Taxonomic identification of "Ludong" fish from the Cagayan River (Philippines)

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"Ludong", also known as the *President's fish*, is a mullet fish belonging to the Mugilidae family that is indigenous to Cagayan River and its tributaries. Because of its rarity and apparent threatened status, it has been the subject of intense research, conservation and management. To date, its accurate taxonomic identification is unclear and only based on conflicting published reports. In this study, we established the identity of "Ludong" using morphological analysis and comparison with pictures of holotypes from the Museum National d'Histoire Naturelle collection-Paris and National Museum of Natural History of the Smithsonian Institution, USA. Results revealed that "Ludong" was confirmed to belong to the genus *Cestraeus* of the Mugilidae family. Furthermore, there were at least two species found in Cagayan River, namely *Cestraeus oxyrhynchus* and *Cestraeus goldiei.* These results established the identity of "Ludong" species which can now be used as baseline information for further studies and conservation.

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INTRODUCTION

"Ludong" is a mullet fish belonging to the Mugilidae family that is indigenous to the Cagayan River and its tributaries. It is thought to be a catadromous fish that traverses 200 to 300 kilometers from the highlands, down to the mouth of the Cagayan River, and on to marine waters to spawn (EC Ame and R Mateo, unpublished report). It is said to be seasonally caught from October to December.

"Ludong" is one of the most expensive edible fish in the country with price ranging from P 4,000 to 5,000 per kilo (Prudencio 2011), or roughly $ 90 to 115 USD (assuming an exchange of about P 44 to $ 1 USD). It is dubbed the *President's fish* and has gained considerable popularity for its rarity, unique taste and high market value. Demand for the fish has gradually increased leading to its heavy exploitation and eventual decrease in natural abundance (EC Ame and JP Ayson, unpublished report). Due to this trend, its research, conservation and management have become a high priority in Region 2, Philippines.

As early as the 1950s, "Ludong" had already been the subject of fisheries management strategies. Fisheries Administrative

KEYWORDS
taxonomy, mugilidae , morphology, mullets, morphomeristics, *Cestraeus*

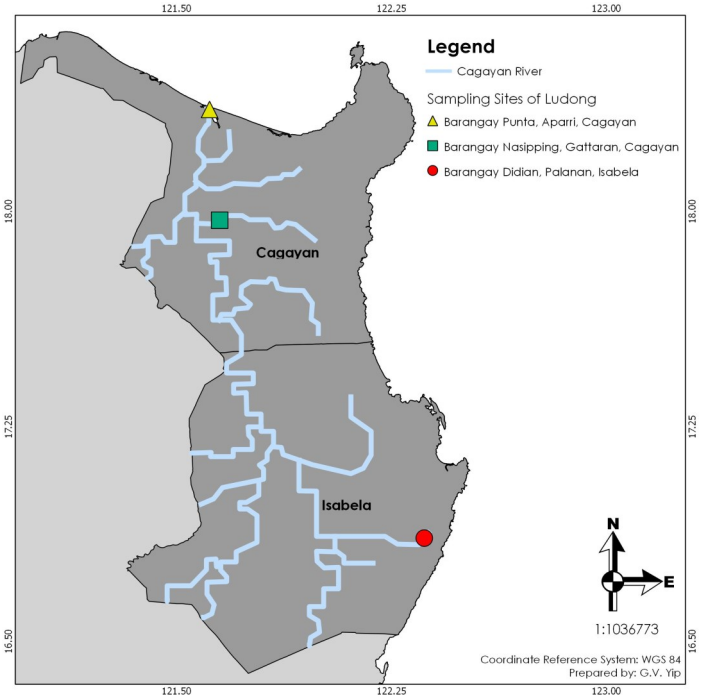

Figure 1. Map showing the sampling sites of the "Ludong" samples used in the study. Due to the lack of coordinates, the sampling site for Alcala, Cagayan, is not shown.

Order #31 Series of 1952 was promulgated to prohibit the capture, purchase, sale preparation and serving of the fish during its spawning season. This was, however, eventually suspended on the basis of a study conducted by Pinzon (EC Ame and JP Ayson, unpublished report). The Province of Isabela in 1997 passed a resolution banning the catching, sale and purchase of "Ludong" for five consecutive years (Resolution No.20, Ordinance No.3, February 1997).

Expectedly, because of its rarity, research efforts towards the conservation of "Ludong" have been directed mainly on aquaculture and artificial breeding to improve production and supply. Commonly reported are studies on the reproductive biology during its spawning season and its population dynamics (EC Ame and JP Ayson, unpublished report; Dela Cruz 2008).

