Florida Museum Undergraduate Internship Progress Report

Florida Museum Fish Collection

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With Dr. Larry Page

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Project Overview, Skills Gained

Originally, my internship under the Florida Museum Fish Collection began with the sorting and revising of certain collections currently being studied by Dr. Page, to assist with the eventual description of the species in his identification and distribution guide of freshwater fishes of a river basin in Thailand. Most notably, I worked with the *Mastacembelidae*, or the freshwater spiny eels, with the objective to more accurately sort what we presumed to be two species of the spiny eels within Thailand's river systems that, due to similarities in both morphology and coloration, could be easily confused: *Mastacembelus tinwini* (Figure 1.1) and *Mastacembelus favus* (Figure 1.2).

As is the case when sorting any organisms, when sorting fishes using variation in morphology (form and structure) and color or pattern, one has to take into account numerous factors to ensure that intraspecific variation between individuals and populations isn't confused with interspecific variation of closely related or similar species. In collections of fishes, there are often very large variations between individuals of a given species due to the event of color loss that often rapidly occurs between when the fish are caught and euthanized for pickling. This effect could be seen prominently in the Florida Museum's collection of *Mastacembelidae*, which often appear with warm tones of yellow and orange when living, and are preserved with much darker, more muted tones and at times even a loss of patterning in the collections. This muting of color can be seen in Figure 1.3. One also has to take into account the possibility of sexual dimorphism, or differences of morphology between different sexes of the same species. This phenomenon along with differences between different populations of a given species and other numerous (and,

sometimes, unknown) causes of variation undoubtedly occur, but given the understudied nature of the genus in the region of Thailand, only certain factors can be consistently accounted for.

My work with the *mastacembelids* in Thailand taught me the basic foundational knowledge of the lab setting and materials, along with fish morphology, diversity, and anatomy I needed to move to my own project. My current research seeks to taxonomically revise and describe freshwater species of fish found in Thailand in the genus *Brachirus*, a clade of flatfish (*Soleidae*). Thus far, I have identified and begun to describe a new species, *Brachirus cf. pan* (Figure 2.1) within the genus that was originally thought to be *Brachirus pan,* which is present in southern Malaysia, the islands of Indonesia west of Thailand, and the Ganges of India (Lapierre, 2007). However, due to geographical and oceanic barriers separating the river systems of Thailand from the Indian Ganges and the islands of Indonesia, respectively, Dr. Page and I determined that the Museum's specimens were likely to be allopatrically speciated from the populations described as *B. pan* by Lapierre (2007). As seen later in this report, further inspection of the case via the collection of meristic data (Figures 2.3, 2.4) supports this hypothesis.

Summary of Activities

Throughout the last several weeks, I have collected meristic data on nearly all of the specimens in the Florida Museum's collections that were originally thought to be *Brachirus pan.* Certain counts are unattainable without the use of CT scanning, including vertebral counts. However, adequate diagnoses could be made by counting dorsal fin rays, anal fin rays, and caudal fin rays, which protrude from the body and can be counted reliably by shining light through the specimen from the bottom of the microscope stage, thus illuminating the bones inside the fish's thin tissue (Figure 2.2). Lateral line scale counts, or counts of the number of scales along the fishes' lateral lines (Figure 2.3), also served to be a useful diagnostic tool. Firstly, the fish were determined immediately to have been within the genus *Brachirus,* rather than the closely related genus *Achiroides,* due to the absence of a distinct, black mark on the blind side (the side on which there are no eyes present) that is consistently visible on specimens of *Achiroides'* mouths (Figure 2.8), even following preservation in ethanol. Secondly, after counting their range of lateral line scales (55-68), the specimens were determined not to be *Brachirus panoides* (82-121), *Brachirus siamensis* (70-90), *Brachirus foliacea* (63-83), or *Brachirus aspilos* (76-117). However, the specimens' counts fell within the range of *Brachirus pan* (56-79). I then counted their range of dorsal fin rays (47-57), a characteristic shown in Figure 2.4. Its range overlaps with that of *Brachirus pan* (53-61) but its distribution shows a clear bimodality (Figure 2.5). Figure 2.6 also demonstrates a bimodal distribution of the overlapping ranges of caudal fin ray counts of *Brachirus pan* (13-15) and *Brachirus cf. pan* (13-17). The presence of these bimodal distributions, along with the geographical barriers that separate the Florida Museum's specimens

