Gender identification in farmed giant gourami (Osphronemus goramy): A methodology for better broodstock management

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Abstract:

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

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

1. Introduction

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

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

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

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

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

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

2. Materials and Methods

2.1. Fish origin and maintenance

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

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

2.2. Experimental procedures

2.2.1. Fish observations and sexing by urogenital cannulation

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

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

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

2.2.2. Description of morphological and behavioral criteria

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

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

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

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

2.3.2. Random forest algorithm

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

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

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

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

3. Results

3.1. Identification of sex-dependent criteria from broodfish observations

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

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

3.2. The most useful criteria for sexing giant gourami broodfish

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

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

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

4. Discussion

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

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

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

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

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

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

5. Conclusion and Recommendations

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

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

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

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References

Abramoff M.D., Magalhaes P.J., 2004, Image Processing with Image J. Biophoton. Internat. 11, 36-42.

Agawa, Y., Iwaki, M., Komiya, T., Honryo, T., Tamura, K., Okada, T., Yagishita, N., Kobayashi, T., Sawada, Y., 2015. Identification of male sex-linked DNA sequence of the cultured Pacific bluefin tuna *Thunnus orientalis*. Fish. Sci. 81, 113–121.

Akkoç, B., Arslan, A., Kök, H., 2017. Automatic gender determination from 3D digital

maxillary tooth plaster models based on the random forest algorithm and discrete cosine transform. Comput. Methods Programs Biomed. 143, 59–65.

Akkoç, B., Arslan, A., Kök, H., 2016. Gray level co-occurrence and random forest algorithm-based gender determination with maxillary tooth plaster images. Comput. Biol. Med. 73, 102–107.

Almeida, I.G., Ianella, P., Faria, M.T., Paiva, S.R., Caetano, A.R., 2013. Bulked segregant analysis of the pirarucu (*Arapaima gigas*) genome for identification of sex-specific molecular markers. Genet. Mol. Res. 12, 6299–6308.

Amornsakun, T., Kullai, S., Hassan, A., 2014a. Some aspects in early life stage of giant gourami, *Osphronemus goramy* (Lacepede) larvae. Songklanakarin J. Sci. Technol. 36.

Amornsakun, T., Kullai, S., Hassan, A., 2014b. Feeding behavior of giant gourami, *Osphronemus gouramy* (Lacepede) larvae. Songklanakarin J. Sci. Technol. 36.

Arifin, O.Z., Imron, I., Asependi, A., Hendri, A., Muslim, N., Yani, A., 2018. Hibridisasi intraspesifik antar dua populasi ikan gurami Galunggung (*Osphronemus goramy*, Lacepede, 1801) (in Indonesian). J. Ris. Akuakultur 12, 315–323.

Azrita, H.S., 2015. Morphological character among five strains of giant gourami, *Osphronemus gouramy* Lacepede, 1801 (Actinopterygii: Perciformes: Osphronemidae) using a truss morphometric system. Int. J. Fish. Aquat. Stud. 2, 344–350.

Breiman, L., 2001. Random Forests. Mach. Learn. 45, 5–32.

Breiman, L., Cutler, A., 2004. Random forests: classification description. https://www.stat.berkeley.edu/~breiman/RandomForests/cc_home.htm (accessed 30 March 2018).

376 BPS, 2013. Jumlah rumah tangga usaha budidaya bukan ikan hias menurut wilayah dan jenis
377 ikan utama (in Indonesian).
378 https://st2013.bps.go.id/dev2/index.php/site/tabel?tid=57&wid=3200000000 (accessed
379 01 May 2018).

380 Budi, D.S., Suprayudi, M.A., 2015. Growth response and feed utilization of giant gourami
381 (*Osphronemus goramy*) juvenile feeding different protein levels of the diets
382 supplemented with recombinant growth hormone. HAYATI J. Biosci. 22, 12–19.

383 Cacot, P., Legendre, M., Dan, T. Q., Tung, L. T., Liem, P. T., Mariojouls, C., Lazard, J. 2002.
384 Induced ovulation of *Pangasius bocourti* (Sauvage, 1880) with a progressive hCG
385 treatment. Aquaculture 213(1-4), 199-206.

