence of black pigmentation; (B)
475 presence of dotted black melanophores (black spots); and (C) very intense black patch (full
476 black).

477 Figure 3. Proportion of males and females in the (A) so-called black broodfish and (B) so-
478 called white broodfish for each level of each sexing criterion taken from the Indonesian
479 National Standard (SNI, 2000b) (*Col.in.pec*, *Col.out.pec*, *Forehead* and *Lower.jaw*) and from
480 farmers' practices (*Caudal.fin*, *Shape* and *Tremor*). For *Forehead* and *Lower.jaw*, the
481 numbers indicated in x-axis refer to developmental stage according to the arbitrary scale
482 presented in the Table 1.

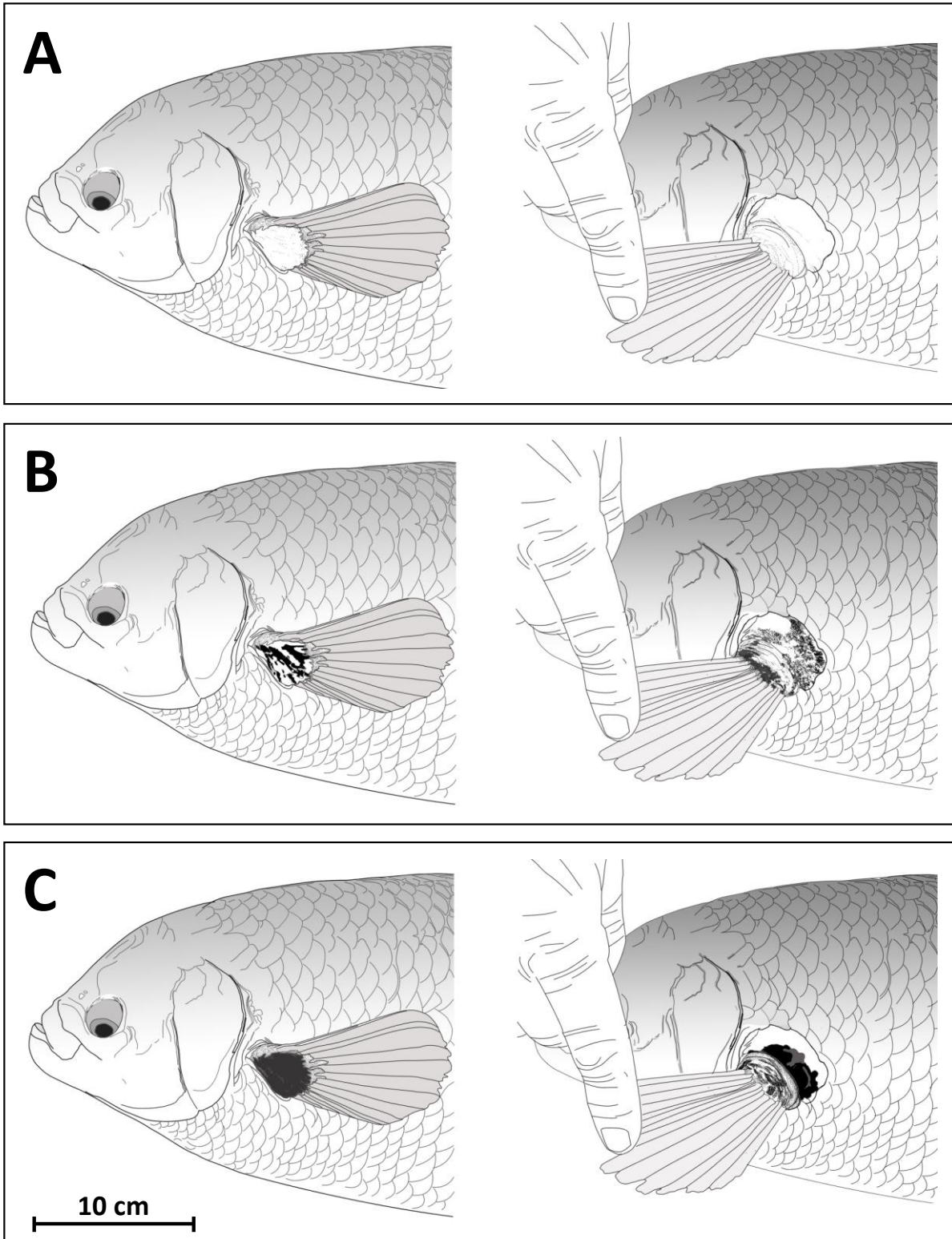
483 Figure 4. Random forest model criteria importance; the mean decrease Gini index. All seven
484 criteria are sorted in ascending order according to their importance in the model built from
485 2000 trees. The most important variables lie between 9.2 and 13.4 for (A) black giant gourami
486 broodfish and between 5.3 and 10.9 for (B) white giant gourami broodfish.