from the specimens used by Lapierre to describe *Brachirus pan,* supports the hypothesized presence of an undescribed species, *Brachirus cf. pan.*

Next Steps, Changes

To adequately describe this new species taxonomically, I must account for several factors and protocols throughout the duration of my research with *Brachirus cf. pan.* Firstly, I plan to finish all other external meristic counts of the fish (such as anal fin ray counts and pelvic fin ray counts), followed by the commencement of my collection of data for selecting a holotype (providing a unique "type" description with scaled anatomical ratios of the new species). The measurements used to collect this data can be seen in Figure 2.7. Though many of these measurements may not significantly differ from those of closely related or similar species such as *Brachirus pan,* they are nonetheless essential to include when describing the species for future identification and experimental replication purposes.

Additional counts that, as previously mentioned, can only be done following CT scanning of the specimens, which will also take place in the future. Moreover, specimens preserved in ethanol cannot be used for genetic sampling and analysis, but tissue samples (small samples containing genetic material, such as pieces clipped from a fin) were taken from certain specimens before being preserved for future genetic analysis. Following genetic analysis of mitochondrial DNA of these specimens' tissues, one can generate phylogenetic trees (exemplified by Figure 1.4) and draw conclusions about the relation of different specimens to one another. This process must be done in a genetics lab, and will certainly take place before I publish a full taxonomic description of the new species, to provide comparative proof of genetic differences between *Brachirus cf. pan* and *Brachirus pan,* as well as other closely related or similar species whose genetic data are available.

Since starting my internship, I have attended one fieldwork excursion for experiential purposes with Adania Flemming and her team, who study *Fundulus escambiae,* or the russetfin topminnow. Despite this, there have thus far been no opportunities for me to participate in fieldwork relevant to my research, given its localization in Thailand and Southeast Asia. However, Dr. Page and I have talked extensively about my potential participation in his upcoming trip to a conference of the Asian Society of Ichthyologists in Malaysia, followed by a fieldwork excursion in Thailand. Should I attend this trip in May and June, as I plan to do, I will gain fieldwork experience while, hopefully, tissuing and adding *Brachirus* specimens to the Florida Museum's collections that will be relevant to my research, which will presumably continue into Fall of 2024.

Closing Remarks

I could not have asked for a better experience under the Ichthyology Lab at the Florida Museum Undergraduate Internship Program. It has helped me build my knowledge, skills, and experience immensely in these past few months, while providing a comfortable educational environment that I looked forward to entering every day I conducted my research. Moreover, my internship has allowed me to form invaluable connections and enabled me to find opportunities within the Florida Museum's research network for a long time to come as I work toward my professional goals. While my project remains unfinished with much still to be answered, I'm excited to continue my work on it through the Fall of 2024, and attend the Lab's upcoming May conference and fieldwork trip abroad in Malaysia and Thailand, where I can continue to hone my experience.