In spite of its popularity, the accurate identification of "Ludong" remained unclear. It has been identified by J.M. Thompson (1982) as *Cestraeus plicatilis* and has since been cited as such in numerous popular reports (Lim 2002, Agasen et al. 2007). However, based on the Food and Agriculture Organization (FAO) Species Identification Guide (edited by Carpenter and Niem 1999), only the mullets *C. oxyrhynchus* and *C. goldiei* are found in the Philippines and not *C. plicatilis*. In addition, Durand et al. (2012) reported in their paper on the molecular systematics of the Mugilidae family that their sample which came from Cagayan River is *C. goldiei*. Finally, archival records of the National Museum of Natural History of the Smithsonian Institution, USA (NMNH/SI), showed that there have been *Cestraeus* specimens from the Philippines collected in 1908, and those specimens were labeled as *C. goldiei*. Hence, the identity of "Ludong" to date is still unclear. In fact, this confusion has also been pointed out in recent reports (Agasen et al. 2007).

This study aims to provide a more detailed examination to establish the identity of the "Ludong" through the use of morphological data. This method has led to successful results both in the identification of new species (Gill and Williams 2011) and in correcting misidentification. A noteworthy example of the latter is the study of Willette and Santos (2013) wherein the correct nomenclature of a sardinella species was established using morphological features and meristic counts, further supported by genetic tools. Willette and Santos were able to show that the widely accepted *Sardinella longiceps* in the country was in fact *S. lemuru.* Results generated from our study will serve as baseline information in support of conservation efforts for biodiversity and the formulation of laws or ordinances for the sustainable resource management of the "Ludong".

MATERIALS AND METHODS

Specimen and Tissue Sampling Procedure

Thirty-four fish samples, whole specimens and muscle tissues/fin only (but with pictures), identified as "Ludong" were collected from the Cagayan River and tributaries. This was made possible with the assistance of BFAR Regional Office II in sampling from different sites presented in Figure 1. The collected "Ludong" samples were then frozen prior to transportation to the Genetic Fingerprinting Laboratory of the National Fisheries Research and Development Institute, Quezon City, Philippines. The whole fish samples were kept frozen for archival purposes.

Morphological Analysis

Identification of the collected "Ludong" samples was done using the FAO Species Identification Guide (edited by Carpenter and Niem 1999). In particular, *Cestraeus spp.* were differentiated from other mullets based on the presence of the ridge of numerous lamellae on the lower jaw of the fish. Morphometric measures of the head and body in millimeters and meristic counts of spines, fin rays and scales were also obtained. All morphometric measurements used for descriptive statistics analysis were standardized prior to analysis using SPSS Statistics for Windows, Version 17.0 (Released 2008; Chicago: SPSS Inc).

Photographs of the holotypes of *C. oxyrhynchus* and *C. plicatilis* from the Museum National d'Histoire Naturelle (MNHN) collection-Paris and a voucher *C. goldiei* specimen from the NMNH/SI were requested and obtained. These photos were used to compare morphological features with the samples in our study. This was carried out by comparing individual shots of the head and medial lobes of the holotype and archived references with those of the "Ludong" samples.

