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Skull shape variation in the Armored Sailfin catfish, *Pterygoplichthys disjunctivus* (Siluriformes: Loricariidae), described using geometric morphometrics

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Abstract

The two-dimensional shapes of the skulls of male and female Armored Sailfin Catfish, *Pterygoplichthys disjunctivus*, were reconstructed in this study using Thin-Plate Spline (TPS) grids. The rationale for doing this is to draw out hypotheses regarding the life history and population dynamics of the Armored Sailfin Fish, which is becoming a nuisance in some freshwater environs. Because of the skull's rigid structure, it offered many stable landmark points that are compatible with geometric morphometric computations and modeling. Through Relative Warp Analysis of forty-nine homologous points, it was observed that the skulls in both sexes differ widely in the length-width aspect ratio (RWI: 38%). The skulls were also asymmetrical with regard to the anterior latero-ethmoidium side of the structure based on analyses of five other relative warps that had a cumulative variance of 38.29% (RW2 to RW6). Because these variations are localized around regions associated with feeding structures, it does not escape our minds that the fishes might differ in their individual feeding habits.

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Introduction

The use of landmark-based Geometric Morphometrics (GM) in the analysis of biological shape is steadily increasing in the scientific literature. This is because the method allows for the description of rigid structures using sets of variables that can be used for statistical hypothesis testing, and to generate graphical representation of shape differences as deformations (Adams, 1999; Rolf *et al.,* 2004). Unlike traditional Morphometrics, GM captures shape nuances using the Cartesian coordinates of landmark points that are assigned to distinct edges, sides, and joints of biological structures. While GM is perceived to be more powerful than its traditional counterpart, however, it can only provide meaningful results if it is fed with morphologically and developmentally equivalent landmarks.

In fishes, the truss network is often used in GM analyses. While the truss is particularly useful as source of landmarks, its use might be challenging for some species such as the Armored Sailfin Fish, *Pterygoplichthys disjunctivus*, whose three-dimensional topology differs widely from other fishes. To transcend beyond this limitation, some studies used alternative parts of the organisms such as the fins, eyes, and skeleton. In the case of the Armored Sailfin Fish, its skull presents rigid spaces and sharp ends that can be assigned homologous landmark points for GM analysis.

In cognizant of the above, this study was conducted to explore utility of the skull as source of landmark points for GM analysis and to draw out hypotheses regarding a population of Armored Sailfin fishes from Iligan City (Jumawan *et al.,* 2010). This fish was chosen in this research because aside from its reputation as being invasive and destructive to freshwater environs in the country, its true taxonomic nature is also sometimes confusing. In fact, in the Philippines, Armored Sailfin fishes are said to belong to either *P. disjunctivus* or *P. pardalis*, depending on the pattern of their body stripes. To address underlying assumptions of sexual dimorphism, males and females were treated separately in the analyses.

Materials and methods

Collection of specimens

A total of thirty-one fish (18 males and 13 females) samples were collected from a fresh water stream in Iligan City, Lanao del Norte. With the help of locales, a total of thirty-one Armored Sailfin Fishes were captured using fishnets, placed in glass containers, and fixed in 10% formalin solution.

Nomenclature and classification

The fishes were examined for diagnostic characters and placed in their taxonomic groups. External morphological features of the samples showed large white spots in the abdomen that were irregularly joined to form a vermiculate pattern. Based from this findings, the fishes were identified as *P. disjunctivus* (Table 1).

Preparation of the skulls

Digital images of the fishes were taken against the backdrop of a ruler for standardization purposes (Fig. 1). Taking the pictures with a ruler was necessary to allow the possibility of taking morphometric measurements of the fishes using an online platform. Scalpels and dissecting needles were then used to separate the skulls from the bodies of the fishes. The skulls were fixed in 10% formalin and later washed with Hydrogen Peroxide.

Landmark selection and placement

Forty-nine landmark points from distinct edges, intersections, and points were manually selected from the Armored Sailfin skull using tpsDig2 ver2 (Rohlf, 2004) (Fig. 2; Table 2). Upon locating landmark points, the tpsDig2 software automatically determined the Cartesian coordinates (X and Y variables) that were used in the statistical analysis (Bookstein, 1991; Rohlf and Marcus, 1993; Dryden and Mardia, 1998).