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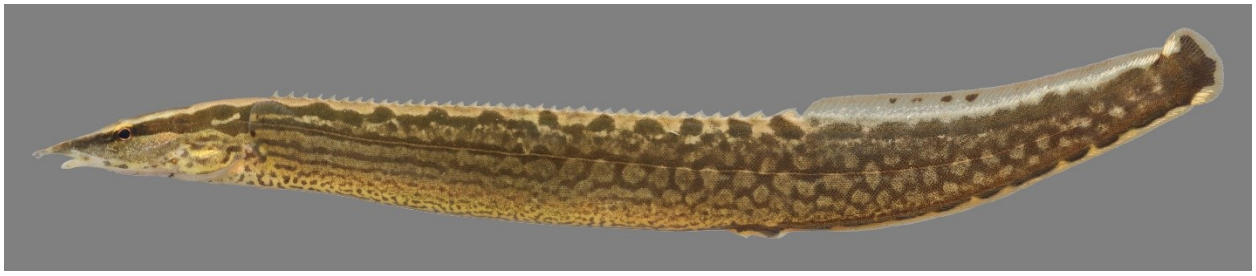

Figure 1.1 *Mastacembelus tinwini*

Figure 1.2 *Mastacembelus favus*

Figure 1.3 *Mastacembelus sp.* on microscope stage