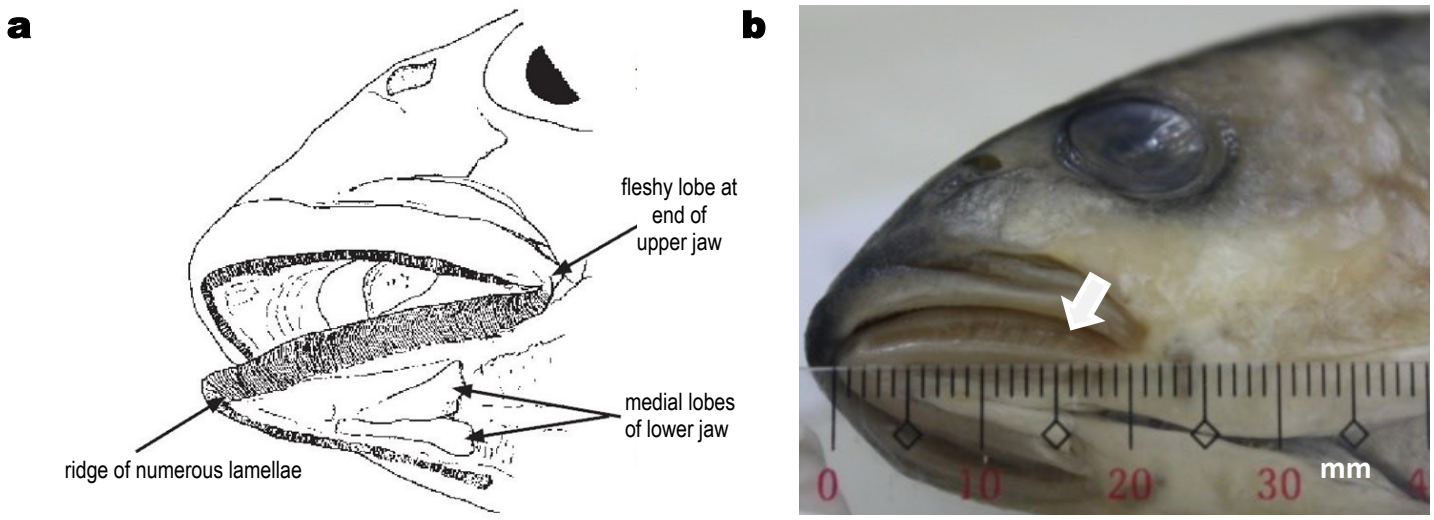

Figure 2. Comparison of **a**, the FAO Guide illustration and **b**, actual "Ludong" sample showing ridge of numerous lamellae bordering the lower jaw and fleshy lobes at posterior end of upper and lower jaws which identify the fish as belonging to the genus *Cestraeus*.