Landmark-based Geometric Morphometric Analyses

The Cartesian coordinates (X & Y values) were subjected to the landmark-based relative warp analysis using the software tpsRelw program version 1.46 (Bookstein, 1998; Rohlf and Marcus, 1993; Adams *et al.*, 2004; Slice, 2005; Rohlf, 2008).

Subjecting the Cartesian coordinates to this analysis returned relative warp scores that were used as morphometric variable in this study. These relative warp scores were summarized as histograms using the Paleontological Statistics (PAST) ver.2.16 software (Hammer *et al*., 2001). For purposes of identifying the most important relative warps, only the axes contributing greater than five percent variance were reported.

Results and discussion

Results of the relative warp analyses provide evidence to the wide latitude of variation in the shapes of the skulls within and between male and female fish samples. Among the relative warps, the first two contributed more to the variance in both sexes.

Table 1. Diagnostic characters used to distinguish the two morphologically indistinguishable species of *Pterygoplichthysis* spp. in Philippines.

P. disjunctivus	*P. pardalis*	Collected specimens
a. Body covered with flexible bony plates	a. Body covered with flexible bony plates	a. Body covered with flexible bony plates
b. Ventral sucking mouth	b. Ventral sucking mouth	b. Ventral sucking mouth
c. Abdomen covered with large spots irregularly joined to form a vermiculate pattern	c. Abdomen covered with large white spots	c. Abdomen covered with large white spots irregularly joined to form a vermiculate pattern
d. 9 to 14 dorsal fin rays with a single spine	d. 9 to 14 dorsal fin rays with a single spine	d. 9 to 14 dorsal fin rays with a single spine
e. Pectoral fins with thick toothed spines	e. Pectoral fins with thick toothed spines	e. Pectoral fins with thick toothed spines

Table 2. Landmark points and their locations in the skull of the *Pterygoplichthys disjunctivus*.

Landmark point	Description
1,2,3,4	Os mesethmoideum
5,6,7,8,9	Left os latero-ethmoideum
33 34,35 36,37	Right os latero-ethmoideum
10,11,12	Left os metapterygoideum
30,31,32	Right os metapterygoideum
13,14	Left os praeoperculare
28,29	Right os praeoperculare
15,16,17,18	Left os operculare
24,25,26,27	Right os operculare
19,20	Left compound pterotic bone
22,23	Right compound pterotic bone
21	Os parieto-supraoccipitale
38,39,40,41,42,43	Left eye
44,45,46,47,48,49	Right eye

As depicted in the Thin-plate spline grids for the first warp, the males posses larger os operculare compared to their female counterparts. The same warp shows differences in the Os mesethmoideum or sucker mouth of the fish samples (Suazo *et. al*. 2008).

The second relative warp, on the other hand, point to asymmetric differences in both anterior and posterior regions of the skull. The rest of the relative warps are described in Table 3 and 4.

Table 3. Patterns of skull shape variation among male *Pterygoplichthys disjunctivus* as described by the first six Relative Warps.

RW	Variance	Negative	Positive
1	38.03%	-shorter length and wider os mesethmoideum -narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect
2	11.31%	-Longer length and narrow os mesethmoideum -More prominent curvature on and shorter in length of os operculare -Narrower in shape -Asymmetric in the anterior aspect	-Shorter length and wider os mesethmoideum -Broader in shape -Asymmetric in anterior and posterior aspect
3	7.98%	-Shorter length and wider os mesethmoideum -Narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect
4	7.27%	-Shorter length and wider os mesethmoideum -Narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect
5	6.28%	-Shorter length and wider os mesethmoideum -Narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect
6	5.45%	-Shorter length and wider os mesethmoideum -Narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect

Table 4. Patterns of skull shape variation among female *Pterygoplichthys disjunctivus* as described by the first six Relative Warps.