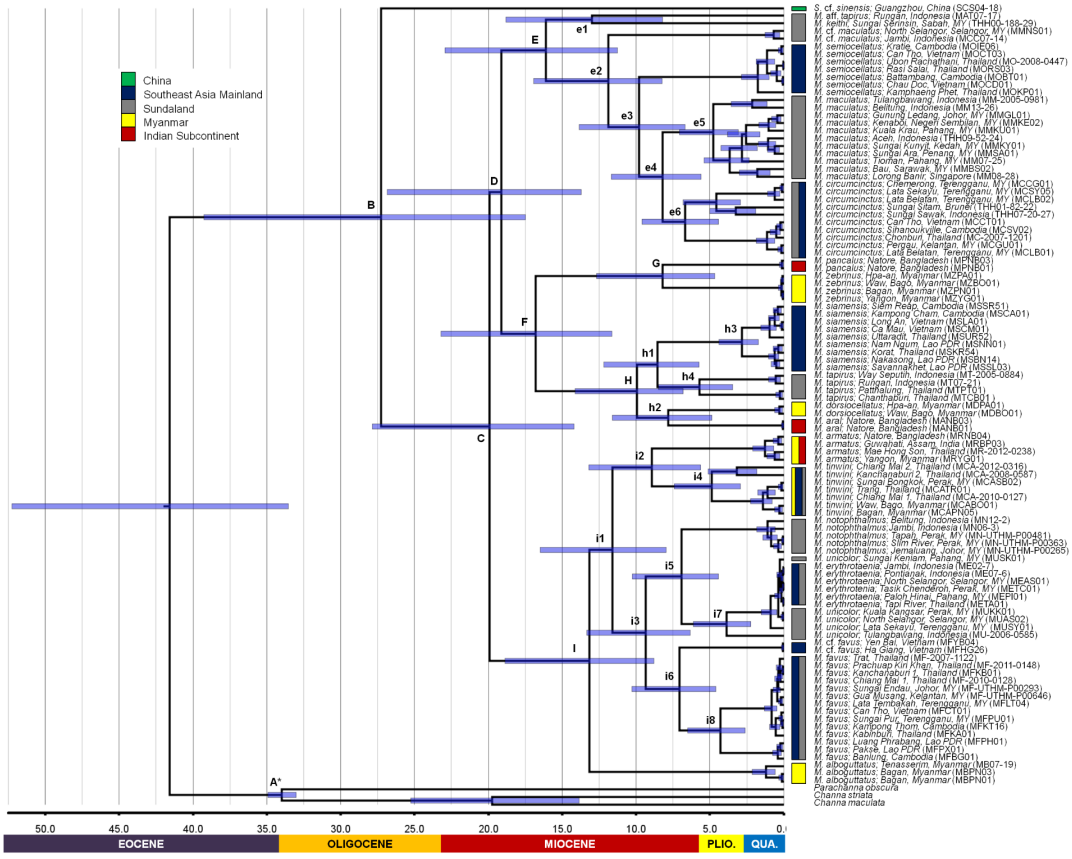

Figure 1.4 Phylogenetic tree of *Mastacembelus* specimens

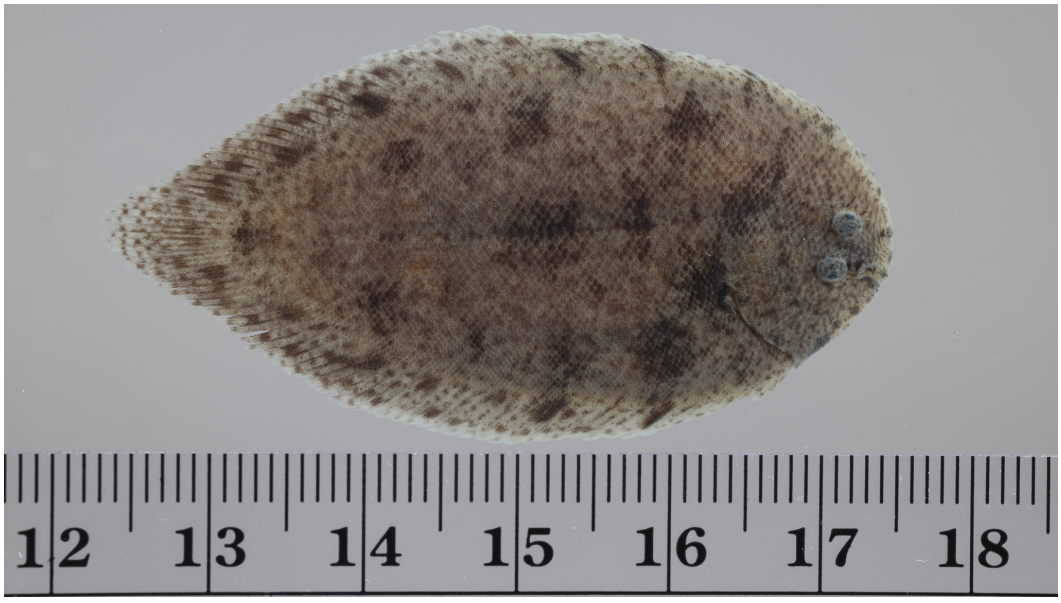

Figure 2.1 Preliminary image of *Brachirus cf. pan*