487 Figure 5. Receiver operating characteristic (ROC) curves of the proposed models including
488 the most contributing criteria (MCC, Table 2) resulting from the random forest analysis on
489 (A) black giant gourami broodfish and (B) white giant gourami broodfish.



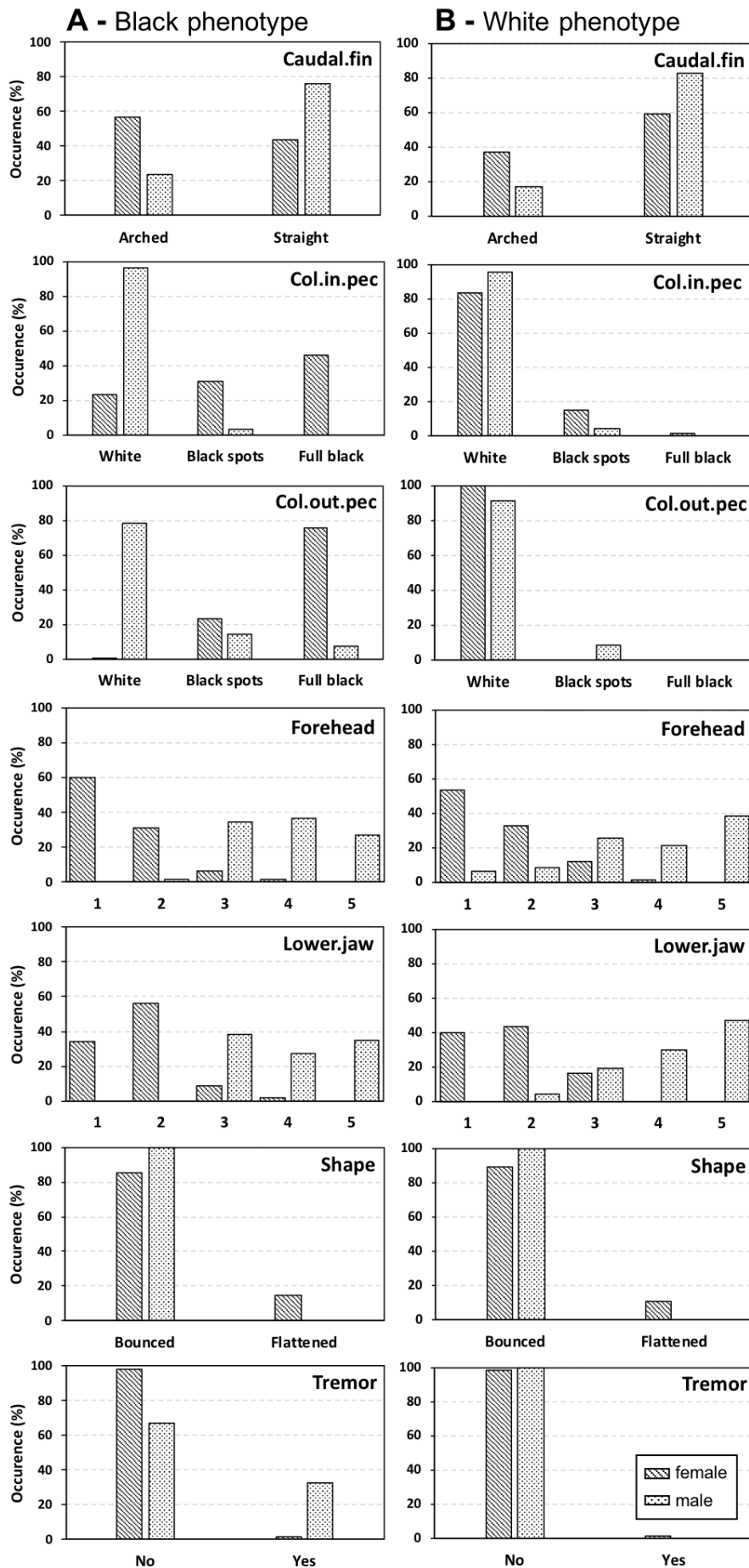
490

491 Figure 1



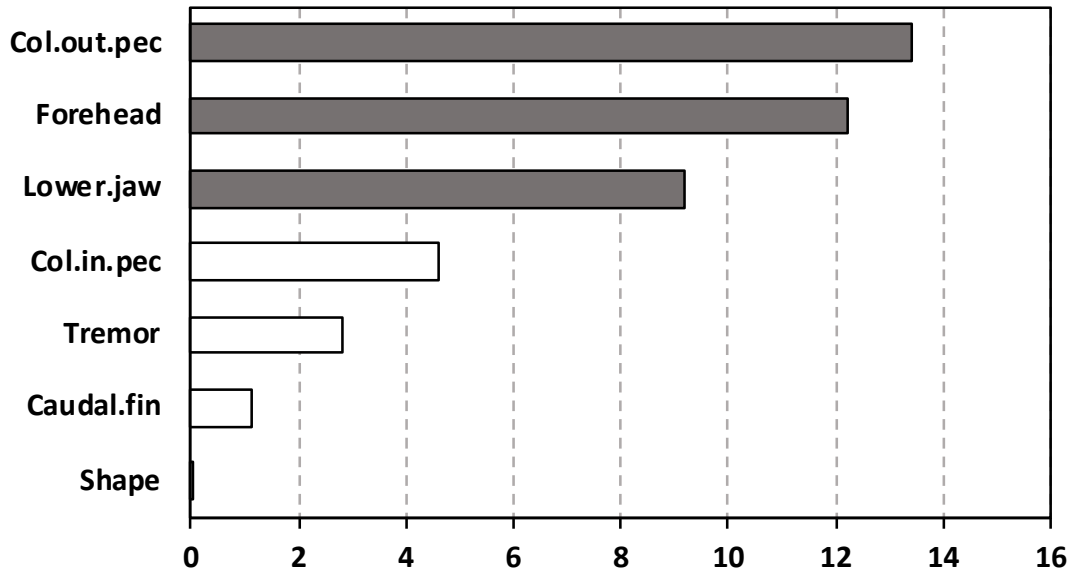
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493 Figure 2