386 Chu-Koo, F., Dugué, R., Aguilar, M.A., Daza, A.C., Bocanegra, F.A., Veintemilla, C.C.,
387 Duponchelle, F., Renno, J.-F., Tello, S., Nunez, J., 2009. Gender determination in the
388 paiche or pirarucu (*Arapaima gigas*) using plasma vitellogenin, 17β-estradiol, and 11-
389 ketotestosterone levels. Fish Physiol. Biochem. 35, 125–136.

390 Falahatkar, B., Akhavan, S.R., Tolouei Gilani, M.H., Abbasalizadeh, A., 2013. Sex
391 identification and sexual maturity stages in farmed great sturgeon, *Huso huso* L. through
392 biopsy. Iran. J. Vet. Res. 14(2), 133-139.

393 Falahatkar, B., Tolouei Gilani, M.H., Falahatkar, S., Abbasalizadeh, A., 2011. Laparoscopy, a
394 minimally-invasive technique for sex identification in cultured great sturgeon *Huso*
395 *huso*. Aquaculture 321, 273–279.

396 FAO, 2017. FishStatJ: software for fishery statistical time series. Roma, Italy.

397 FAO, 2016. Planning for aquaculture diversification: the importance of climate change and
398 other drivers (No. Fisheries and Aquaculture Proceedings 47). Food and Agriculture

399 Organization of the United Nations, Roma, Italy.

400 Feist, G., Van Eenennaam, J.P., Doroshov, S.I., Schreck, C.B., Schneider, R.P., Fitzpatrick,
401 M.S., 2004. Early identification of sex in cultured white sturgeon, *Acipenser*
402 *transmontanus*, using plasma steroid levels. Aquaculture 232, 581–590.

403 Garcia, L. M. B., 1989. Development of an ovarian biopsy technique in the sea bass, *Lates*
404 *calcarifer* (Bloch). Aquaculture 77(1), 97-102.

405 Holm, J., 1994. Ultrasonography, a non–invasive method for sex determination in cod (*Gadus*
406 *morhua*. J. Fish Biol. 44, 965–971.

407 Legendre, M., Satyani, D., Subandiyah, S., Pouyaud, L., Baras, E., Slembrouck, J. 2012.
408 Biology and culture of the clown loach *Chromobotia macracanthus* (Cypriniformes,
409 Cobitidae): 1-Hormonal induced breeding, unusual latency response and egg production
410 in two populations from Sumatra and Borneo Islands. Aquat. Living Resour. 25(2), 95-
411 108.

412 Lewis, R.M., 1962. Sexual maturity as determined from ovum diameters in striped bass from
413 North Carolina. Trans. Am. Fish. Soc. 91, 279–282.

414 Martin, R.W., Myers, J., Sower, S.A., Phillips, D.J., Mcauley, C., 1983. Ultrasonic imaging, a
415 potential tool for sex determination of live fish. North Am. J. Fish. Manag. 3, 258–264.

416 Martin-Robichaud, D.J., Rommens, M., 2001. Assessment of sex and evaluation of ovarian
417 maturation of fish using ultrasonography. Aquac. Res. 32, 113–120.

418 Masoudifard, M., Vajhi, A.R., Moghim, M., Nazari, R.M., Naghavi, A.R., Sohrabnejad, M.,
419 2011. High validity sex determination of three years old cultured beluga sturgeon (*Huso*
420 *huso*) using ultrasonography. J. Appl. Ichthyol. 27, 643–647.

421 Micera, E., Zupa, R., Zarrilli, A., Camarda, A., Moramarco, A.M., Acone, F., De Metrio, G.,
422 Corriero, A., 2010. A rapid latex agglutination test for gender identification in the
423 Atlantic bluefin tuna, *Thunnus thynnus* (Linnaeus). Aquac. Res. 41, 1396–1401.

