A reply to a comment on Brito et al., 2024, A Saharan fossil and the dawn of the Neotropical Armoured catfishes in Gondwana by Britz, Pinion, Kubicek and Conway

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Afrocascudo saharaensis Brito et al., 2024 is an armoured actinopterygian fish from the early Late Cretaceous (Cenomanian, ~100–95 million years) Jbel Oum Tkout locality (OT1) of Morocco. The fossil is known by a single specimen, fragmented in three pieces that have been prepared using resin transfer and removal of the clayey matrix. Optical microscopy observations and state-of-the-art imaging techniques including synchrotron X-ray tomography allowed Brito et al. (2024) to recognise a number of synapomorphies unequivocally assigning the fossil to Loricarioidei and Loricariidae catfishes, a result further supported by phylogenetic analyses. Britz et al. (2024) question several of Brito et al.'s anatomical interpretations and argue that the fossil *A. saharaensis* is not a loricariid or even a teleost but an holostean. Here, we show how their arguments are based on erroneous anatomical reinterpretations of some of Brito et al.'s data. Therefore, and this is herein reinforced, *A. saharaensis* still stands as the earliest known occurrence of an armoured catfish, and more generally of a catfish, also providing evidence that loricarioids have diversified before the separation of Africa and South America.

By predating the predicted origin of Loricariidae to the “Mid"Cretaceous and revealing that this iconic group of freshwater catfishes, considered to have originated and be endemic of the Neotropical region (South and Central America), actually has an African rather than South American origin (Brito et al. 2024), *A. saharaensis* significantly changes our understanding of their evolutionary history. It was anticipated that such a discovery would rise controversy, especially among some fish neontologists. Britz et al. immediatly refuted these conclusions, without examining the specimen themselves, by challenging the fidelity of the artistic reconstruction, the structuring and associated initial hypothesis of the paper, and the accuracy of the 3D segmentation and orientation of the caudal fin skeleton. Based on reinterpretations of these features, Britz et al. reassess *A. saharaensis* as a juvenile lepisosteiform, most likely of the fossil gar genus *Obaichthys* Wenz and Brito, 1992.

First of all, Britz et al. object, among other things, regarding our inclusion of an anal fin and the position of the dorsal fin in our drawing and forget that these were included merely to give shape to a fossil that is fragmented into three distinct parts. We note here that in our text, we make it clear that the anal fin is not preserved (although here we acknowledge the error in Fig. S5a) and that only the impression of four rays in the resin were detected. Briefly our drawning was simply an artistic reconstruction to adorn the cladogram.

Secondly, Britz et al. criticize the way the article was put together, starting with the fact that, in our introduction, we stated that the structure of the caudal fin clearly indicates that it is a teleost fish and insinuating that the original description of *Afrocascudo* was given with an *a priori* body plan of Loricariidae in mind. Based on this argument, and stating that the posterior part of the specimen would have been depicted upside down, they propose reinterpretations of some cranial bones, the unpaired fins, the caudal endoskeleton and the dermal plates on the flank of the body, leading to the conclusion that the fossil in question would be instead a juvenile specimen of the gar *Obaichthys* Wenz and Brito, 1992, as gars also occurs in locations in North Gondwana, including the Douira Formation of the Kem Kem Group (that they call Kem Kem Formation), and the overlying Asfla Member of the Turonian Akrabou Formation (Cooper et al., 2023). We fully understand the difficulties that non-paleontologists can face in interpreting fossils, especially fossils that were flattened and broken during fossilization, excavation and preparation. When dealing with material exhibiting such preservation, a key step is to examine all other taxa found in the locality. The first thing that caught our attention in the *Afrocascudo* specimen was the occurrence of odontodes on the skull, which reminded us of the obaichthyid species *Dentilepisosteus laevis* (Wenz and Brito, 1992) (see an osteology of the skull in Grande, 2010 and Brito et al., 2016). We also considered the polypteriformes from the Kem Kem group (see Dutheil, 1999). These initial examinations led us to perform a histological analysis using thin sections of both cranial and dermal plates of *A. saharaensis*. The observed histological features, which have been entirely overlooked by Britz et al., themselves refutes their taxonomic assignment to holosteans. The presence of osteocytes with branched cellular extensions that are extremely well preserved (Brito et al., 2024, fig. S6) and the absence of Williamson's canaliculi, an important holostean character (Thomson and McCune, 1984) undoubtedly identify the bone of *A. saharaensis* as non-holostean bone. In any case, if the specimen was an obaichthyid gar and considering its degree of ossification, it would possess the respective layers of ganoine and dentine on its "scales" (see Brito et al., 2000) as ganoine scales appear for the first time on the the flanks between the dorsal-anal fins and the caudal fin area (Grande, 2010, Scherrer et al., 2017).