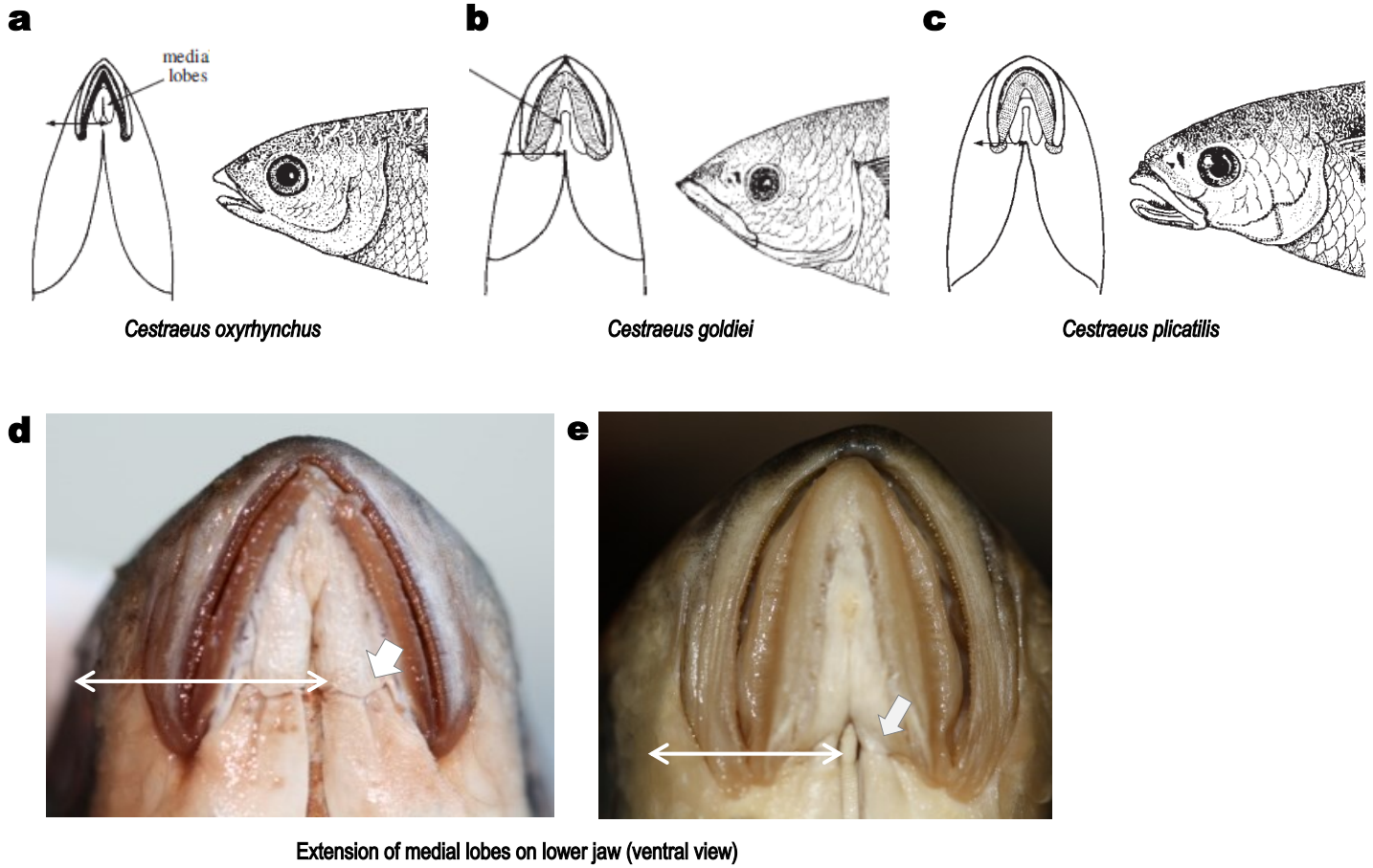

Figure 3. Comparison of the extension of medial lobes in the FAO Guide illustration (**a**-**c**) and of an actual "Ludong" sample (**d** and **e**). Based on the FAO Guide, samples with medial lobes not reaching the end of the mouth were identified as *C. oxyrhynchus* (**a** and **d**) while those with medial lobes reaching the end of the mouth were identified as either *C. goldiei* or *C. plicatilis* (**b**, **c** and **e**).

Table 1. Morphological features and meristic count averages for 5 specimens of *C. oxyrhynchus* and *C. goldiei* each compared with FAO descriptions of *Cestraeus* spp.

Morphology/meristics	FAO Identification Guide Descriptions			*Cestraeus* Samples (Archived)	
	Cestraeus goldiei	*Cestraeus oxyrhynchus*	*Cestraeus plicatilis*	Identified *Cestraeus oxyrhynchus*	Identified *Cestraeus goldiei*
eye diameter vs head length (%)	14-22	22-29	21-25	23.98 (± 0.48)	23.30 (± 0.83)
anal fin spines	3	3	3	2	2
pectoral fin soft rays (free from fin membrane)	>12	4-5	4-5	10 (± 1.44)	9.8 (± 1.46)
ctenoid scales: longtitudinal	36-43	41-47	40-45	44.6 (± 0.6)	44.2 (± 0.66)
ctenoid scales: transverse	12-14	12-14	13-14	12.8 (± 0.37)	12.8 (± 0.48)
ctenoid scales: longtitudinal anterior to origin of 2nd dorsal fin	23-26	26-29	25-26	26.4 (± 0.67)	26.2 (± 0.48)
ctenoid scales: transverse entirely around caudal peduncle	22	22	22-23	21.2 (± 0.58)	20 (± 1.54)
dorsal and caudal fins: uniformly speckled brown and black	no	yes	yes	yes	no
anal fin: pale and speckled brown distally	no	yes	yes	no	no
pectoral fins: weakly speckled	no	yes	no	no	no
pelvic fins: pale	yes	yes	no	yes	yes
pectoral fins: well speckled	no	no	yes	yes	no
lower jaw shape	pointed	pointed	rounded	pointed	pointed
medial lobes extension (vs corner of mouth)	reaching level	not reaching level	reaching level	not reaching level	reaching level
pectoral fin vs standard length (%)	23-30	18-20	19-21	20.90 (± 0.23)	20.66 (± 0.33)
pectoral fin: shorter than head length (% of head length)	no	yes;75-85	83-95	95.69 (± 2.54)	94.08 (± 2.01)
medial lobes ratio	-	-	-	0.68 (± 0.04)	0.74 (± 0.34)

Quantitative values reported as Average (± SE). Ratios are listed as percentages. Qualitative feature "yes" indicates majority of specimens possessed feature.

RESULTS AND DISCUSSION

"Ludong" samples were identified using morphological characteristics based on the dichotomous key for the Mugilidae family published in the FAO Species Identification Guide (edited by Carpenter and Niem 1999). Furthermore, holotype comparisons using pictures from the MNHN collection-Paris and the NMNH/SI were also used.

Based on the presence of the ridge of numerous lamellae on the lower jaw and the fleshy lobes at the posterior end of the upper and lower jaws (Figure 2), initial identification revealed that out of the 34 "Ludong" samples collected, 20 belonged to the genus *Cestraeus*. The other 14 samples were also mullets but of a different species locally known as "Porong" (*Liza* or *Mugil* species).

The characteristic ridge of numerous lamellae bordering the lower jaw is unique to the *Cestraeus* genus and serves to identify the *Cestraeus spp.* from the rest of genera in the Mugilidae family. *Cestraeus spp.* are further differentiated based on the extension of the medial lobes on the lower jaw, whether it reaches the corner of the mouth or not. According to the FAO Guide, those with medial lobes not reaching the corner of mouth are identified as *C. oxyrhynchus* (Figure 3: a and d), while those with medial lobes reaching the corner of the mouth are identified as either *C. goldiei* or *C. plicatilis* (Figure 3: b, c and e). Given these criteria, of the 20 *Cestraeus spp.*, 7 were initially identified as *C. oxyrhynchus* and 13 were identified as *C. goldiei* or *C. plicatilis*.