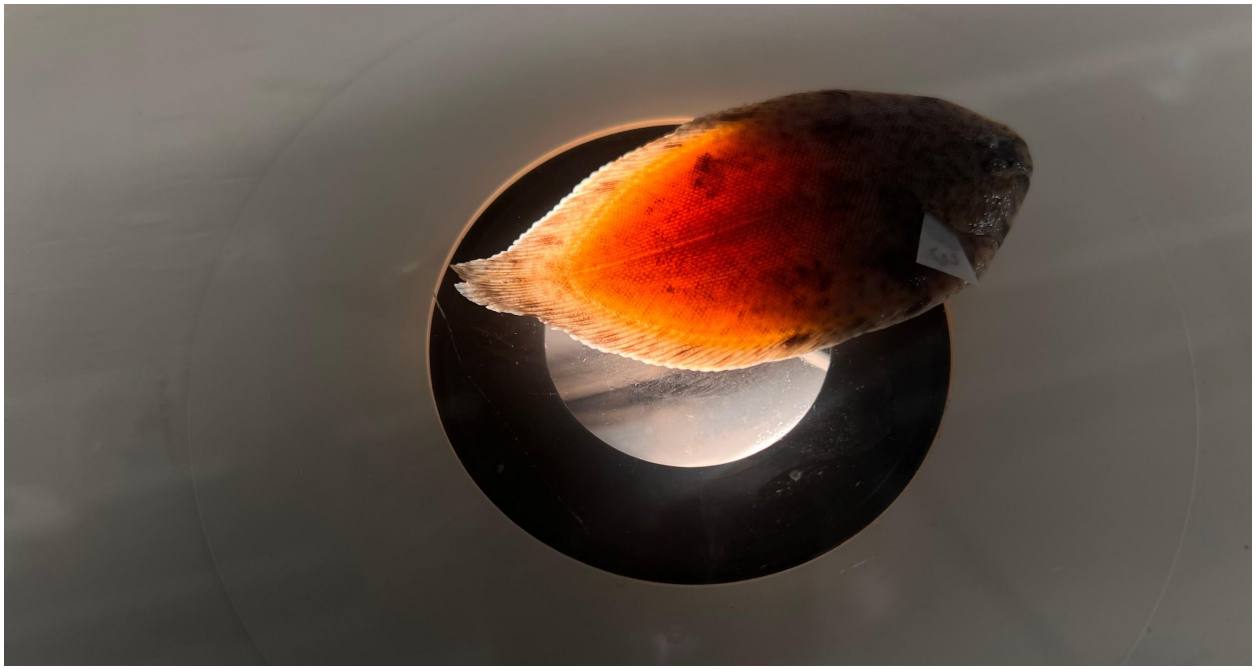

Figure 2.2 Light shone through *Brachirus cf. pan* specimen to count rays

Figure 2.3 Lateral line scale count of *Brachirus cf. pan* specimen, as indicated by white dots marking scales