RW	Variance	Negative	Positive
1	34.68%	-Shorter length and wider os mesethmoideum -Narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect
2	17.41%	-Longer length and narrow os mesethmoideum -More prominent curvature on and shorter in length of os operculare -Narrower in shape -Asymmetric in the anterior aspect	-Shorter length and wider os mesethmoideum -Less prominent curvature and shorter in length of os operculare -Broader in shape
3	11.32%	-Shorter length and wider os mesethmoideum -Narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect
4	9.00%	-Shorter length and wider os mesethmoideum -Narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect
5	6.42%	-Shorter length and wider os mesethmoideum -Narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect
6	5.72%	-Shorter length and wider os mesethmoideum -Narrower in shape -More prominent curvature on and shorter in length of os operculare -Prominent anterior aspect	-Longer length and narrow os mesethmoideum -Broader in shape -Less prominent curvature and shorter in length of os operculare -Prominent posterior aspect

While there are noticeable variations in the shapes of the skulls, it can also be observed that the sexes share one common thing – that is, the left and the right sides of the skull differ in shape. The phenomenon of asymmetry between the left and right sides of biological structures has already gained attention in scientific literature. In fact, studies have suggested that asymmetry can be interpreted in various ways.

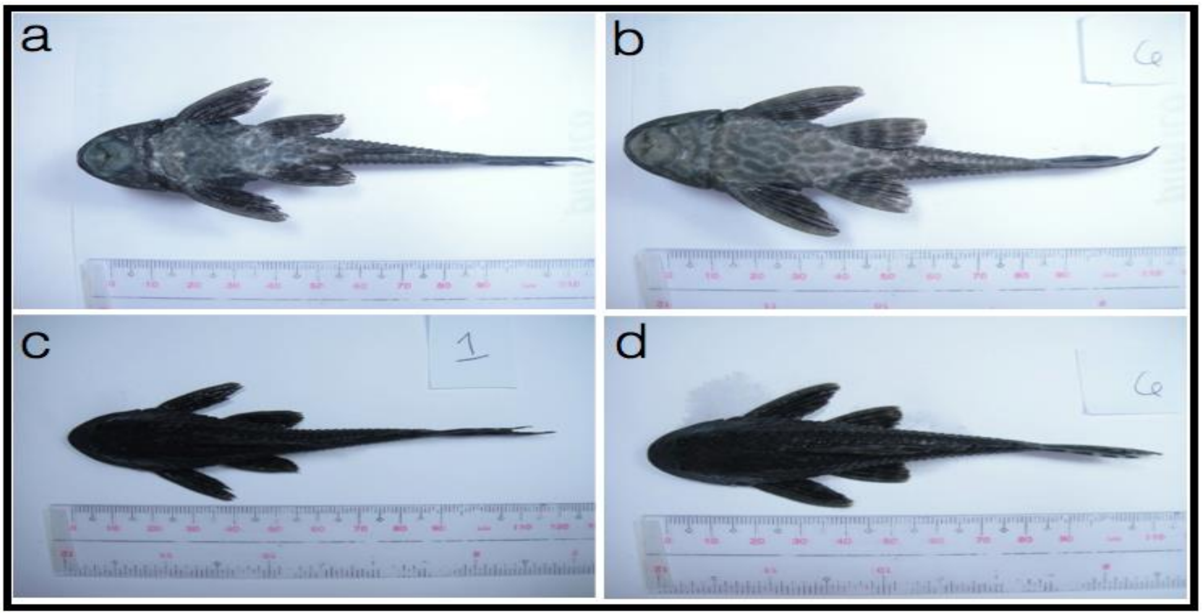

Fig. 1. Representative images of the *Pterygoplichthys disjunctivus*: ventral view of one (a) male and one (b) female; dorsal view of one (c) male and one (d) female.