Morphometric measures and meristic counts were collected from the available 10 archived *Cestraeus* samples. Comparative matrix of the features using FAO Guide descriptions for the *Cestraeus spp.* and the archived *Cestraeus* samples are presented in Table 1. It can be observed that overlap of the measurements among the *Cestraeus spp.* is a common feature that renders the use of morphomeristic data more difficult in differentiating between species. Nevertheless, the data provide evidence for

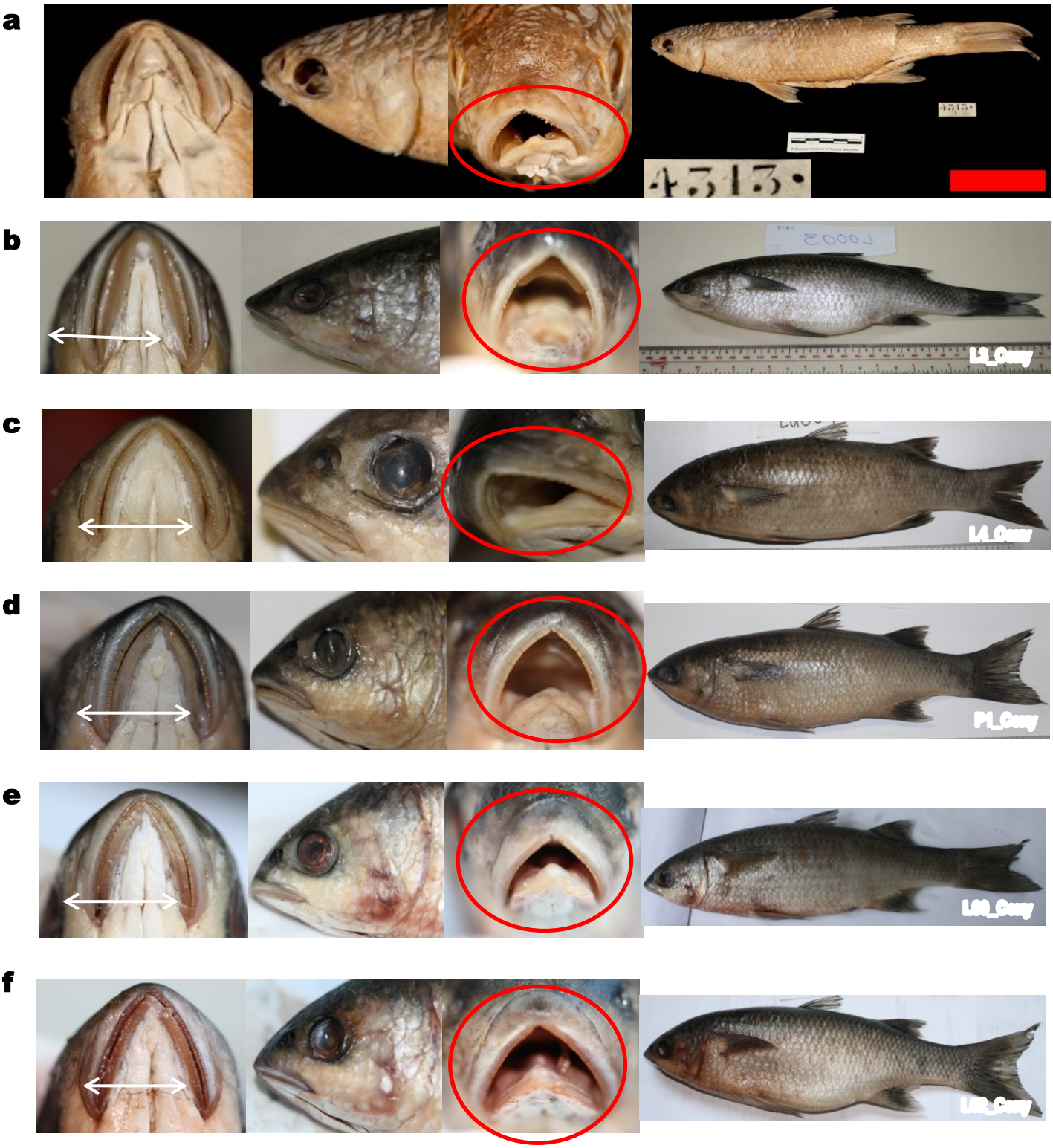

Figure 4. Holotype comparison of the *C. oxyrhynchus* (MNHN A-4313) specimen from MNHN collection-Paris (**a**) with the FAO-identified *C. oxyrhynchus* samples (**b** - **f**). Medial lobes extension (horizontal arrow) and mouth morphology (shape and teeth (red circle)) of samples (**b** - **f**) show similarities with holotype (**a**).