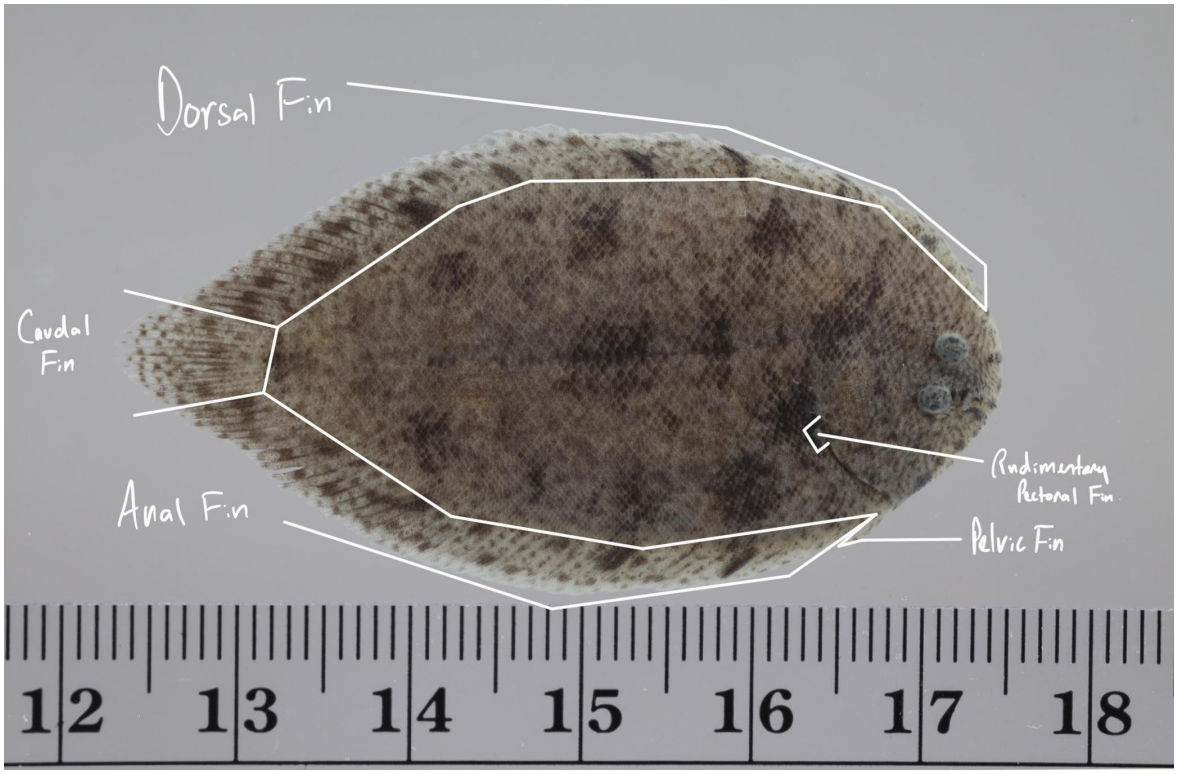

Figure 2.4 The external anatomy of *Brachirus cf. pan*

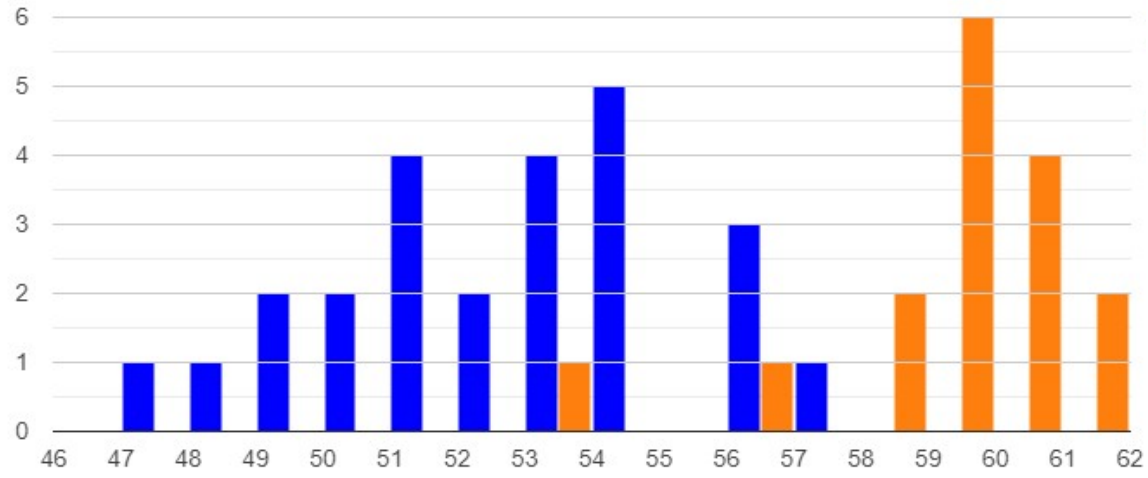

Figure 2.5 Preliminary histogram demonstrating bimodal distribution of dorsal fin ray count (x-axis) ranges and number of individuals (y-axis) of *Brachirus cf. pan* (blue) and *Brachirus pan* (orange)