Both the dorsal and lateral surfaces of the head and body of *A. saharaensis* are entirely covered by bony plates coated by numerous odontodes which, as we saw above, do not exhibit any holostean characters. Britz et al. also reinterpreted odontodes described by Brito et al. (2024) on the dorsal fin as fringing fulcra. It must be, however, considered that fringing fulcra are defined as paired structures associated with the leading rays of paired and unpaired fins (Arratia, 2008). In the specific case of the structures anterior to the dorsal fin spine in *A. saharaensis*, they present a random distribution, often unpaired, which leads us to describe them as odontodes, a rationale that also applies for the upper caudal ray (Brito et al., 2024, fig. 2d). Moreover, Britz et al. attempt to compare the pointed median projection that ends in a spine-shaped posterior projection in *Obaichthys* with the odontodes found in the post-cranial plates of *A. saharaensis*. These structures are completely different as in *Obaichthys* the median projections resemble a keel that forms part of the body of the scale, covered by ganoine, and extending almost through its entire length (see Grande, 2010, figs. 479b, 480a,b). In contrast, the odontodes of *A. saharaensis*, even the posterior and most protruding ones in the series, are conical structures constituted by enamel surrounding a central pulp cavity that communicate with the vascular network of the bony plate (Sire and Meunier, 1993; Brito et al., 2024, fig. S6).

Thirdly, with these points clarified and the certainty that *A. saharaensis* is not an obaichthyid, we hereby address the criticism regarding the suggested dorsoventral inversion of the caudal fin skeleton. As explicitly stated in the text, a dorsal fin spine, preceded by its anterior spinelet and presenting a clear connecting bone is present and clearly visible in *A. saharaensis* (Brito et al., 2024, fig. 1e). In all gars, including the obaichthyids, all principal rays of the median fins, even the first one, are segmented and branched (Grande, 2010), which is definitely not the case with the dorsal fin of *A. saharaensis.*

Fourthly, Britz et al. were not able to reproduce the 3D rendering of the caudal endoskeleton of *A. saharaensis* presented in Brito et al.'s figs. 2e and S4 a,b using the data (tomographic slices) made accessible alongside the original publication. Therefore, they doubt the accuracy of our interpretations regarding the fusion of hypural plates 3, 4 and 5, the fusion of parhypural and hypural plates 1 and 2, and the neural spine of second preural centrum (respectively hy3-5, ph + hy (1 + 2), and nsPu2 in Brito et al. 2024, fig. 2e and S4c, d). We concur with Britz et al. that the remains are heavily compressed and extremely thin in this region, and that density varies only very slightly between the different preserved bones. In line with Britz et al.'s second criticism, we also concur that due to the fact that these features are not easily distinguished from each other we should probably have described the observed morphology (i.e. two rectangular bony complexes anterior to the caudal fin, the dorsal one in contact anteriorly with a trapezoidal bone) before interpreting them anatomically. However, it is essential to emphasize that these features are internal. Consequently, Britz et al. were unable to visualize them by only producing a quick surface rendering, as depicted in their figure 1b. This surface image, depending on the position in which the fossil is positioned for observation, shows varied impressions. In their surface rendering, which they present dorsoventrally inverted (see discussion above), Britz et al. pointed out the presence of possible hypurals, features that we instead interpret as the internal view of dermal plates and flattened fin rays. In contrast to Britz et al., our approach involved days of meticulous observations of the tomographic slices under various reslicing angles. We focused not only on density contrasts but, more importantly, on bone margins and contacts, as well as on the position of microstructural details (well visible owing to our high-resolution and high-contrast synchrotron data), which allowed us to segment the more accurately possible these features. To substantiate our segmentation and demonstrate that we did not arbitrarily draw the bones, as alleged by Britz et al., we have shared our dataset and presented a couple tomographic slices in our fig. S4c,d. These slices clearly show that these features are indeed present in *A. saharaensis* and can be seen in the data. The outline of these features is, nevertheless, not easily discernible, in most individual tomograms, but they become distinctly apparent when several tomograms are combined into intensity projections (Fig. 2). The anatomical identification of these remains may be subject to debate, but their existence is unquestionable.