424 Pengseng, P., Claude, E.B., 2011. Assessment of fertilizer application intervals for giant
425 gourami (*Osphronemus goramy* Lacepede) in ponds. Walailak J. Sci. Technol. WJST 8,
426 33–40.

427 R Development Core Team, 2016. R: a language and environment for statistical computing. R
428 Foundation for Statistical Computing, Vienna, Austria.

429 Rimmer, M.A., Sugama, K., Rakhmawati, D., Rofiq, R., Habgood, R.H., 2013. A review and
430 SWOT analysis of aquaculture development in Indonesia. Rev. Aquac. 5, 255–279.

431 Ross, R.M., 1984. Catheterization: a non-harmful method of sex identification for sexually
432 monomorphic fishes. Progress. Fish-Cult. 46, 151–152.

433 Ross, L.G., Martinez Palacios, C.A., Morales, E.J., 2008. Developing native fish species for
434 aquaculture: the interacting demands of biodiversity, sustainable aquaculture and
435 livelihoods. Aquac. Res. 39, 675–683.

436 Saint-Paul, U., 2017. Native fish species boosting Brazilian's aquaculture development. Acta
437 Fish. Aquat. Resour. 5, 1–9.

438 Slembrouck, J., Komarudin, O. M. A. N., & Maskur, L. M. 2004. Technical manual for the
439 artificial propagation of the Indonesian Catfish, *Pangasius djambal*. IRD-BRKP, Paris,
440 Jakarta.

441 SNI, 2000a. SNI 01-6485.3-2000: Produksi benih ikan gurame (*Osphronemus goramy*, Lac)
442 kelas benih sebar (in Indonesian). Badan Standardisasi Nasional (BSN), Jakarta,

Indonesia.

SNI, 2000b. SNI 01-6485.1-2000: Induk Ikan Gurami (*Osphronemus gouramy*, Lac) kelas Induk Pokok (Parent Stock) (in Indonesian). Badan Standardisasi Nasional (BSN), Jakarta, Indonesia.

Sendjaja JT, Rizki MH, 2002. Usaha Pembenihan Gurami (in Indonesian). Penebar Swadaya. Jakarta.

Smith, G.H., Murie, D.J., Parkyn, D.C., 2014. Nonlethal sex determination of the greater amberjack, with direct application to sex ratio analysis of the Gulf of Mexico stock. Mar. Coast. Fish. 6, 200–210.

Sularto, S., Febrianti, R., Suharyanto, S., 2017. Perbandingan jenis kelamin dan dimorfisme seksual pada pertumbuhan ikan gurami (*Osphronemus goramy*) serta implikasinya terhadap strategi seleksinya (in Indonesian). J. Ris. Akuakultur 11, 307–312.

Torati, L.S., Varges, A.P.S., Galvao, J.A., Mesquita, P.E., Migaud, H., 2016. Endoscopy application in broodstock management of *Arapaima gigas* (Schinz, 1822). J. Appl. Ichthyol. 32, 353–355.

Webb, M.A., Feist, G.W., Foster, E.P., Schreck, C.B., Fitzpatrick, M.S., 2002. Potential classification of sex and stage of gonadal maturity of wild white sturgeon using blood plasma indicators. Trans. Am. Fish. Soc. 131, 132–142.