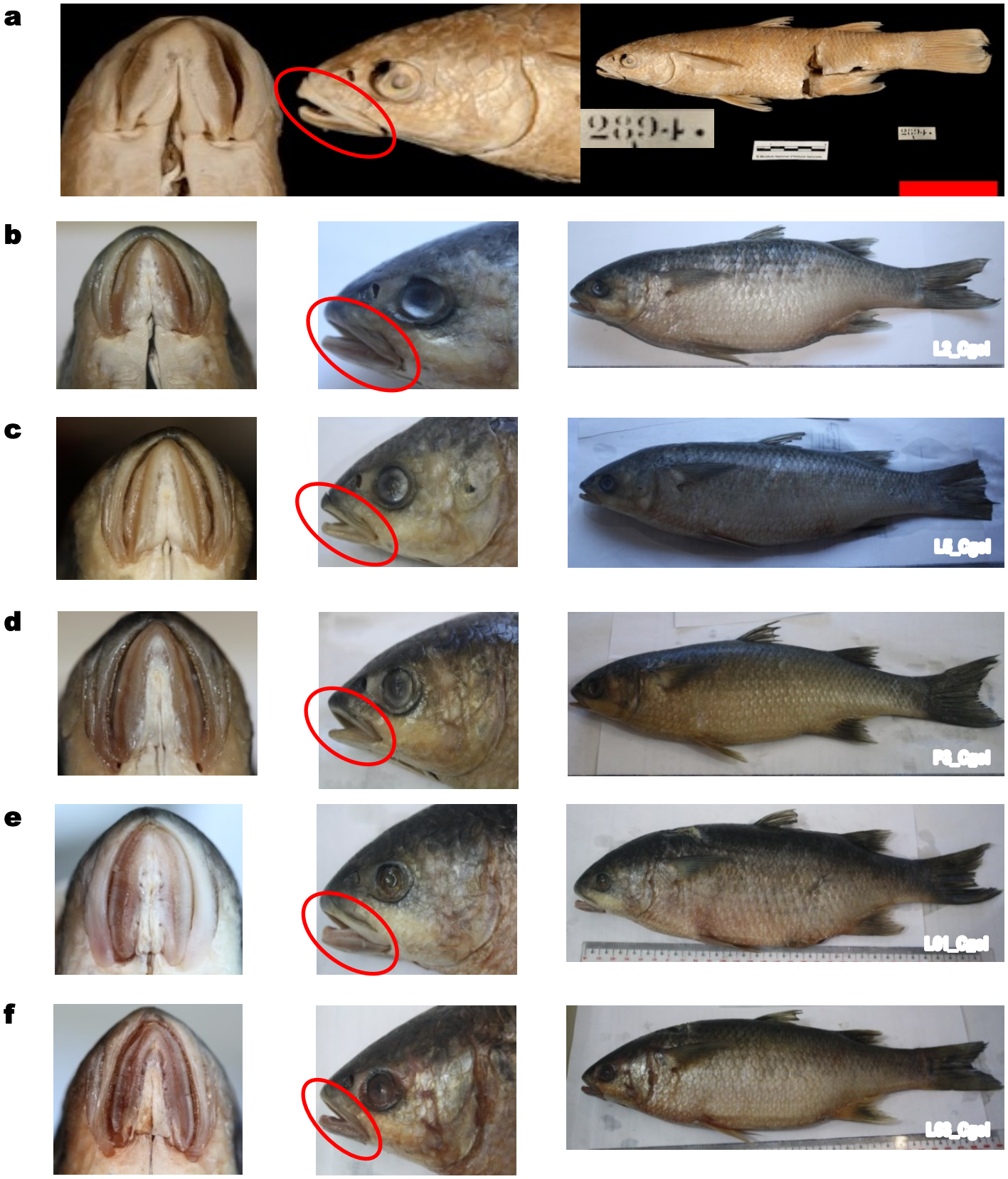

Figure 5. Holotype comparison of the *C. plicatilis* (MNHN A-2894) specimen from MNHN collection-Paris (**a**) with the FAO-identified *C. goldiei* or *C. plicatilis* samples (**b** - **f**). Difference in the shape of the mouth (red circle) of samples (**b** - **f**) with holotype specimen (**a**) makes *C. goldiei* as the more likely identity.