Finally, we must make it clear that the scientific foundation for organisms identification and classification (systematics) relies on the presence of derived features shared within a group of organisms (i.e. synapomorphies) to hypothesize phylogenetic relationships. This scientific approach was followed in Brito et al., pointing out a number of synapomorphies shared between *A. saharaensis* and Loricarioidei and Loricariidae, which were statistically supported through a phylogenetic analysis (even with the small error in character 2 where *Danio* was coded with state 0 and not state 1). It is important to remember that the phylogenetic position outlined in this study is a hypothesis of relationships and that, like any scientific hypotheses, might one day be refuted based on new evidence, but it cannot be refuted *a priori* without such evidence being presented. Britz et al. did not follow this procedure, and instead propose a change in the taxonomic status of the new species based on simplistic comparisons without testing any hypothesis. In sum we think the argument presented above, starting with histology, allows us to emphasize that *A. saharaensis* is not an obaichthyid, and remains the earliest known occurrence of an armoured catfish, and more generally of a catfish with significant implications for our understanding of the early evolutionary history and geographic origin of Loricariidae.

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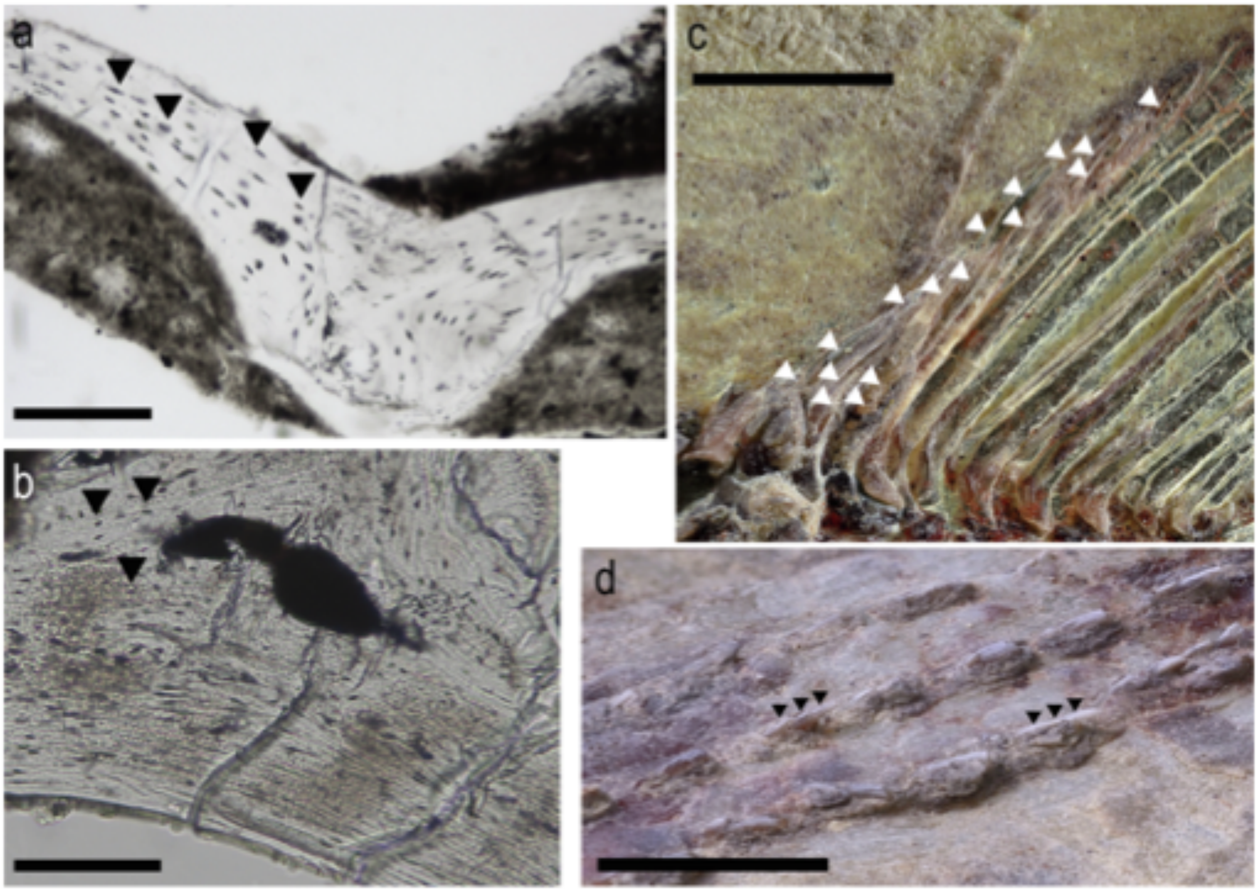