Captions to figures

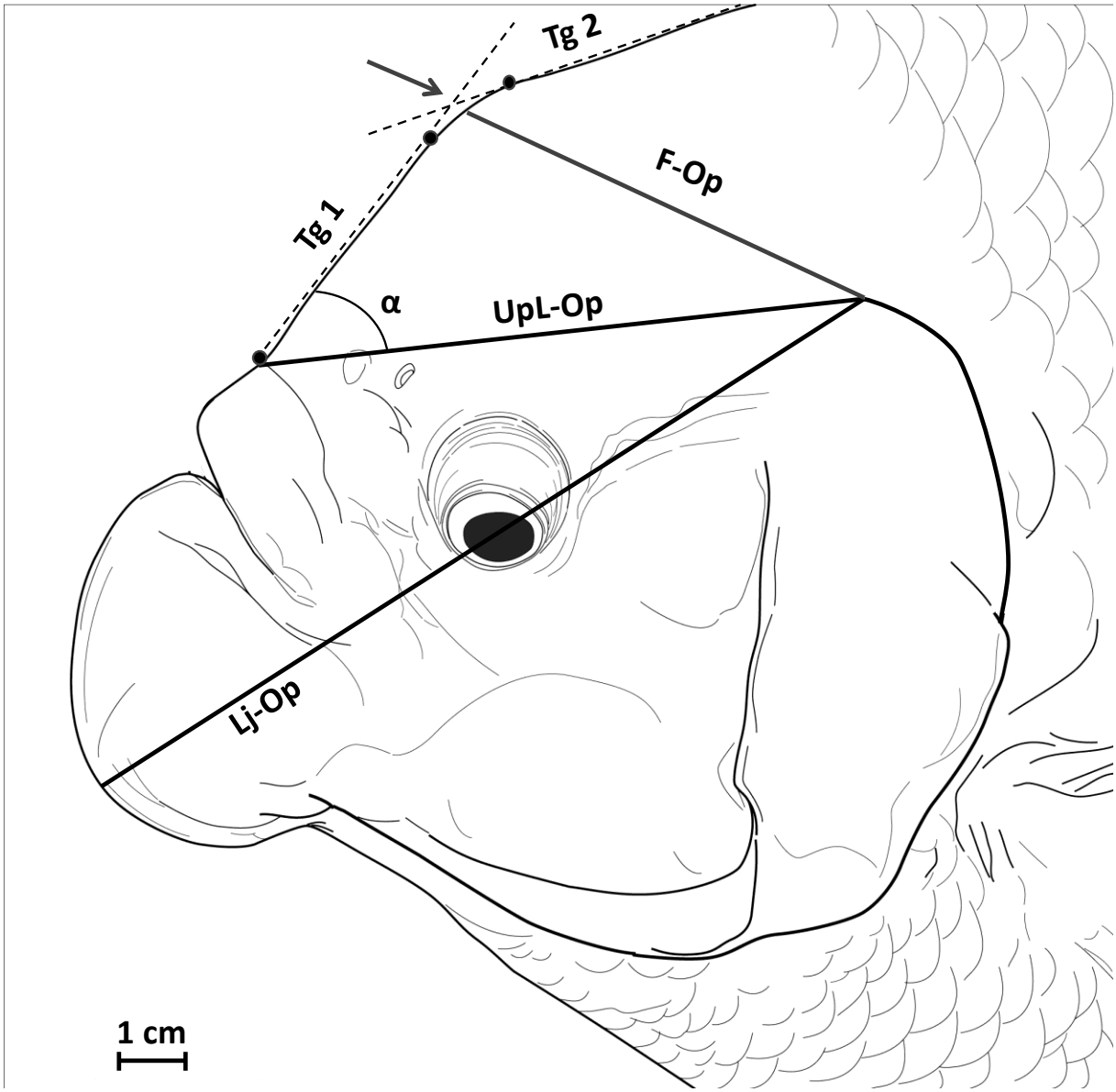
Figure 1. Morphometric measurements made to score the development of the humping of the forehead and the thickening of the lower jaw in broodfish of the giant gourami, *Osphronemus goramy*. F-OP: distance between the top of the hump and the upper end of the opercula opening. The top of the hump (indicated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