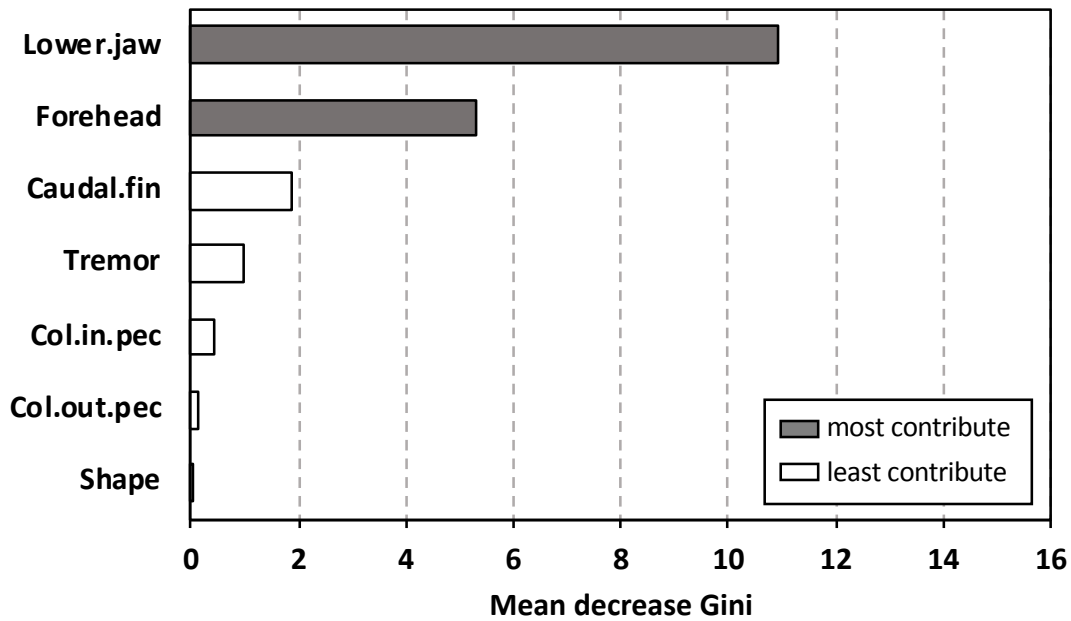


494 Figure 3

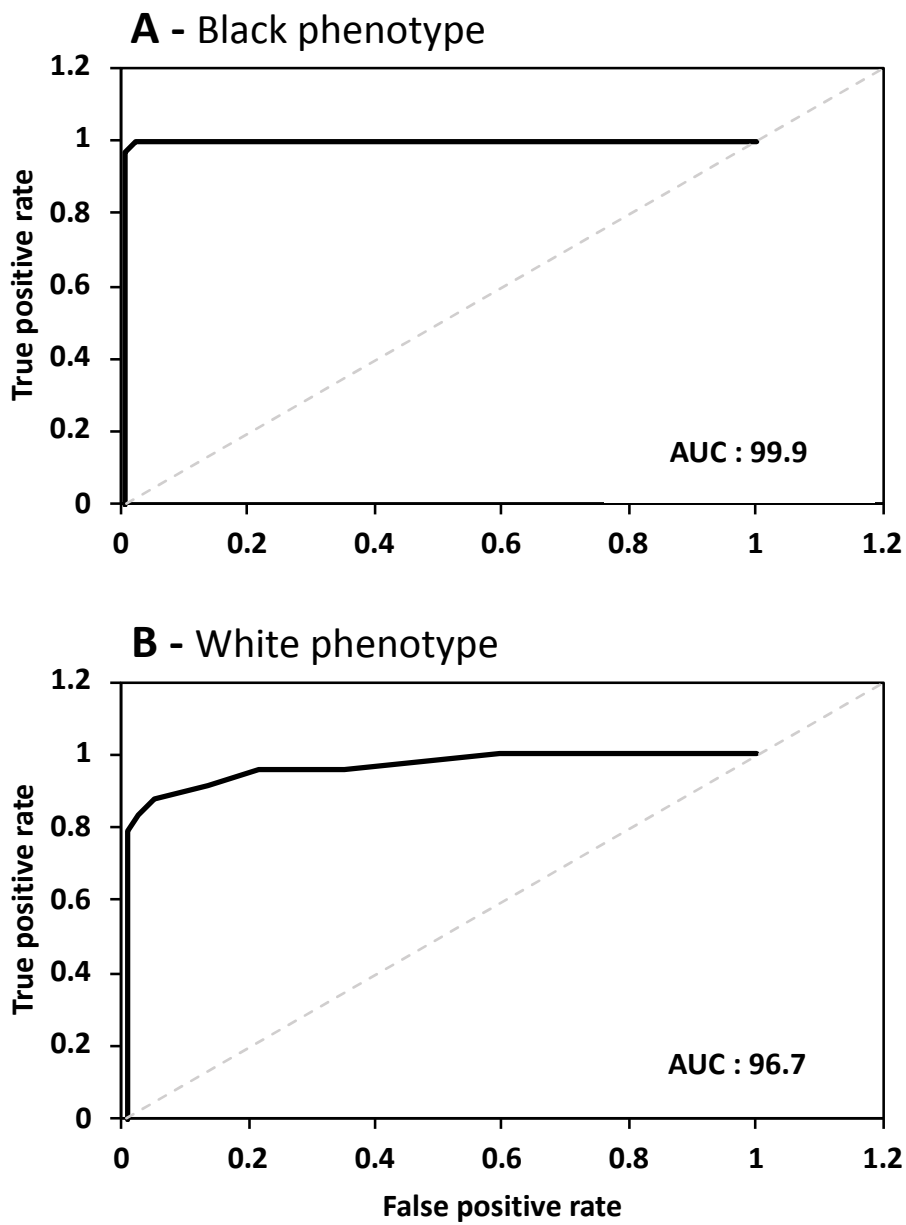
A - Black phenotype



B - White phenotype



495 Figure 4



496 Figure 5

497 Table 1. Arbitrary scale used to score the morphometric measurements taken to quantify the
 498 development of the humping of the forehead and the thickening of the lower jaw in giant
 499 gourami broodfish. F-OP: distance between the top of the hump and the upper end of the
 500 opercula opening. Lj-OP: distance between the upper end of the opercula opening and the
 501 furthest point of the lower jaw (passing through the center of the eye). Upl-OP: distance
 502 between the upper end of the opercula opening and the top of the upper lip. α : angle between
 503 Upl-OP and the tangent to the hump from the top of the upper lip.

Stage	Description	F-Op / Upl-Op (%)	Lj-Op / Upl-Op (%)	α (degree)
1	No particular hump or thickening visible	≤ 62	≤ 121	≤ 33
2	Hump or thickening slightly marked	62 - 66	120 - 127	33 - 37
3	Hump or thickening clearly marked but moderately developed	64 - 69	126 - 130	38 - 42
4	Hump or thickening well developed	68 - 71	128 - 134	41 - 45
5	Hump or thickening strongly developed	≥ 70	≥ 135	≥ 45

504

505 Table 2. Results of sexing in giant gourami broodfish (black and white phenotypes) from
506 random forest analysis. Several models including different criteria were tested: AC = All
507 criteria, MCC = Most contributing criteria (see Fig. 4) and criterion tested one by one. Data
508 are expressed as the percentage of correct sexing for males and females. OOB = out-of-the-
509 bag.

Criteria included	Dataset	Phenotype	Correct sexing (%)		OOB error (%)
			Female	Male	
AC	Creation	Black	100	96	< 1
		White	93	89	13
	Validation	Black	> 99	96	1
		White	100	96	2
MCC	Creation	Black	98	93	3
		White	95	89	13
	Validation	Black	97	96	3
		White	100	96	2
<i>Col.out.pec</i>	Creation	Black	> 99	82	4
	Validation	Black	> 99	75	5
<i>Forehead</i>	Creation	Black	94	100	5
		White	84	75	20
	Validation	Black	> 99	74	5
		White	89	78	15
<i>Lower.jaw</i>	Creation	Black	97	64	10
		White	92	75	15
	Validation	Black	92	100	7
		White	100	88	8