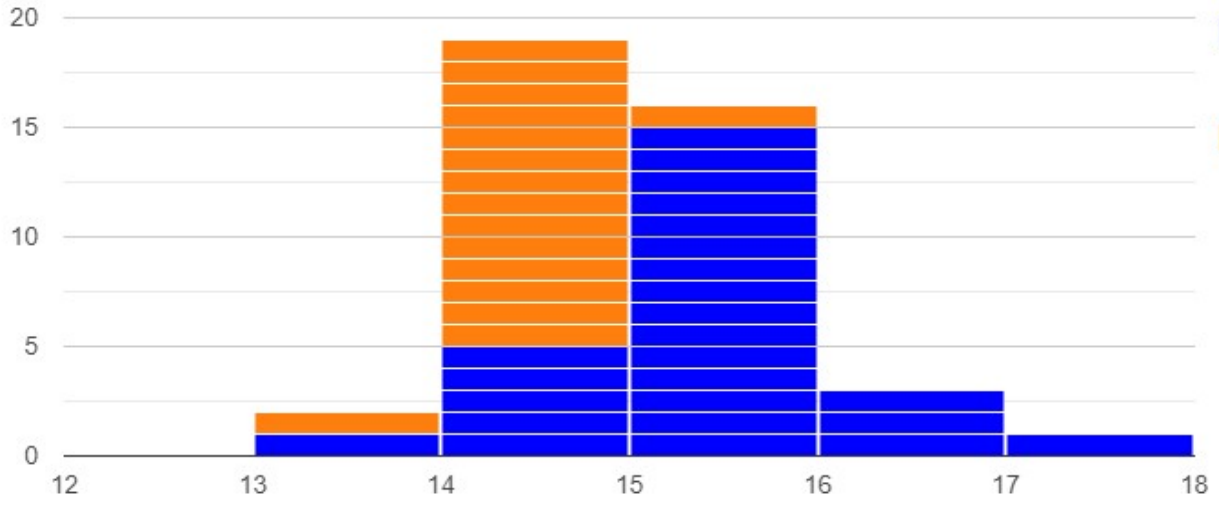

Figure 2.6 Preliminary histogram demonstrating bimodal distribution of caudal fin ray count (x-axis) ranges and number of individuals (y-axis) of *Brachirus cf. pan* (blue) and *Brachirus pan* (orange)

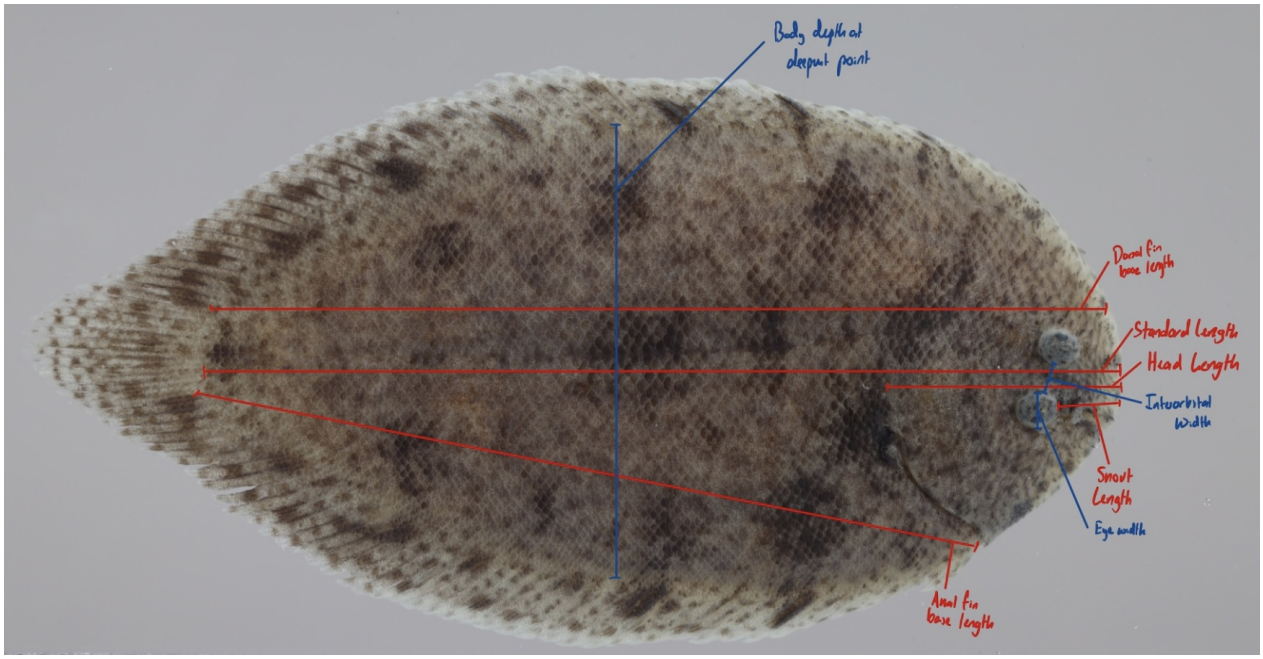

Figure 2.7 External measurements of *Brachirus cf. pan* used for holotype data

Figure 2.8 Image depicting distinct black mark on mouth of *Achiroides melanorhynchus* specimen (blind side)