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

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

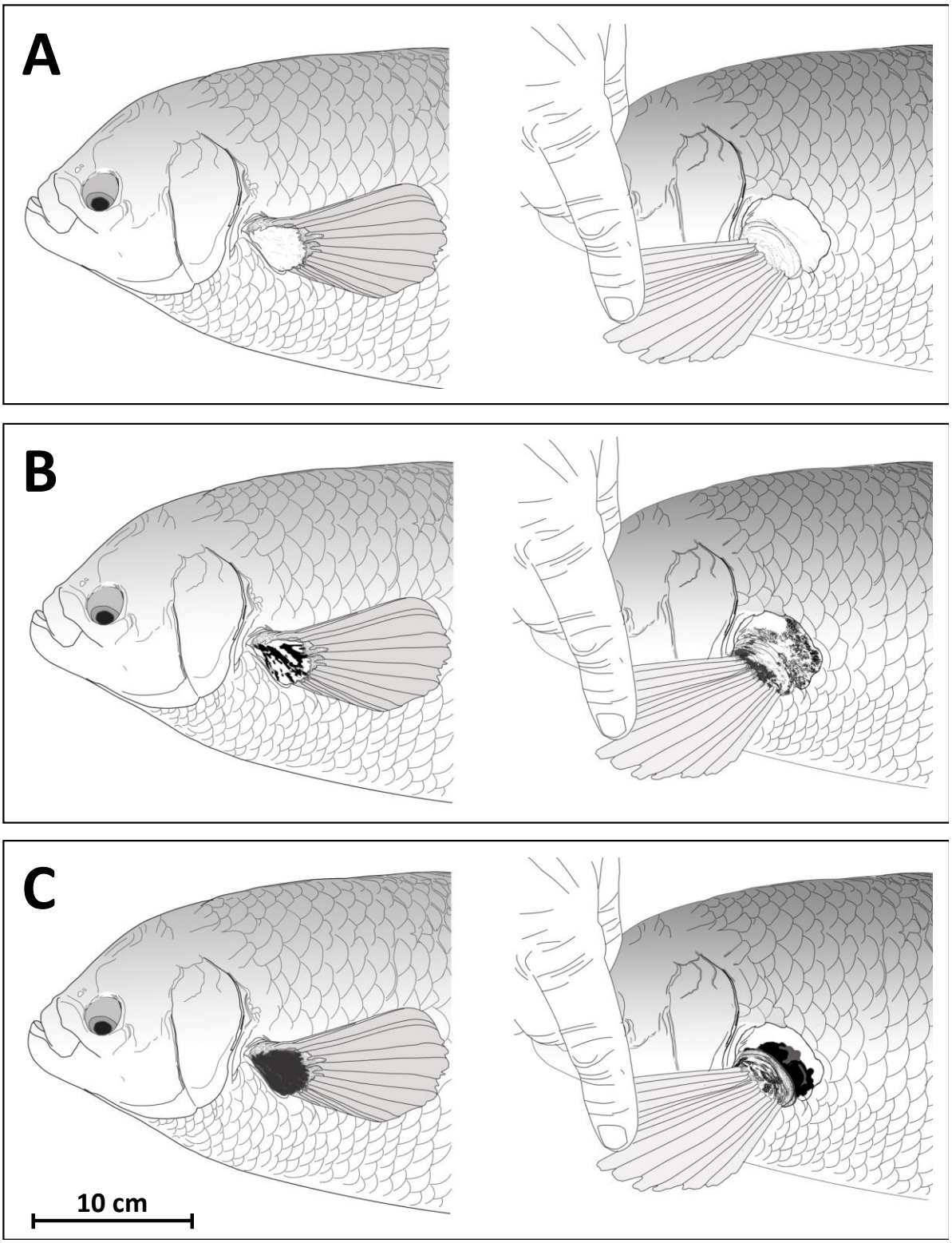
Figure 4. Random forest model criteria importance; the mean decrease Gini index. All seven criteria are sorted in ascending order according to their importance in the model built from 2000 trees. The most important variables lie between 9.2 and 13.4 for (A) black giant gourami broodfish and between 5.3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