Figure 6. Comparison of FAO-identified *C. goldiei* samples (**b** -**f**) with *C. goldiei* USNM_191860 (**a**) archived in the NMNH/SI, USA. Mouth morphology shows closer resemblance to *C. goldiei* than to *C. plicatilis*.

Table 2. Comparison of the group statistics of *C. oxyrhynchus* and *C. goldiei* using medial lobes measurements.

	FAO guide identification based on medial lobes	N	Mean	Median	Min / Max	Std. Deviation	Std. Error Mean
ratio	*C. oxyrhynchus*	5	0.689757	0.705882	0.5294117 / 0.7857142	0.1054831	0.0471735
	C. goldiei	5	0.743816	0.750000	0.65 / 0.8421052	0.0770658	0.0344649

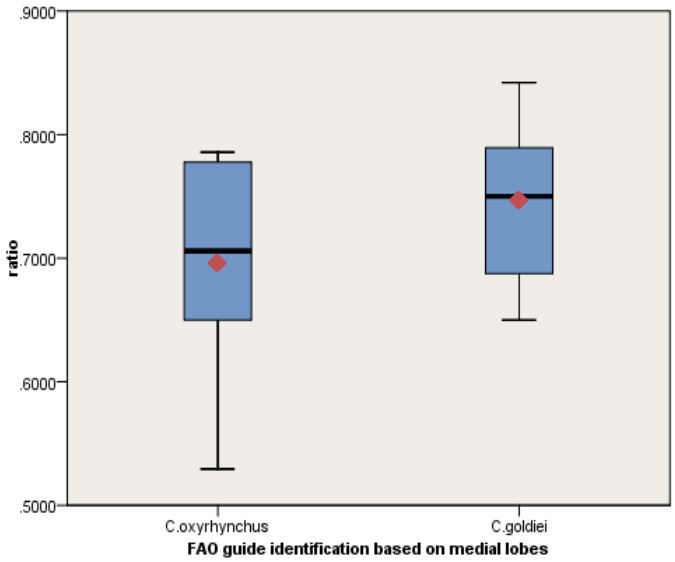

Figure 7. Comparison of the average medial lobes lengths of 5 *C. oxyrhynchus* and 5 *C. goldiei* samples. Box = 25th and 75th quartile; bars = max and min values; heavy bar inside box = median; red diamond = mean.

supporting the identification of two species: *C. oxyrhynchus* and *C. goldiei*. *C. oxyrhynchus* was identified using the differentiating character for this group, which is that the medial lobe extension does not reach the corner of the mouth. *C. goldiei* is more difficult to separate from *C. plicatilis* because of their extensive overlaps. Still, a number of morphometric characters points to this group as *C. goldiei* including: (1) 10 pectoral fins soft rays (free of membrane), (2) dorsal and caudal fins not uniformly speckled brown and black, (3) anal fin not pale and speckled brown distally, (4) pelvic fins pale, (5) pelvic fins not well speckled, and (6) pointed lower jaw shape.

The *C. oxyrhynchus* identification was further confirmed through similarities observed in comparison with the *C. oxyrhynchus* holotype (MNHN A-4313) from MNHN collection-Paris (Figure 4). These observed similarities include the extension of the medial lobes, shape of the mouth, thick-edged lower lip directed forward and upper jaw with an outer row of closed packed teeth (Figure 4: red circle).

The *C. goldiei*/*C. plicatilis* identification was also further refined using comparisons with the available *C. plicatilis* holotype (MNHN A-2894) from MNHN collection-Paris (Figure 5) and the *C. goldiei* specimen from NMNH/SI, USA (Figure 6). Comparison of the *C. plicatilis* holotype with 5 of the 13 samples showed differences in the shape of the mouth, specifically the shape of the upper jaws (Figure 5: red circle). On the other hand, comparison of the *C. goldiei* specimen from the NMNH/SI, USA, with the same 5 samples revealed closer resemblance of the shape of the mouth (Figure 6). This makes the 5 samples more likely to be *C. goldiei* than *C. plicatilis.*

Taking into account the medial lobes as the differentiating character for species identification for the *Cestraeus* genus, comparison of the ratios of the medial lobes in relation to the length of the mouth was further examined. Descriptive statistics (Table 2) and Boxplot (Figure 7) showed that there is observable difference in the mean measurements of the medial lobes between the *C. oxyrhynchus* group and the *C. goldiei* group. This result helps to support the notion that indeed the medial lobe is the distinguishing characteristic that could be used to separate the *Cestraeus spp.*