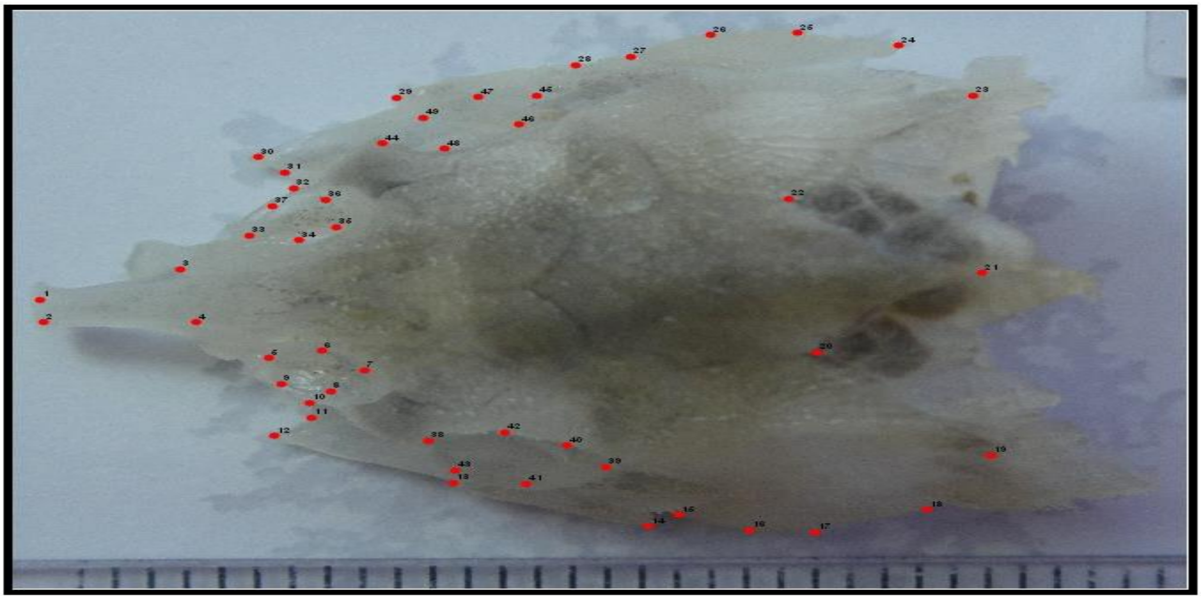

Fig. 2. Locations of the landmark points collected from the skull of the *Pterygoplichthys disjunctivus*.

Asymmetry can be directional if one side is consistently different than the other side. Asymmetry can also be fluctuating if neither the left nor the right side of the structure is consistently different. In the case of fluctuating asymmetry, differences in the shapes of the left and right sides of biological structures are said to emanate because of developmental noises or stressors in the ontogeny of individuals.