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



490

491 Figure 1



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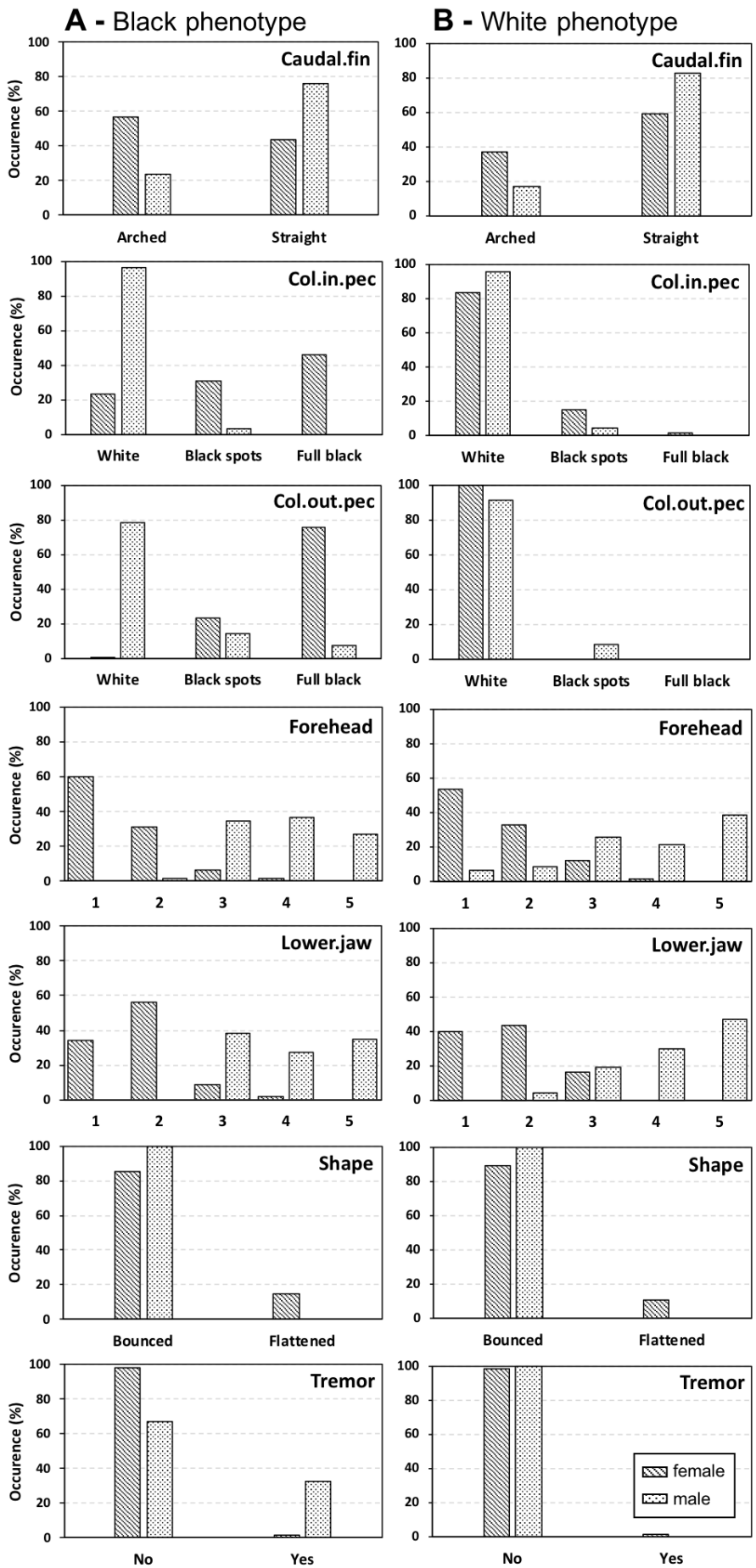
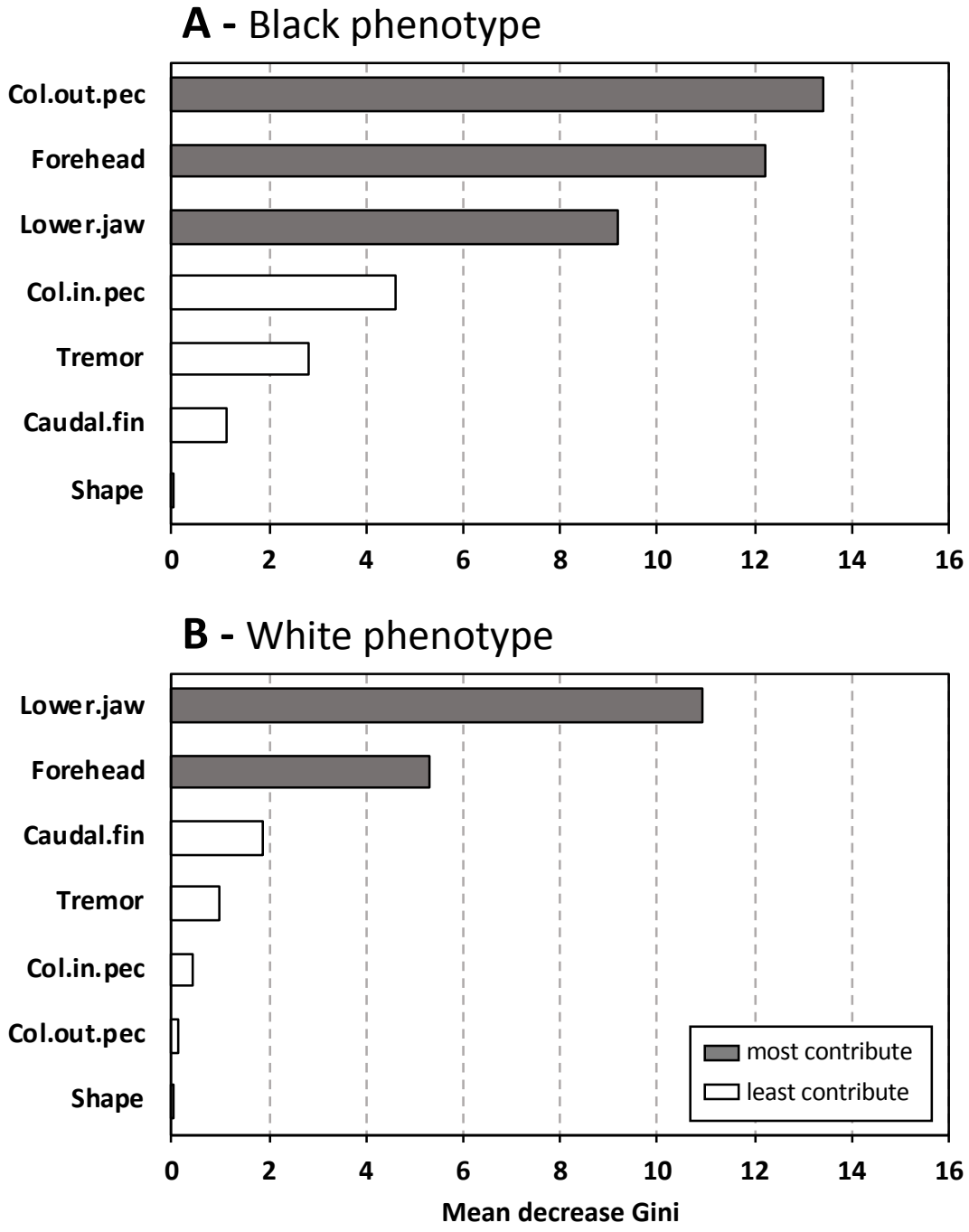
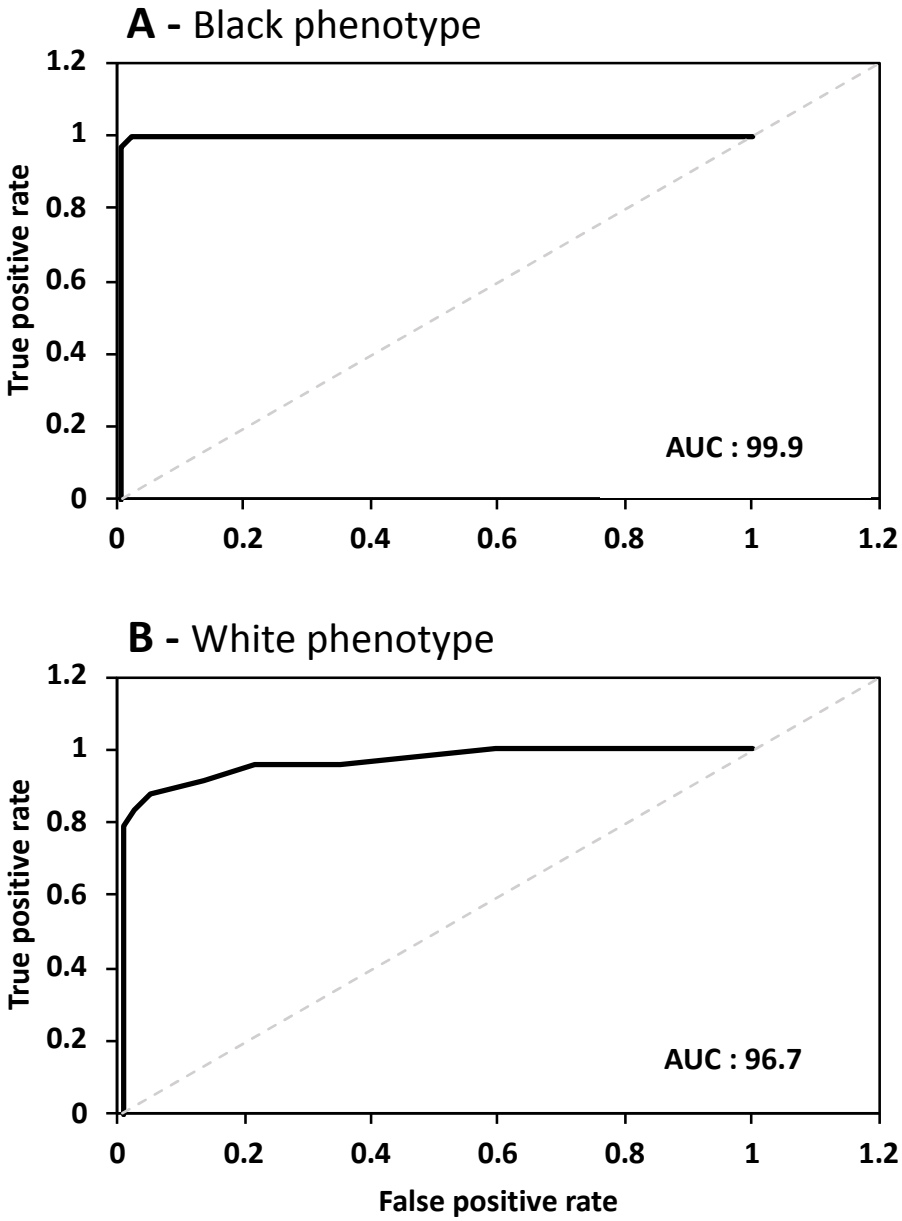
493 Figure 2


A - Black phenotype
B - White phenotype
Caudal.fin
Occurence (%)
Arched
Straight
Col.in.pec
White
Black spots
Full black
Col.out.pec
Forehead
1
2
3
4
5
Lower.jaw
Shape
Bounced
Flattened
Tremor
No
Yes
female
male

494 Figure 3


A - Black phenotype
Col.out.pec
Forehead
Lower.jaw
Col.in.pec
Tremor
Caudal.fin
Shape
0
2
4
6
8
10
12
14
16
B - White phenotype
Lower.jaw
Forehead
Caudal.fin
Tremor
Col.in.pec
Col.out.pec
Shape
most contribute
least contribute
0
2
4
6
8
10
12
14
16
Mean decrease Gini

495 Figure 4


A - Black phenotype
True positive rate
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
AUC : 99.9
B - White phenotype
True positive rate
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
False positive rate
AUC : 96.7

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
Col.out.pec	Creation	Black	> 99	82	4
	Validation	Black	> 99	75	5
Forehead	Creation	Black	94	100	5
		White	84	75	20
	Validation	Black	> 99	74	5
		White	89	78	15
Lower.jaw	Creation	Black	97	64	10
		White	92	75	15
	Validation	Black	92	100	7
		White	100	88	8

510