Employing morphological analysis in the identification of Mugilidae species is not uncommon. Trape et al. (2012) in their recent report showed that morphological characteristics can be used in distinctly identifying the mullet *Liza bandialensis*, and even provided an identification key in identifying mullet species of the Eastern Central Atlantic. Some morphological studies make use of morphomeristic data alone or in combination with morphological features or characteristics. Studies by Ibañez-Aguirre et al. (2006) showed that two populations of *Mugil curema* in the Gulf of Mexico and Pacific Ocean could only be differentiated based on eye diameter and body width. In 2011, Turan et al. published their systematic relationship study of four Mugilidae genera, namely *Mugil, Liza, Chelon* and *Oedalechilus* of the Mediterranean Sea, where they showed that meristic data were more discriminative than morphometrics data. Further, Antovic and Somonovic (2006) showed that morphometric characteristics of visceral skeleton, and dermatocranium and jaw can be used in distinguishing *Mugil* and *Liza* species.

It is not surprising that difficulties in identifying *Cestraeus spp.* were encountered. In fact, the observation is shared with other genera where morphological confusion exists (Semina et al. 2007, Aurelle et al. 2008, Heras et al. 2006). This observation was noted as well by the FAO Guide, wherein it is stated that many species belonging to the Mugilidae family are morphologically similar and even exhibiting conspecificity. For certain mullets, where anatomical differences are weak, it has been reported that the use of genetic analysis, such as the employment of DNA

barcodes, particularly 16S, 12S and CO1, was very helpful in species identification, discrimination of fry samples, identification of commercially important mullets in processed products and phylogeography analysis (Erguden et al. 2010, Durand et al. 2012, Caldara et al. 1996, Imsiridou et al. 2007, Murgia et al. 2002, Livi et al. 2011, Fraga et al. 2007).

Those observations, however, should not undermine the validity of taxonomic identification based on morphological characteristics. The utility of this technique, particularly in the Philippines, is highlighted by the discoveries of new species, such as the report on new species of *Pseudochromidae* from Northern Palawan and Mindoro, wherein the proclamation was based solely on coloration details and meristic data (Gill and Williams 2011), while Pedrosa-Gerasmio et al. (2012) reported the use of liver morphology in discriminating between Yellowfin and Bigeye tunas, which are difficult to differentiate at the juvenile stage. Moreover, morphology, meristics and molecular genetic tools helped in discovering that the widespread applied nomenclature for a sardine species in the country was incorrect and that the previously thought *S. longiceps* was instead *S. lemuru* as reported by Willette and Santos (2013).

In this study, the use of morphological data is highlighted, showing that "Ludong" from Cagayan River is composed of at least two species: *C. oxyrhynchus* and *C. goldiei.* Additionally, our results serve as baseline information regarding the taxonomical identity of "Ludong", which was collectively identified before as *C. plicatilis.* These findings, however, do not necessarily claim that *C. plicatilis* does not exist in the country, but they emphasize the need for further research regarding the species composition of the Cagayan River and its tributaries in which published reports are few and far between (Asis et al. 2013, Herre AWCT 1958). As for "Ludong", the need for increased sample size and the use of other morphological methods and genetic tools is recommended.

CONCLUSION

The "Ludong", an indigenous fish caught in the Cagayan River and its tributaries, is taxonomically identified in this study through the use of morphological analysis. The "Ludong" samples investigated in this study were identified as belonging to the genus *Cestraeus* based on the presence of numerous lamellae on the lower jaw. They were further differentiated into two species, namely *C. oxyrhynchus* and *C. goldiei*, based on the following: (1) extension of the medial lobes on the lower jaw, in which samples with medial lobes not reaching the corner of the mouth were identified as *C. oxyrhynchus*, while those with medial lobes reaching the corner of the mouth were identified as *C. goldiei*, (2) comparison of the "Ludong" samples with holotype (MNHN collection-Paris) and archived (NMNH/SI, USA) samples that showed similarities and differences of mouth morphology, and (3) distribution ranges of *Cestraeus spp.* as reported in the FAO Guide. Our results show that the "Ludong" is composed of at least two species.

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CONFLICT OF INTEREST

None.

CONTRIBUTION OF INDIVIDUAL AUTHORS

Samples were collected by Evelyn Ame. Framing of hypotheses and experimental design, laboratory work, data analysis and interpretation and preparation of the manuscript were contributed by Minerva Ventolero, Billy Catacutan and Mudjekeewis D. Santos.

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