Figure 1. *Afrocascudo saharaensis*, holotype MHNM-KK-OT 36. a, b, transversal ground section of a bone basal plate showing osteocytes (arrow heads); Note the total absence of canalicules of Williamson. c, close-up of posterior plates (arrow heads point to odontodes), d, detail of dorsal fin (arrow heads point to odontodes). Scale bars, 100 µm (a), 50 µm (b), 5 mm(c), 5 mm(d).

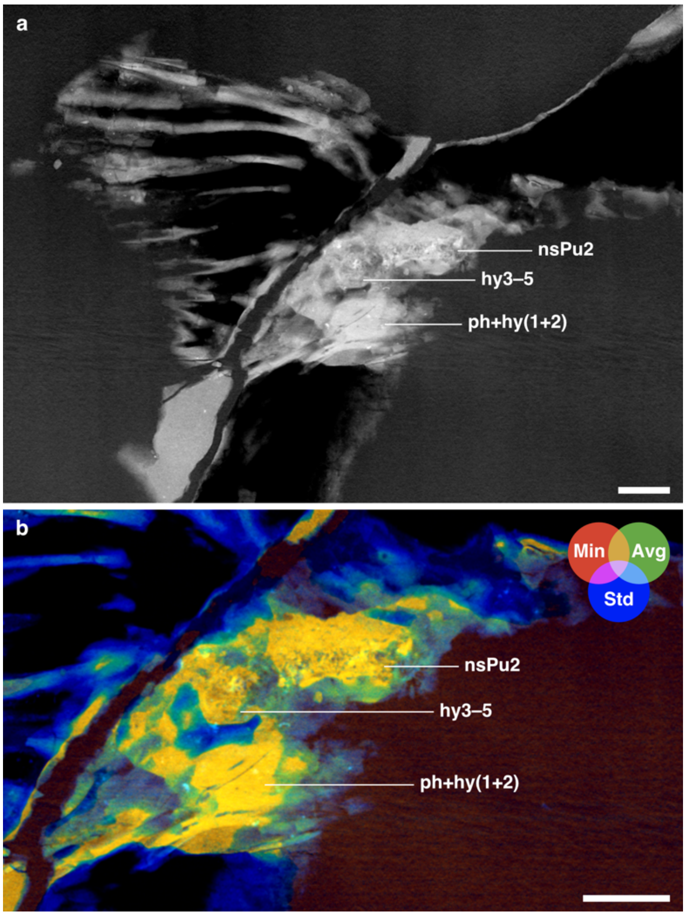

Figure 2. Intensity projections of 10 sagittal tomograms through the caudal region of *Afrocascudo saharaensis*, holotype MHNM-KK-OT 36c, produced from the synchrotron X-ray microtomography dataset shared in Brito et al. a, Average intensity projection. b, False-color overlay of the minimum (Min; red), average (Avg; green) and standard deviation (Std; blue) intensity projections for the “hypurals/ parahypural/ neural spine” region. These images were generated in ImageJ using the “Reslice [/]…” and “Z project…” functions to display the sagittal tomograms (the dataset was shared with frontal tomograms) and to compute the intensity projections, respectively. Sagittal tomograms 96 to 105 were utilized. Abbreviations: hy3–5, fusion of hypural plates 3, 4 and 5 (plus uroneural and epural); nsPu2, neural spine of second pretrial centrum; ph+hy(1+2), fusion of parhypural and hypural plates 1 and 2. Scale bars 1 mm.