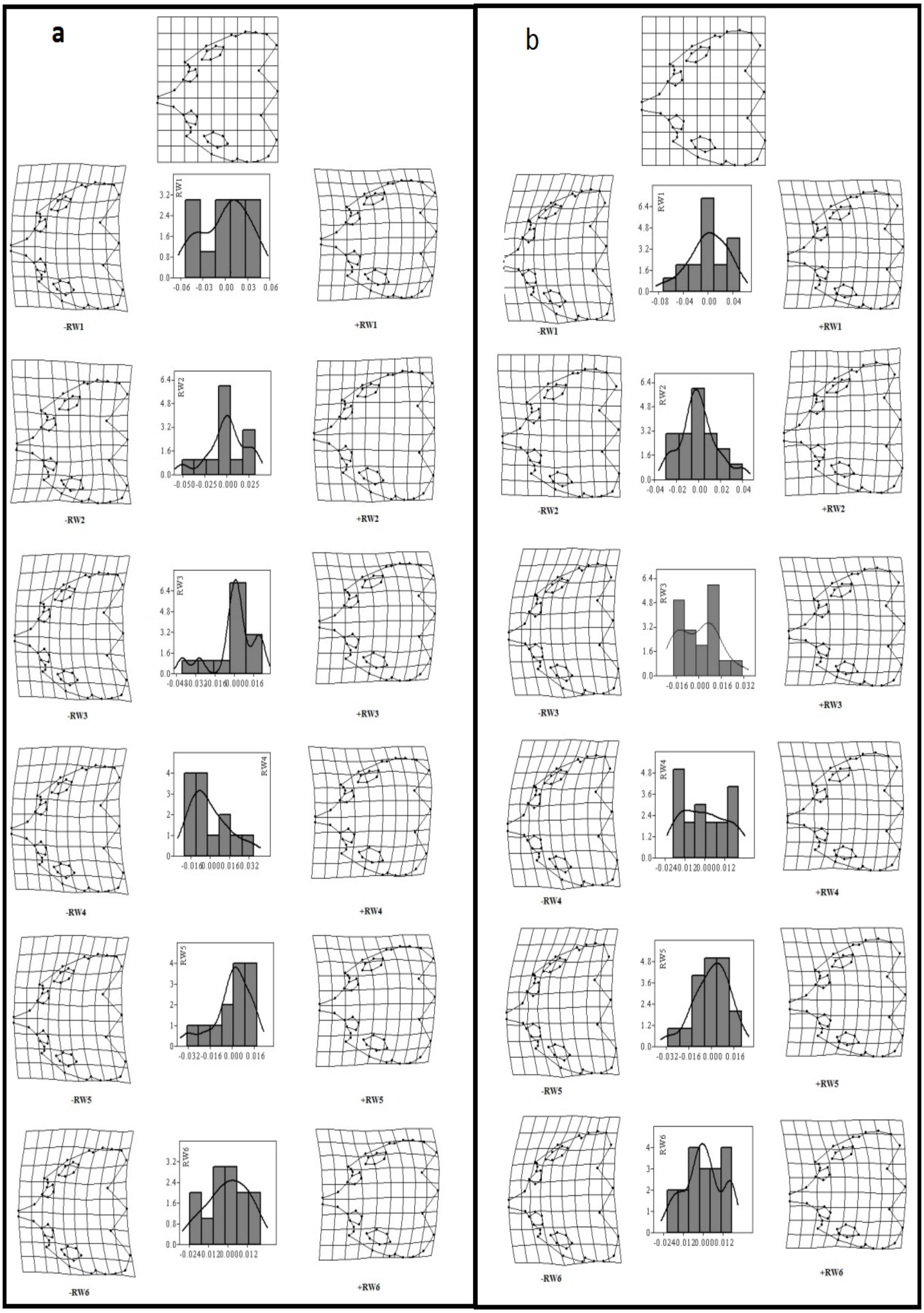

Fig. 3. Thin-plate spline transformation grids locating sources of variation in the shape of the skull in *Pterygoplichthys disjunctivus*: a) male (b) female.

To put things into a simple perspective, data from scientific literature suggests that there is an association between levels of fluctuating asymmetry and the health of populations of organisms (Jumawan *et al*., 2016). In the case of fishes, studies have shown that fluctuating asymmetry in populations of organisms is indicative of the presence of stressors, which can either be endogenous or exogenous in nature. Endogenous stressors can result from inbreeding depression or lack of genetic variation in populations of fishes (Jumawan *et al*., 2016). This happens when individual organisms are only able to mate with genetically similar individuals resulting to the expression of rare recessive genes coding for disorders.

In this study, inbreeding depression within the population of janitor fishes can be attributed to mating among genetically similar founder individuals introduced into the stream. Interviews among the locales suggest that the fishes either came from ponds of enthusiasts or from the aquariums of pet owners.

Conclusion

This study shows the utility of the skull in the morphometric analyses of shape variation in *Pterygoplichthys disjunctivus*. The presence of edges, points of articulation, and distinctive structures makes the skull an important object for Geometric Morphometic (GM) Analysis. Aside from quantifying shape variation, GM effectively located nuances in the shapes between the male and female catfishes as shown in the Thin-Plate spline grids. By applying multivariate statistics, it was also possible to determine patterns of inter-individual variation. The method of GM also was able to describe dissimilarities between the left and right sides of the skull, which is now becoming a prominent feature of biological structures. To provide deeper understanding of the factors responsible for the observe shape variation, further studies can be directed towards differentiating more than one population. There can also be platforms to test for the genetic bases of the observed shape variation. Other aspects of the skull can also be explored to gain appreciation of the nature of populations of the Armored Sailfin Catfishes.

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